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Expression and molecular analysis of *DsRed* and *gfp* fluorescent genes in tobacco (*Nicotiana tabacum* L.)

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ABSTRACT

Agrobacterium-mediated transformation of tobacco leaf disks with *Agrobacterium tumefaciens* (*A. t.*) strain LBA4404 and two plasmids (pCAMBIA1390-DsRed and pART27 2mgfp5-ER) was used for introducing red fluorescent gene (*DsRed*), green fluorescent gene (*gfp*) and corresponding selection genes (*hptII* for resistance to antibiotic hygromycin and *nptII* for resistance to kanamycin) into leaf discs of tobacco (*Nicotiana tabacum* L.). Epifluorescent microscopy with the appropriate set of filters did not reveal phenotypic expression of the *DsRed* gene in 6.9 % of regenerants and the *gfp* gene in 1.3 % of regenerants that were successfully grown on selective medium. The duplex PCR method also did not confirm the presence of fragments specific to *DsRed* or *gfp* genes in these regenerants, while the presence of fragments characteristic of selection genes *hptII* and *nptII* was confirmed. A built-in *nptII* gene mutation, a deletion, was detected in one regenerant. Out of the 139 regenerants generated after the transformation of *A. t.*-pCAMBIA1390-DsRed, 38 or 25.5 % successfully grew only on non-selective medium; after transformation with *A. t.*-pART27 2mgfp5-ER 9 or 5.4 % of the 161 generants grew successfully. PCR analysis confirmed in all regenerants the presence of fragments characteristic of both transgenes, which were not expressed or were silenced. The effectiveness of transformation after infection with *A. t.*-pCAMBIA1390-DsRed was 93.1 %, and 98.7 % after infection with *A. t.*-pART27 2mgfp5-ER. We established that both fluorescent genes are suitable for setting up a transformation system. The antibiotics hygromycin and kanamycin successfully prevented the growth of untransformed tissues, but the antibiotic timentin successfully prevented the growth of bacteria *A. t.* after the transformation.

Key words: *Nicotiana tabacum*, fluorescent genes, selection genes, transformation, exspression of transgenes, DNA analysis

IZVLEČEK

IZRAŽANJE IN MOLEKULSKA ANALIZA *DsRed* IN *gfp* FLUORESCENTNIH GENOV PRI TOBAKU (*Nicotiana tabacum* L.)

Z metodo posredne transformacije z vektorskim sistemom *Agrobacterim tumefaciens* (*A. t.*) sev LBA4404 in dvema plazmidoma (pCAMBIA1390-DsRed in pART27 2mgfp5-ER) smo v listne izsečke tobaka (*Nicotiana tabacum* L.) vnesli fluorescentni markerski gen za rdečo (*DsRed*) oz. zeleno (*gfp*) fluorescenco ter selekcijska gena za odpornost na antibiotik higromicin (*hptII*) oz. kanamicin (*nptII*). Z epifluorescentnim mikroskopom in ustreznim setom filtrov nismo zasledili fenotipskega izražanje *DsRed* gena pri 6,9 % regenerantih in *gfp* gena pri 1,3 % regenerantih, ki so uspešno rastli na selekcijskem gojišču. Pri teh regenerantih tudi z dupleks PCR metodo nismo potrdili prisotnosti fragmentov značilnih za *DsRed* oz. *gfp* gen, medtem ko smo potrdili prisotnost fragmentov značilnih za selekcijska gena *hptII* in *nptII*. Pri enem regenerantu smo v vgrajenem *nptII* genu zasledili mutacijo in sicer delecijo. Od 139 nastalih regenerantov, po transformaciji z *A. t.*-pCAMBIA1390-DsRed, jih je 38 oz. 25,5 % uspešno rastlo le na neselekcijskem gojišču, po transformaciji z *A. t.*-pART27 2mgfp5-ER je bilo takih 9 oz. 5,4 % od 161 nastalih. Pri vseh smo s PCR analizo potrdili prisotnost fragmentov značilnih za oba transgena, ki se nista izražala oz. sta bila utišana. Učinkovitost transformacije po okužbi z *A. t.*-pCAMBIA1390-DsRed je bila 93,1 %, po okužbi z *A. t.*-pART27 2mgfp5-ER pa 98,7 %. Ugotovili smo, da sta oba fluorescentna gena primerna za vzpostavitev transformacijskega sistema. Antibiotika higromicin in kanamicin sta uspešno preprečila rast netransformiranih tkiv, antibiotik timentin pa je uspešno preprečil rast bakterije *A. t.* po transformaciji.

Ključne besede: *Nicotiana tabacum*, fluorescentni geni, selekcijski geni, transformacija, izražanje transgenov, DNA analiza

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1 INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) has been shown to be a very suitable model plant for genetic transformation because it grows quickly and successfully in tissue culture. Regeneration from leaf explants is fast and efficient (Stolarz *et al*., 1991). Tobacco was the first transformed plant. In 1983, the gene for resistance to the antibiotic kanamycin was inserted in tobacco (Horsch *et al*., 1984) by the indirect method of transformation using the soil phytopathogenic bacterium *Agrobacterium tumefaciens* (*A. t.*). There are currently two authorizations for commercial production of tobacco with tolerance to the herbicide oxynil in the European Union and for tobacco with a reduced content of nicotine in the United States of America (CERA, 2012).

The development of plant regeneration procedures and the discovery of new techniques of gene transfer in plant cells have provided opportunities for practical application of genetic engineering to modify and improve important agricultural crops. Genetic transformation has become useful in improving plant properties and for the detection of gene functions in plants (Rao *et al*., 2009).

In most cases, only a small proportion of plant cells transform, so it is necessary to enter a selection gene together with the desired gene, by which transformed cells can be distinguished from non-transformed ones. Selection can be positive or negative (Miki and McHugh, 2004). The correct concentration of antibiotic or selection agent must be made, which completely prevents regeneration of non-transformed cells and, at the same time, minimizes the number of non-transformed regenerants that develop in cultured explants due to the detoxification activity of the surrounding transformed cells (Park *et al*., 1998).

Using selection genes for antibiotic resistance and resistance to herbicides gives rise to most concerns in the commercial use of transgenic plants. DNA transfer between transgenic plants and other organisms is unlikely. *NptII* gene does not signify any risk to human and animal health (Fuchs *et al*., 1993). Nevertheless, the complete removal of the selection gene is desirable because selection genes are no longer required after selection. It would probably also contribute to the greater acceptance of genetically modified plants. There are quite a few successful methods of removing these genes (Afolabi, 2007). European regulations governing the release of genetically modified plants in the field prohibit the inclusion of genes for resistance to antibiotics.

Test or marker genes are genes whose gene product can be visually identified and their location determined. They enable the quick identification of transformed tissues. Marker genes that can be detected through other senses, such as taste or smell, can also be useful (Witty, 1989).

Genes for the synthesis of fluorescent proteins have advantages over other marker genes because they can be visually detected in living cells without the use of invasive procedures using substrates and products that could diffuse within or between cells. Transformed cells, in which these genes express, can be identified shortly after the transformation and it can be determined whether they are dividing (Harper *et al*., 1999). Fluorescent proteins in fusion with any proteins allow monitoring of the location, movement and activities of proteins in living cells. They can be used as markers for tracing and tracking proteins, discovering interactions between proteins and tracking the destiny of proteins in the cell (Lippincott-Schwartz and Patterson, 2003). Fluorescent proteins can also be used to monitor the destiny of transgenes introduced into cultivated plants and their impact on the environment (Stewart, 2005).

The best known fluorescent protein is the green fluorescent protein (GFP) from the jellyfish (*Aequorea victoria*) (Haseloff and Amos, 1995), which emits green fluorescence under illumination with long-wave UV light. The wild-type *gfp* gene was modified in such a way that it effectively reflects in plants and the spectral properties and fluorescence were changed and improved (Reichel *et al*.,1996; Haseloff *et al*., 1997).

Red fluorescent protein DsRED was isolated from coral (*Discosoma* sp.) and, using appropriate filters, can be more easily separated from autofluorescent chlorophyll (Matz *et al*., 1999) than GFP. *DsRed* gene is used as a marker gene for transient and stable transformation of tobacco and,

in combination with the *gfp* gene, is suitable for simultaneous monitoring of the expression of the two genes (Jach *et al*., 2001).

Many fluorescent proteins that are useful for studies of genetic transformations have been discovered. Orange fluorescent proteins have proved to be very successful as marker genes, especially TdTomato-ER, which fluoresces the brightest of all fluorescent proteins, followed by Morang-ER (Mann *et al*., 2012).

In this study, we monitored the phenotypic expression of *DsRed* and *gfp* fluorescent genes and selection genes *hpII* and *nptII*, as well as molecular analysis of their insertion into the genome of tobacco.

2 MATERIALS AND METHODS

2.1 Plant material

The leaves of micropropagated tobacco variety Havana 38 were used for transformation.

2.2 Bacteria and plasmids

The commercial bacterium *A. t*. strain LBA4404 was chosen for gene insertion, in which modified plasmid pCAMBIA1390-DsRed (Cambia, 1997; Škof, 2008) or plasmid pART27 2mgfp5-ER was introduced by electroporation (Gleave, 1992).

Plasmid pDsRed-Express contains the gene *DsRed-Express*, which is a form of red fluorescent protein DsRED. For preparation of the plasmid vector with the *DsRed* marker gene, the gene for DsRED-Express protein from plasmid pDsRed-Express (BD Bioscience Clontech, Palo Alto, USA) was used, which was equipped with a constitutive CaMV35S promoter from the vector pBIN m-gfp5-ER and included in the plasmid vector pCAMBIA1390 (Cambia, Canberra, Australia) (Škof, 2008). In addition to the *DsRed* marker gene, the plasmid contained the plant selection *hptII* gene for resistance to the antibiotic hygromycin for selection of transformed plant tissues and the *nptII* selection gene for resistance to the antibiotic kanamycin for selection of transformed bacteria (Table 1).

Plasmid pART27 2mgfp5-ER is a binary vector, which was prepared in the laboratory of Prof. Dr. C. C. Eady (Institute of Crop and Food Research, Christchurch, New Zealand), in such a way that two repetitions of mgfp5-ER gene from the vector pBIN m-gfp5-ER were included in the plasmid vector pART27 at location *Spe*I of the multiple cloning site (MCS). pART27 vector contains the selection gene *spec* for resistance to the antibiotic spectinomycin for selection of transformed bacteria and the *nptII* gene for resistance to the amino glycoside antibiotics geneticin and kanamycin for selection of transformed plant tissues (Table 1) (Gleave, 1992).

Table 1. Plasmids with bacterial and plant selection and fluorescent genes

Plasmid	Bacterial selection	Gene	Plant selection	Gene	Fluorescent protein	Gene
pCAMBIA1390-DsRed	kanamycin	*nptII*	hygromycin	*hptII*	DsRED	*DsRed*
pART27 2mgfp5-ER	spectinomycin	*spec*	kanamycin, geneticin	*nptII*	GFP	*gfp*

2.3 Agrobacterium-mediated transformation

Transformation of tobacco with *A. t*. was performed using a slightly modified method of transformation of leaves after Horsch *et al*. (1985) and Fisher and Guiltinan (1995). Tobacco leaves were cut under sterile conditions to explants of about 1 cm^2. For plasmid pCAMBIA1390-DsRed 105 leaf explants were prepared and for plasmid pART27 2mgfp5-ER 103 explants.

Bacterial suspensions of *A. t*., with the appropriate plasmid included, were incubated overnight at 28 °C and shaken at 120 rev./min. in *YEB* medium

[sucrose 5 g/l, peptone 5 g/l, beef extract 5 g/l, yeast extract 1 g/l, $MgSO_4 \times 7H_2O$ 1 g/l; pH 7.0]. Bacterial suspensions were centrifuged at 5000 rpm for 5 min. The supernatant was removed and the *Agrobacterium* pellet was resuspended in ½*MS* liquid basal medium (Murashige and Skoog, 1962) at an optical density of OD_{600nm} = 0.5 (5×10^6 cells/ml). Tobacco leaf explants were incubated in Petri dishes for approximately 20 min in the *A. t.* suspension with the appropriate plasmid and then gently dried on sterile filter paper in a laminar flow cabinet and co-cultivated on *MSr* medium with the addition of [Fe-Na_2-EDTA 0.1 mg/l, thiamine 0.1 mg/l, BAP 1.0 mg/l, NAA 0.1 mg/l, acetosyringone 100 µM, agar 8 g/l; pH 5.8] (Stolarz et al., 1991). After three days of co-cultivation, they were washed twice in a solution of antibiotic timentin 200 mg/l [100:1 (w/w) ticarcillin: clavulanic acid] and air-dried.

Then, the leaf explants were transferred onto selective *MSr* medium without acetosyringone and with the addition of timentin 150 mg/l to prevent the growth of *A. t.* bacteria and an appropriate selection antibiotic (Table 1). The minimum effective concentration of selection antibiotics was chosen, i.e., 25 mg/l hygromycin antibiotic for the selection of tobacco transformants after infection with *A. t.*-pCAMBIA1390-DsRed and 300 mg/l of the antibiotic kanamycin after infection with *A. t.*-pART27 2mgfp5-ER. Explants were cultured in a growth chamber at a 16/8 hour photoperiod and a temperature of 24 ± 1 °C, illuminated with about 40 µmol/m^2s. After five weeks, the explants were transferred or sub-cultured on the appropriate fresh selective *MSr* medium. The resulting regenerants were transferred onto *MSm* medium with the addition of the appropriate selection antibiotic, without timentin. After five weeks, the regenerants that had successfully grown were transferred to the appropriate *MS* selective medium. Regenerants that had grown poorly or had begun to decay were transferred to *MSm* medium without selection antibiotics in order to determine the presence of the selection transgene and its expression.

2.4 Expression of *DsRed* and *gfp* genes

Expression of fluorescent marker genes in the regenerants was observed after infection at the beginning of regeneration in the rising stages of pessaries or inception. Transformed tobacco explants were examined by epifluorescent microscope (Nikkon SMZ 1000) at 20× magnification and appropriate filters for the detection of the red fluorescence *DsRed* gene and the green fluorescence *gfp* gene. For the detection of red fluorescent protein DsRED-Express (plasmid pCAMBIA1390-DsRed), which has an excitational maximum at 557 nm and emission maximum at 579 nm, a set of filters with EX 546/10 nm, DM 575 nm and BA 620 nm was used. For the detection of green fluorescent protein m-GFP5-ER (plasmid pART27 2mgfp5-ER), which has an excitational maximum at 484 nm and emission maximum at 510 nm, a set of filters with EX 480/40 nm, DM 505 nm and BA 535/50 nm was used.

2.5 Molecular analysis of plant material by PCR method

For DNA analysis of the presence of transgenes in tobacco regenerants, the complete DNA was isolated, the overall concentration of isolated DNA was measured, dilutions to 20 ng/µl were prepared, and polymerase chain reaction (PCR) and fragment analysis amplified with agarose gel electrophoresis were performed.

Isolation of DNA from plant tissue

Overall genomic DNA from the leaves of non-transformed tobacco were isolated - negative control and transformed regenerants, as well regenerants that had only prospered on non-selective mediums without antibiotics, according to the method of Kump *et al*. (1992).

Measuring the concentration of DNA by fluorimeter

The concentration of isolated DNA in solution was measured using a DNA fluorimeter DyNA Quant™ 200 (GE Healthcare). A working solution was prepared from 10×TNE buffer [0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA; pH 7] and colorant Hoechts 33258 added in a final concentration of 0.1 µg/ml. Calf thymus DNA (1 mg/ml DNA in 1×TNE buffer) was used for calibration of the fluorimeter. For each sample of DNA, 2 ml of the working solution and 2 µl DNA sample were added to the cuvette, the mixture stirred and the concentration of DNA then measured. DNA samples were diluted to 20 ng/µl.

Polymerase chain reaction (PCR)

Specific multiplication of *DsRed* and *gfp* genes was carried out in duplex PCR reactions using two pairs of primers (Table 2). For analysis of the inclusion of *DsRed* and *hptII* genes in the plant genome after transformation with *A. t*. and the plasmid pCAMBIA1390-DsRed, a combination of REDfor/RED2right and HPTII-for/HPTII-rev1 primers was used. A combination of GFP1a/GFP1b and NPTII1a/NPTII1b primers was used for analysis of the inclusion of mgfp5-ER and *nptII* genes after transformation with *A. t*. and the plasmid pART27 2mgfp5-ER.

PCR reaction mixtures were prepared in a laminar flow cabinet. A 5 µl DNA sample was pipetted into the PCR microfuge. Samples were centrifuged at 1000 rpm/min and 1×PCR buffer [10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet P40] (Fermentas), 2 mM $MgCl_2$, 0.2 mM of each deoxynucleotide (dATP, dGTP, dCTP, dTTP), 4×0.5 µM suitable primer and 0.5 units of enzyme Taq DNA polymerase (Fermentas) were added. The final volume of the reaction mixture in which multiplication of DNA was conducted, was 25 µl. The PCR reaction was carried out in a cyclical thermostat GeneAmp PCR System 9700 (PE Applied Biosystems, USA) using the modified temperature model (Lakshmi *et al*., 1998):

- initial denaturation of 5 min at 94 °C,
- 33 repeated cycles: - denaturation of DNA 1 min at 94 °C,

- annealing of primers 1 min at 58 °C,
- synthesis of DNA fragments 1.5 min at 72 °C,

- final incubation 7 min at 72 °C.

Samples were stored at 12 °C until further analysis.

Table 2: DNA nucleotide sequences of primers for an individual transgene and the expected length of the amplified fragment

Primer	The nucleotide sequence 5' - 3'	Expected length of the fragment (bp)
GFP1a	AGT GGA GAG GGT GAA GGT GAT G	422
GFP1b	TTG TGG CGG GTC TTG AAG TTG G	
REDfor	AGG ACG TCA TCA AGG AGT TCA T	211
RED2right	GTG CTT CAC GTA CAC CTT GGA G	
HPTII-for	ATG ACC GCT GTT ATG CGG CCA TTG	641
HPTII-rev1	AAA AAG CCT GAA CTC ACC GCG ACG	
NPTII1a	GAG GCT ATT CGG CTA TGA CTG	650
NPTII1b	ATG GGG AGC GGC GAT ACC GTA	

Analysis of DNA fragments by agarose gel electrophoresis

For the separation of DNA fragments, horizontal electrophoresis was used on a 1.4 % gel [1.4 % SeaKem LE agarose (Cambrex, USA), 1×TBE buffer, Ethidium bromide 0.5 µg/ml], which was installed in an electrophoretic tank (Bio-Rad Sub-Cell, model 192) immersed in 1×TBE buffer [890 mM Tris, 890 mM boric acid, 10 mM EDTA]. Five µl dispensing dye BPB [12.5% (w/v) ficol 400, 0.2% (w/v) bromophenol blue, 6.7% (v/v) 10×TBE] were added to the samples, which were stirred and 17 µl of sample was applied on the agarose gel. In addition to the samples, on the gel were also applied: DNA isolated from control (non-transformed tobacco), corresponding pure plasmid (isolated from *E. coli*), a blind sample (all components of the reaction mixture except the DNA; instead of adding 5 µl of water) and a size standard (GeneRulerTM 100 bp DNA Ladder Plus (Fermentas) with 14 fragments: 3000, 2000, 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp). Electrophoresis was carried out at 140 V in the anode direction for about 1 hour and 30 min. The gel contained 0.5 µg/ml ethidium bromide, which, in a complex with double stranded DNA molecules, allowed their detection under UV light (302 nm). Gels were observed using a transilluminator TMF-30 (UVP Inc., UK) and photographed with a digital camera.

3 RESULTS AND DISCUSSION

3.1 Regeneration of tobacco leaf explants and phenotypic transgene expression

Leaf explants, after incubation with *A. t.* and an appropriate plasmid, were co-cultivated on *MSr* medium with added acetosyringone 100 μM, in order to increase the infection, as described by Sunilkumar *et al*. (1999). In nature, phenolic substances such as acetosyringone, which are released on wounding of plant tissue, trigger the activation of genes for virulence (*vir* genes) in infection with *Agrobacterium* (Gelvin, 2003). We obtained a high percentage of transformed regenerants, which can be attributed to the acetosyringone attached to the *MSr* medium in the period of co-cultivation. After the completion of co-cultivation, timentin 150 mg/l was added to the *MSr* medium, which effectively inhibited the growth of the *A. t.* bacteria but did not adversely affect regeneration. The regenerants on the medium with timentin were distinctly dark green. Nauerby *et al*. (1996) reported that timentin in this concentration completely prevented the multiplication of *A. t.* and positively impacted on the regeneration of leaf and cotyledon explants of tobacco. Similarly, Cheng *et al*. (1998) emphasized that timentin is just as effective as carbenicillin and cefotaxime and does not have an inhibitory effect on the regeneration of shoots in tobacco and Siberian elm.

Germs of the first regenerants occurred after 10-12 days, regardless of the built-in genetic construct. Regeneration was mostly direct, without an intermediate callus, as noted by Stolarz *et al*. (1991).

After five weeks, a large number of regenerants was observed. Regenerants from the leaf explants, in which phenotypic expression of the inserted fluorescent genes was observed, were transferred onto *MSm* medium with the addition of an appropriate selection antibiotic. After five weeks, regenerants that had grown poorly were transferred to *MSm* medium without added antibiotics, other regenerants were transferred to appropriate fresh *MSm* selective medium. The percentage of surviving and failed regenerants is given in Table 3.

Table 3: Percentage of surviving and failed regenerants of tobacco in the appropriate selective or non-selective *MSm* media after transformation with *A. t.* and plasmid pCAMBIA1390-DsRed or plasmid pART272mgfp5-ER

Plasmid	Percentage of regenerants on the medium				
	selective		non-selective		
	survived	failed	transferred	survived	failed
pCAMBIA1390-DsRed	67.8	6.7	25.5	25.5	0
pART27 2mgfp5-ER	90.4	1.2	8.4	5.4	3.0

After transformation with *A. t.*-pCAMBIA1390-DsRed, 149 regenerants were obtained from 105 explants. After sub-cultivation on selective *MSm* medium with 25 mg/l hygromycin, 101 or 67.8 % of regenerants grew successfully, and 10 or 6.7 % failed. On non-selective medium, all 38 regenerants (25.5 % out of 149), were successfully grown. With *A. t.* pART272mgfp5-ER, 103 tobacco explants were transformed and 168 regenerants were obtained. On the selective *MSm* medium containing 300 mg/l kanamycin, 152 or 90.4 % of regenerants successfully grew, and 2 or 1.2 % failed. Fourteen or 8.4 % of regenerants that had grown poorly or had begun to deteriorate on the selective medium, were transferred to non-selective medium. Out of them, 9 or 5.4 % grew successfully, while 5 or 3 % failed (Tables 3 and 4).

With 6.9 % of regenerants examined by epifluorescent microscope, no red fluorescence DsRED protein was observed and with 1.3 % of

regenerants no green fluorescence m-GFP5-ER protein was detected, despite the fact that they had successfully grown on the selection media (Tables 4 and 5). There had been non-expression or silencing of the fluorescent genes.

3.2 Molecular analysis of transgene integration

DNA analysis was performed on all 300 surviving regenerants, whether or not they had been transferred to non-selective *MSm* medium (Tables 4 and 5).

Table 4: Number and percentage of regenerants and transgenes of tobacco after transformation with *A. t.* - pCAMBIA1390-DsRed on selective and non-selective *MSm* medium.

Number of regenerants on *MSm* media	Number or percentage of transgenes					
	DsRed in *hptII*		*DsRed*		*hptII*	
	number	percentage	number	percentage	number	percentage
101 on selective	94	93.1	0	0.0	7	6.9
38 on non-selective	38	100	0	0.0	0	0.0
139 together	132	95.0	0	0.0	7	5.0

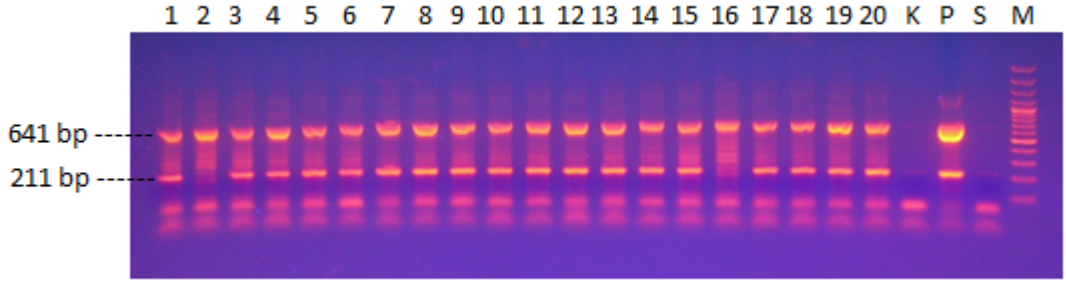

Figure 1: Multiplied DNA fragments in duplex PCR reaction with the pair of primers for the *DsRed* gene (211 bp) and the pair of primers for the *hptII* gene (641 bp). The figure shows only the first 20 regenerants of 139. (1-101 - transformed regenerants of tobacco grown on selective medium, 102-139 - transformed regenerants of tobacco grown on non-selective medium), K - control - non-transformed tobacco, P - plasmid pCAMBIA1390-DsRed, S - blind sample M - size standard

In all regenerants of tobacco transformed with *A. t.*-pCAMBIA1390-DsRed that were grown on selective medium (regenerants 1-101) and regenerants that, due to poor growth or decay were transferred to non-selective medium (regenerants 102-139), the presence of fragment length 641 bp (selection *hptII* gen) was detected. In regenerants 2, 16, 49, 51, 53, 61 and 78, only the presence of fragment length 641 bp was detected, but not the presence of fragment length 211 bp, which would have confirmed the presence of marker *DsRed* gene. On the selective medium, 93.1 % of regenerants included both transgenes from the genetic construct, thus both *DsRed* and *hptII,* and 6.9 % regenerants only part of the genetic construct, with the *hptII* gene (Figure 1 - only 20 out of 139 regenerants are presented, Table 4).

For all 38, or 25.5% of regenerants that were transferred to the non-selective medium, molecular analysis determined the presence of both transgenes from the gene construct (Table 4). Epifluorescent microscopy revealed the presence of protein DsRED in all regenerants, at least mosaic expression. Despite the confirmed presence of selection gene *hptII*, which should disintegrate the hygromycin added to the medium and allow normal growth and development of regenerants, they decayed. This suggests that gene *hptII* phenotypically did not express or was silenced (Figure 1 - only 20 out of 139 regenerants are presented, Tables 3 and 4).

Table 5: Number and percentage of regenerants and transgenes of tobacco after transformation with *A. t.*-pART27 2mgfp5-ER on selective and non-selective *MSm* medium

Number of regenerants on *MSm* medium	Number or percentage of transgenes					
	gfp in *nptII*		*gfp*		*nptII*	
	number	percentage	number	percentage	number	percentage
152 on selective	150	98.7	0	0.0	2	1.3
9 on non-selective	9	100	0	0.0	0	0.0
161 together	159	98.8	0	0.0	2	1.,2

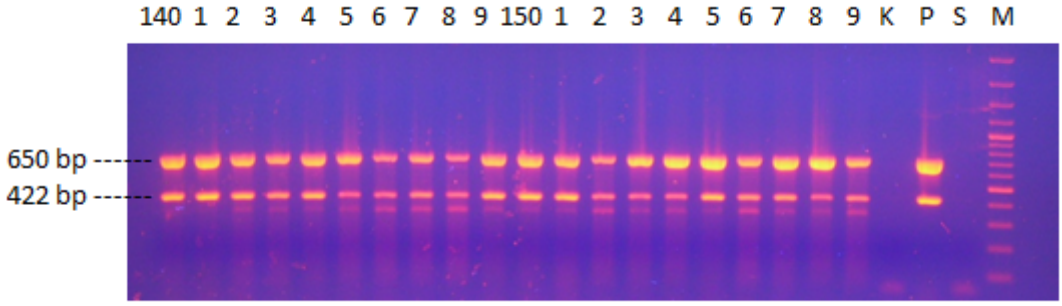

Figure 2: Multiplied DNA fragments in duplex PCR reaction with the pair of primers for the *gfp* gene (422 bp) and the pair of primers for the *nptII* gene (650 bp). The figure shows only the first 20 regenerants of 161. (140-291 - transformed regenerants of tobacco grown on the selective medium, 292-300 - transformed regenerants of tobacco grown on the non-selective medium), K - control - non-transformed tobacco, P - plasmid pART27 2mgfp5-ER, S - blind sample M - size standard.

In the regenerants of tobacco transformed with *A. t.*-pART27 2mgfp5-ER, which were grown on the selective medium (regenerants 140-291), with the exception of regenerants 225 and 245, in which only a fragment length of 650 bp (*nptII* selection gene) was multiplied, the presence of both transgenes (*gfp* and *nptII*) was confirmed. With regenerant 245, a slightly shorter replicate fragment of 650 bp specific to the *nptII* gene was multiplied. This was probably the result of mutation, the deletion of an individual nucleotide or nucleotides. The deletion of embedded transgenes was reported in a small number transformed plants by Hiei *et al.* (1994) in rice, Yao *et al.* (1995) in apple, Mercuri *et al.* (2000) in African violets, Atkinson and Gardner (1991) in *Solanum muricatum* and Atkinson and Gardner (1993) in tamarillo. On the selective medium, 98.7 % of regenerants included both transgenes from the genetic construct, with *gfp* and *nptII* genes, only 1.3 % of the genetic construct with *nptII* gene, (Figure 2 - shows only 20 of 161 regenerants and Table 5). No data were found in the literature on only part-installation of the genetic construct.

In all 9 or 5.4% regenerants of tobacco grown on non-selective medium (regenerants 292-300), the presence of fragment length 422 bp (marker *gfp* gene) and 650 bp (*nptII* selection gene) was found. On the non-selective medium, 3.0% of regenerants failed (Table 3).

Non-expression of the selection transgene, which was observed in regenerants that, due to degradation were transferred from the selective to non-selective medium but the presence was confirmed by molecular analysis, may be the result of installation of the transgene on the range of the plant chromosome that is transcriptionally inactive, mutations or gene silencing. Other authors have also reported that some regenerants that were negative a marker enzyme of β-glucuronidase (GUS), had the presence of the *gus* gene confirmed by hybridization (Hiei *et al.*, 1997) or by PCR analysis (Yao *et al.*, 1995, Mercuri *et al.*, 2000).

4 CONCLUSION

Timentin at a concentration of 150 mg/l completely prevented the growth of *A. t*. LBA4404 bacteria with included plasmid pCAMBIA1390-DsRed or pART27 2mgfp5-ER and had no negative impact on regeneration or the growth and development of regenerants. The success of transformation, with the confirmed presence of both transgenes from the gene construct, both marker *DsRed* gene and selection *hptII* gene, was 93.1 %, while the genetic construct with marker *gfp* gene and selection *nptII* gene was 98.7 %. In regenerant designated 245, the fragment multiplied slightly less than the expected 650 bp specific to the *nptII* gene. This was probably the result of mutation, the deletion of an individual nucleotide or nucleotides. With the transfer of regenerants that had grown poorly on the selective medium to the non-selective medium, we confirmed the non-expression or silencing of selection transgenes, the presence of which was confirmed on the DNA level.

5 REFERENCES

Afolabi A.S. 2007. Status of clean gene (selection marker-free) technology. African Journal of Biotechnology, 6: 2910-2923

Atkinson R.G., Gardner R.C. 1991. *Agrobacterium*-mediated transformation of pepino and regeneration of transgenic plants. Plant Cell Reports, 10: 208-212

Atkinson R.G., Gardner R.C. 1993. Regeneration of transgenic tamarillo plants. Plant Cell Reports, 12: 347-351

Cambia. 1997. pCAMBIA vector release manual version 3.05. Camberra, Center for the application of molecular biology to international agriculture: 6 p.

CERA. 2012. GM crop database. Center for Environmental Risk Assessment (CERA). Washington D.C. ILSI Research Foundation http://cera-gmc.org/index.php?action=gm_crop_database

Cheng Z.M., Schnurr J.A., Kapaun J.A. 1998. Timentin as an alternative antibiotic for suppressin of *Agrobacteriu tumefaciens* in genetic transformation. Plant Cell Reports, 17: 646-649

Gelvin S.B. 2003. *Agrobacterium*-mediated plant transformation: the biology behind the "gene-jockeying tool". Microbiology and Molecular Biology Reviews, 67: 16-37

Gleave A.P. 1992. A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Molecular Biology, 20: 1203-1207

Fisher D.K., Guiltinan M.J. 1995. Rapid, efficient production of homozygous transgenic tobacco plants with *Agrobacterium tumefaciens*: a seed-to-seed protocol. Plant Molecular Biology Reporter, 13, 3: 278-289

Fuchs R.L., Ream J.E., Hammond B.G., Naylor M.W., Leimgruber R.M., Berberich S.A. 1993. Safety assessment of the neomycin phosphotransferaseII (NPTII) protein. Bio/Technology 11: 1543-1547

Harper B.K., Mabon S.A., Leffel S.M., Halfhill M.D., Richards H.A., Moyer K.A., Stewart C.N. 1999. Green fluorescent protein as a marker for expression of a second gene in transgenic plants. Nature Biotechnology, 17: 1125-1129

Haseloff J., Amos B. 1995. GFP in plants. Trends in Genetics 11: 328-329

Haseloff J., Siemering K.R., Prasher D.C., Hodge S. 1997. Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. Proceedings of the National Academy of Science of the United States of America, 94: 2122-2127

Hiei Y., Ohta S., Komari T., Kumashiro T. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant Journal, 6: 271-282

Hiei Y., Komori T., Kubo T. 1997. Transformation of rice mediated by *Agrobacterium tumefaciens*. Plant Molecular Biology, 35: 205-218

Horsch R.B., Fraley R.T., Rogers S.G., Sanders P.R., Lloyd A., Hoffmann N. 1984. Inheritance of functional foregin genes in plants. Science, 223: 496-498

Horsch R.B., Fry J.E., Hoffmann N.L., Eichholtz D., Rogers S.G., Fraley R.T. 1985. A simple and

general method for transferring genes into plants. Science, 227: 1229-1231

Jach G., Binot E., Frings S., Luxa K., Schell J. 2001. Use of red fluorescent protein from *Discosoma* sp. (dsRED) as a reporter for plant gene expression. The Plant Journal, 28: 483-491

Kump B., Svetek S., Javornik B. 1992. Izolacija visokomolekularne DNA iz rastlinskih tkiv. Zbornik Biotehniške fakultete Univerze v Ljubljani - Kmetijstvo, 59: 63-66

Lakshmi Sita G., Sreenivas G.L., Bhattacharya A. 1998. *Agrobacterium* mediated transformation of sandalwood (*Santalum album* L.) a tropical forest tree. Plant Tissue Culture and Biotechnology, 4, 3-4: 189-195

Lippincott-Scgwartz J., Patterson G.H. 2003. Development and use of fluorescent protein markers in living cells. Science, 300, 5616: 87-91

Mann D.G.J., Abercrombie L.L., Rudis M.R., Millwood R.J., Dunlap J.R., Stewart C.N. 2012. Very bright orange fluorescent plants: endoplasmatic reticulum targeting of orange fluorescent proteins as visual reporters in transgenic plants. BMC Biotechnology, 12: 17 p.

Matz M.V., Fradkov A.F., Labas Y.A., Savitsky A.P., Zaraisky A.G., Markelov M.L., Lukyanov S.A. 1999. Fluorescent proteins from nonbiluminescent *Anthozoa* species. Nature Biotechnology, 17: 969-973

Mercuri A., De Benedetti L., Burchi G., Schiva T. 2000. *Agrobacterium*-mediated transformation of African violet. Plant Cell, Tissue and Organ Culture, 60: 39-46

Miki B., McHugh S. 2004. Selectable marker genes in transgenic plants: applications, alternatives and biosafety. Journal of Biotechnology, 107: 193-232

Murashige T., Skoog H. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-479

Nauerby B., Billing K., Wyndaele R. 1996. Influence of the antibiotic timentin on plant regeneration compared to carbenicillin and cefotaxime in concentrations suitable for elimination of *Agrobacterium tumefaciens*. Plant Science, 123: 169-177

Park S.H., Rose S.C., Zapata C., Srivatanakul M., Smith R.H. 1998. Cross-protection and selectable marker genes in plant transformation. In Vitro Cellular and Developmental Biology Plant, 34, 2: 117-121

Rao A.Q., Bakhsh A., Kiani S., Shahzad K., Shahid A.A., Husnain T., Riazuddin S. 2009. The myth of plant transformation. Biotechnology Advances, 27: 753-763

Reichel C., Mathur J., Ecke P., Langenkemper K., Koncz C., Schell J., Reiss B., Maas C. 1996. Enhanced green fluorescence by the expression of an *Aequorea victoria* green fluorescent protein mutant in mono- and dicotyledonous plant cells. Proceedings of the National academy of Sciences of the United States of America, 93: 5888-5893

Stewart C.N. 2005. Monitoring the presence and expression of transgenes in living plants. Trends in Plant Science, 10: 390-396

Stolarz A., Macewicz J., Lörz H. 1991. Direct somatic embryogenesis and plant regeneration from leaf explants of *Nicotiana tabacum* L. Journal of Plant Physiology, 137: 347-357

Sunilkumar G., Vijayachandra K., Veluthambi K. 1999. Preincubation of cut tobacco leaf explants promotes *Agrobacterium*-mediated transformation by increasing *vir* gene induction. Plant Science, 141: 51-58

Škof S. 2008. Izražanje markerskih genov pri hmelju (*Humulus lupulus* L.) in tobaku (*Nicotiana tabacum* L.). Doktorska disertacija. Ljubljana, Biotehniška fakulteta, Oddelek za agronomijo: 119 p.

Witty M., 1989. Thaumatin II: a simple marker gene for use in plants. Nucleic Acids Research, 17: 3312

Yao J.L., Cohen D., Atkinson R., Richardson K., Morris B. 1995. Regeneration of transgenic plants from the commercial apple cultivar Royal Gala. Plant cell Reports, 14: 407-412

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Effect of cytokinin type and concentration, and source of explant on shoot multiplication of pineapple plant (*Ananas comosus* 'Queen') *in vitro*

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ABSTRACT

The current study was conducted to test cytokinin type and concentration, and source of explant on shoot multiplication of pineapple plant (*Ananas comosus* 'Queen') *in vitro* during 22/6/2009 to 1/5/2010. The results of the first experiment showed that axillary buds delayed to grow during 4-5 months of culturing in MS medium supplemented with different concentrations of BA (1.0, 1.5, 2.0, 2.5 or 3.0 mg l^{-1}). The MS medium supplemented with 1.0 mg l^{-1} BA has led to the vegetative growth of axillary buds. The other concentrations of BA added to MS medium led to callus growth. The results showed that MS medium supplemented with 1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA gave adventitious shoots directly after two months from culturing of callus. The results of the second experiment showed that nodal segments cultured in MS medium supplemented with 1.0 mg l^{-1} kinetin was a significantly superior on other treatments studied (0.5 mg l^{-1} kinetin, 0.5 and 1.0 mg l^{-1} BA) in number of shoots/explant, leaf length, number of leaves/shoot and leaf area of formation shoot which reached 18.60 shoot/explant, 5.38 cm, 10.60 leaves/shoot and 3.64 cm^2, respectively. The results showed that kinetin was a significant superior in all vegetative characteristics of shoots compared with BA.
Abbreviation: BA: 6-benzyl adenine.
NAA: α-naphthalene acetic acid.
MS: Murashige and Skoog salts (Murashige and Skoog, 1962)

Key words: *in vitro*, benzyl adenine, axillary buds, kinetin, naphthalene acetic acid, shoot multiplication

IZVLEČEK

UČINEK VRST IN KONCENTRACIJ CITOKININOV TER VIRA STEBELNIH IZSEČKOV NA *IN VITRO* RAZMNOŽEVANJE ANANASA (*Ananas comosus* 'Queen')

Raziskava je bila izvedena z namenom testiranja različnih vrst in koncentracij citokininov ter vira stebelnih izsečkov na *in vitro* razmnoževanje ananasa (*Ananas comosus* 'Queen') v obdobju 22/6/2009 do 1/5/2010. Rezultati prvega poskusa so pokazali, da je rast zalistnih brstov zaostajala prvih 4-5 mesecev gojenja na MS gojišču, ki so mu dodali različne koncentracije BA (1,0, 1,5, 2,0, 2,5 ali 3,0 mg l^{-1}). MS gojišče, ki so mu dodali 1,0 mg l^{-1} BA je vzpodbudilo rast zalistnih brstov. Druge koncentracije BA, dodane MS gojišču so vodile le k rasti kalusa. Nadaljnji rezultati so pokazali, da so se razvili na MS gojišču, ki sta mu bila dodana 1,0 mg l^{-1} BA in 0,2 mg l^{-1} NAA poganjki neposredno iz kalusa po dveh mesecih gojenja. Rezultati drugega poskusa so pokazali, da so bili nodijski izsečki, gojeni na MS gojišču, ki so mu dodali 1,0 mg l^{-1} kinetina značilno boljši kot drugi postopki, izvedeni v tej raziskavi (0,5 mg l^{-1} kinetina, 0,5 in 1,0 mg l^{-1} BA) v številu poganjkov/izseček, dolžini listov, številu listov/poganjek in listni površini nastalih poganjkov. Nastalo je 18,60 poganjkov/izseček, 10,60 listov/poganjek, dolžine 5,38 cm in 3,64 cm^2 površine. Rezultati so pokazali, da je dalo obravnavanje s kinetinom boljše rezultate glede vrednosti vseh merjenih vegetativnih lastnosti poganjkov v primerjavi z BA.
Okrajšave: BA: 6-benzil adenin.
NAA: α-naftalen ocetna kislina.
MS: Murashige in Skoog bazalno gojišče (Murashige in Skoog, 1962)

Ključne besede: *in vitro*, benzil adenin, zalistni brsti, kinetin, naftalen ocetna kislina, razmnoževanje s stebelnimi izsečki

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1 INTRODUCTION

The pineapple plant (*Ananas comosus* (L.) Merr.) belongs to bromeliad family (Bromeliaceae), which contains 50 genera and about 2500 known species (Duval *et al*., 2003). It is a perennial, monocotyledonous plant having a terminal inflorescence and a terminal multiple fruit (OGTR, 2003). South America is the original home of this plant, which has spread from it through immigration to Central and North America (Bertoni, 1919). Crown, slips, suckers and stem sections have all been commonly utilized for vegetative multiplication of the pineapple (OGTR, 2003). Many studies showed million of pineapple plants can be produced by tissue culture of the crown or shoot tip per year. They reported the rate of multiplication and total number of plantlets produced using plant growth regulators (Sripaoraya *et al*., 2003; Be and Debergh, 2006; Hamad and Taha, 2008). Khan *et al*. (2004) found that the terminal buds of pineapple plant cultured on full and half strength MS medium supplemented with 5.0 mg.l^{-1} BA gave better from other studied concentrations in the number and length of shoots per explant. Abul-Soad *et al*. (2006) got similar results when culturing *in vitro* axillary buds of pineapple plant on full strength MS medium supplemented with 1.0 mg l^{-1} BA and kinetin. Also, that the terminal buds cultured on MS medium supplemented with BA at 2.0 mg.l^{-1} gave high number and length of shoots (Al-Saif *et al*., 2011). The aim of present study is testing of axillary buds and nodal segments as explants for the propagation of pineapple plant by shoot multiplication technique.

2 MATERIALS AND METHODS

2.1 The first experiments: Effect of different concentrations of BA on shoots formation from axillary buds culture

The fresh pineapple fruits with bright green crowns were used for the experiment. Then The green crowns anatomized for the purpose of obtaining the axillary buds at Plant Tissue Culture Laboratory, College of Agriculture, University of Basrah. As the axillary bud excises with the part of leaf base. These explants were put in antioxidant solution consisting of citric acid (150 mg l^{-1}) and ascorbic acid (100 mg l^{-1}) in the refrigerator for 24 hours to avoid phenolic compounds exudation during explants culturing. These explants were then rinsed with sterile distilled water for 3 times and surface sterilized with 20% commercial chlorax solution containing 1.05% sodium hypochlorite, and a drop of tween 20 for 15 minutes. The explants were rinsed in sterile distilled water for 3 times. Immediately after the sterilization process, the explants cultured on full strength of MS medium (Murashige and Skoog, 1962) supplemented with the organic components referred in table 1. The cultures were grown in a growth room at 27±1 °C and 16 hours light and 8 hours dark. The axillary buds did not grow after 4-5 months of culture. There have been re-cultured in the same medium used in first culture.

Table 1: The chemical composition additives to MS medium used for axillary buds culture.

Seq.	Chemical material	Quantity (mg l^{-1})
1	Sucrose	30000
2	Poly vinyl pyrolodine	2000
3	Thiamin-HCl	2
4	Biotin	2
5	Glycine	2
6	Adenine sulphate	40
7	Agar	6000
8	Naphthalene acetic acid	0.2
9	Benzyl adenine	1.0, 1.5, 2.0, 2.5 or 3.0
10	Activated charcoal	500

2.2 The second experiments: Effect of cytokinin type and concentration on shoots formation from nodal segment culture

In this experiment used the same of the last medium components with two cytokinins (benzyl adenine and kinetin) tested at two concentrations (0.5 and 1.0 mg l^{-1}) for each of them. The nodal segments used were taken from shoot produced from indirect organogenesis of callus induced in the first experiment. They were 1.0 cm length and contain 2-3 nods with removal of shoot tips (Figure1 D). The data records after eight months from culture.

The characteristics studied:
1. Number of shoot/explant
2. Leaf length (cm)
3. Number of leaves/shoot
4. Leaf area (cm^2).

Statistical analysis:
Data were statistically analyzed in a completely randomized design with five replicates. Mean values were compared using revised LSD at 5% (Snedecor and Cochran, 1986).

3 RESULTS AND DISCUSSION

3.1 Effect of different concentrations of BA on shoots formation from axillary buds culture

Axillary buds did not grow during 4-5 months of culturing in the MS medium prepared for the shoot multiplication. There growth has been slow because of the buds dormancy. This result is in accordance with Roy *et al*. (2000), when they were propagated *in vitro* pineapple plant. But the axillary buds grew after re-cultured on the same MS medium after two months (Table 2).

Results in the table 2 showed that axillary buds were vegetative growth without callus induction after two months from culturing on full strength MS medium supplemented with 1.0 mg l^{-1} BA. The other MS mediums with different concentrations of BA led to stopped growth of axillary buds, but the growth of callus occurred. Amount of callus increased with increasing concentration of BA added to MS medium except for concentration of BA at 3.0 mg l^{-1} which led to browning of axillary buds and death with very small amount of callus (Table 2). The results showed that the concentration of BA at 2.5 mg l^{-1} added to MS medium are given a very large amount of callus compared to other studied concentrations of BA. The callus induction without shoots formation was related to the explant, type and concentration of cytokinin used in the experiment that stimulated cell division and callus formation without producing lateral shoots (Chanana and Gill, 2008; Al-Taha *et al*., 2012).

The results of the present study are agreement with the results of other studies related to the culturing of *in vitro* pineapple buds taken from crowns of fruits in the full strength MS medium supplemented with different concentrations of BA (Akbar *et al*., 2003; Amin *et al*., 2005; Al-Taha *et al*., 2012). That the reason to increase the amount of callus culturing from MS medium supplemented with 2.5 mg l^{-1} BA due to be supra-optimal for cell division stimulation and callus formation (Al-Taha, 2008).

The callus grown in the MS medium supplemented with 1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA induced indirect shoots growth after eight weeks from culture(Figure 1 A and B). The nodal segments (explants) taken from shoots obtained by organogenesis were used in the second experiment (Figure 1 C). Organogenesis was induced by the components of MS medium that led cells dedifferentiation of callus that had grown and developed into vegetative shoots (Thorpe, 1978). These results are in accordance with results of other studies on indirect organogenesis from callus of pineapple plant cultured on MS medium supplemented with cytokinin and auxin (Akbar *et al*., 2003; Khan *et al*., 2004; Al-Taha *et al*., 2012).

Table 2: Effect of different concentrations of BA added to MS medium prepared for growth of axillary buds of pineapple plant.

Conc. of BA (mg l^{-1})	Notes	Amount of callus
1.0	Axillary bud grew vegetative and it produced small shoot.	-
1.5	Axillary bud grew slightly with be callus greenish color.	*
2.0	Axillary bud did not grow and be callus grainy whitish green.	**
2.5	Axillary bud did not grow and be callus grainy whitish green.	***
3.0	Axillary bud died and brown discoloration and callus be very small amount.	*

(-): Mean not grow callus.
(*): Means the growth of a small amount of callus.
(**): Means the growth of a large amount of callus.
(***): Means the growth of a very large amount of callus.

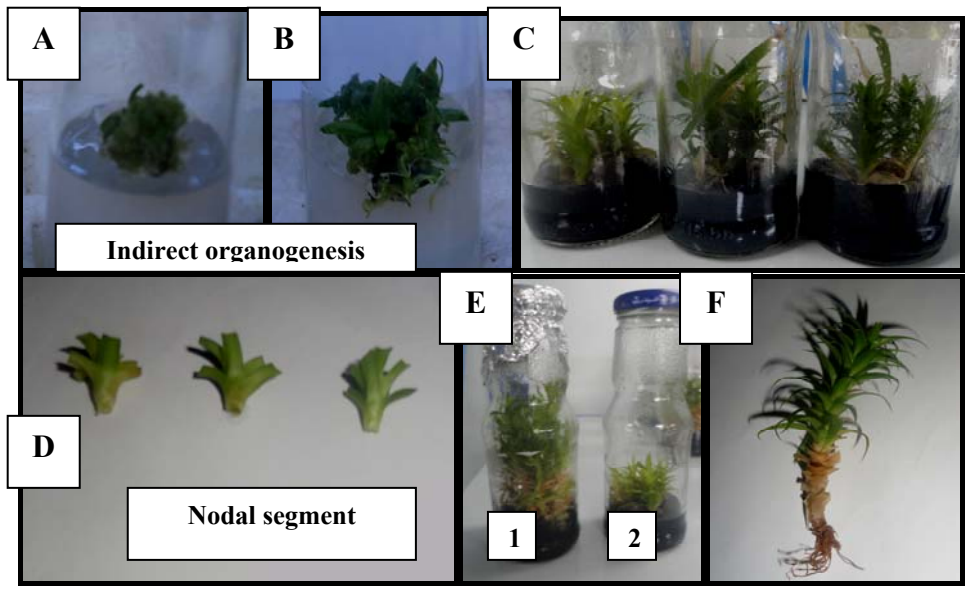

Figure 1: Effect of source of explant and cytokinin on formation shoots of *in vitro* propagation of pineapple plant (*Ananas comosus* 'Queen').

A, B and C: The stages of indirect shoots from culturing of callus of axillary buds on MS medium supplemented with (1.0 mg l^{-1} BA and 0.2 mg l^{-1} NAA) after two months.
D: Nodal segments (2-3 nodes) used in the second experiment of shoots multiplication.
E: Formation shoots cultured on MS medium supplemented with 1.0 mg l^{-1} kinetin (1) 0.5 mg l^{-1} BA (2).
F: Shoot produced from shoots multiplication of nodal segment cultured on MS medium supplemented with 1.0 mg l^{-1} kinetin.

3.2 Effect of cytokinin type and concentration on shoots formation from nodal segments culture

Table 3 showed the effect of BA and kinetin at (0.5 or 1.0 mg l^{-1}) added to full strength MS on vegetative characteristics of induction shoots from nodal segments after eight weeks from culturing. Number of multiplication shoots/explant, leaf length (cm), number of leaves/shoot and leaf area (cm^2) were increased with increasing cytokinin (BA or kinetin) added to MS medium. This result

is in accordance with results of other studies on effect of cytokinins on shoots multiplication of *in vitro* propagation of pineapple plant (Kiss *et al*., 1995; Bhatia and Ashwath, 2002; Abul-Soad *et al*., 2006; Be and Debergh, 2006).

The results of Table 3 showed that MS medium supplemented with kinetin at 1.0 mg l^{-1} was a significant superior than other treatments of MS media. It gave highest rate in vegetative characteristics of formation shoots (18.60 shoots/explant, 5.38 cm of leaf length (Figure 1 F), 10.60 leaves/shoot and 3.64 cm^2 of leaf area) except leaf area which did not significant different with leaf area of MS medium supplemented with 1.0 mg l^{-1} BA. While the MS medium supplemented with 0.5 mg l^{-1} BA led to a significant decline in all vegetative characteristics reached (7.00 shoots/explant, 2.72 cm, 4.80 leaves/shoot and 1.74 cm^2), respectively when compared with other treatments of MS media (Figure 1 E1 and 2).

The same table showed that MS medium supplemented with kinetin at (0.5 or 1.0 mg l^{-1}) was a significant superior on BA in all vegetative characteristics of formation shoots except the treatment of kinetin at 1.0 mg l^{-1} which was not significantly different from 1.0 mg l^{-1} BA in leaf area. The reason for this is that kinetin was probably more effective than BA in stimulation of adventitious shoots production.

Table 3: Effect of cytokinin type and concentration on vegetative characteristics of formation shoots from nodal segments of pineapple plant culture in full strength MS medium after eight weeks.

Treatment (mg l^{-1})		No. of shoots/explant	Leaf length (cm)	No. of leaves/shoot	Leaf area (cm^2)
BA	0.5	7.00	2.72	4.80	1.74
	1.0	13.60	4.12	9.40	3.42
Kinetin	0.5	10.60	3.80	7.60	2.94
	1.0	18.60	5.38	10.60	3.64
Revised-LSD (5%)		1.912	0.382	1.175	0.355

4 CONCLUSIONS

The source of explant used in tissue culture of pineapple plant has an important role in shoot multiplication. The nodal segments as explants gave adventitious shoots while axillary buds did not develop into the shoots but grew callus. As we can deduce from the results of this study kinetin was more effective than BA in shoots multiplication.

5 REFERENCES

Abul-Soad, A. A.; Boshra, E. S. and Ali, H. S. (2006). An improved protocol for the micropropagation of pineapple (*Ananas comosus* (L.) Merr.). Assiut J. Agric. Sci., 37(3): 13-30.

Akbar, M. A.; Karmarka, B. and Roy, S. (2003). Callus induction and high frequency plant regeneration of pineapple (*Ananas comosus* (L.) Merr.). Plant Tiss. Cult., 13(2): 109-116.

Al-Saif, A. M.; Sharif Hossain, A. B. M. (2011). Effects of benzyl amino purine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* (L.) Merr.) *in vitro*. Afr. J. Biotech., 10(27): 5291-5295.

Al-Taha, H. A. (2008). The use of plant tissue culture technique in micropropagation of salt tolerant plants of local orange trees. (Ph. D. Thesis). College of Agriculture, University of Basrah, Iraq, pp. 192.

Al-Taha, H. A.; Ibrahim, M. A. and Saleh, A. M. (2012). Regeneration of plantlets from callus induced from shoot tips of pineapple (*Ananas comosus* (L.) Merr. cv. Del Monte by tissue culture technique. Kuf. J. Agric. Sci., (In Press).

Amin, M. N.; Rahman, M. M.; Rahman, K. W.; Ahmed, R.; Hossain, M. S. and Ahmed, M. B. (2005). Large scale regeneration *in vitro* from derived callus

cultures of pineapple (*Ananas comosus* (L.) Merr. cv. Giant Kew). Bang. Inter. J. Bot., 1(2): 128-132.

Be, L. V. and Debergh, P. C. (2006). Potential low cost micropropagation of pineapple (*Ananas comosus*). Sou. Afr. J. Bot., 72: 191-194.

Bertoni, M. S. (1919). Contributions al'etude botanique des plantes cultivees. I. Essai d'une monographie du enre *Ananas*. Anales Cientificos Paraguayos (Ser.II), 4: 250-322.

Bhatia, P. and Ashwath, N. (2002). Development of a rapid method for micropropagation of a new pineapple (*Ananas comosus* (L.) Merr.) clone "Yeppoon Gold". Acta Hort., 575: 125-131.

Chanana, Y. R. and Gill, M. I. S. (2008). Propagation and nursery management. Cited from: http://www.usdl.niscai.res.in /bitstre/123456789/472/1/pdf. On: 26/3/2009.

Duval, M. F.; Buso, G. C.; Ferreira, F. R.; Noyer, J. L.; Coppens, E. G.; Hamon, P. and Ferreira, M. E. (2003). Relationships in *Ananas* and other related genera using chloroplast DNA restriction site variation. Genome, 46(6): 990-1004.

Hamad, A. M. and Taha, R. M. (2008). The effect of sequential different hormones on *in vitro* proliferation of pineapple (*Ananas comosus* (L.) Merr. Cv. Smooth Cayenne) shoot-tip culture. Pak. J. Biol. Sci., 11(3): 386-391.

Khan, S.; Nasib, A. and Saeed, B.A. (2004). Employment of *in vitro* technology for large scale multiplication of pineapples (*Ananas comosus*). Pak. J. Bot., 36(3): 611-615.

Kiss, E.; Kiss, J.; Gyuali, G. and Heszky, L. E. (1995). A noval method for rapid micropropagation of pineapple. HortScience, 30: 127-129.

Murashige, T. and Skoog, F. A. (1962). A revised medium of rapid growth and bioassay with tobacco tissue culture. Physiol. Plant, 15: 473-497.

Office of the Gene Technology Regulator. (2003). The Biology and Ecology of Pineapple (*Ananas comosus* var. *comosus*) in Australia. April: 1-24.

Roy, S. K.; Rahman, M. and Haque. (2000). Mass propagation of pineapple through *in vitro* culture. In: Transplant Production in the 21st Century. Cubota C. and Chun (eds.). Kluwer Academic Publishers. The Netherlands. pp. 279-283.

Snedecor, G. M. and Cochran, W. G. (1986). Statistical Methods. 9th ed.; The Iowa State Univ., Press. Amer. Iowa, U.S.A., 507 p.

Sripaoraya, S.; Merchant, R.; Power, J. B. and Davey, M. R. (2003). Plant regeneration by somatic embryogenesis and organogenesis in commercial pineapple (*Ananas comosus* L.). In Vitro Cell Dev. Biol. Plant, 39: 450-454.

Thorpe, T. A. (1978). Physiological and biochemical aspects of organogenesis *in vitro*. In: Thorpe, T. A. (ed.). Frontiers of Plant Tissue Culture. Univ. Calgary, Alberta, Canaela: 49-58.

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Genetic variation among accessions of *Lathyrus inconspicuous* (L.) as revealed by SDS Polyacrylamide Gel Electrophoresis

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ABSTRACT

Eighteen *L. inconspicuous* accessions collected from different countries were evaluated for variations of seed weight, seed protein content, and electrophoretic patterns of the total seed proteins analyzed under reducing conditions. They exhibited a reasonable genetic variability for the evaluated traits. This genetic variability revealed that improvement through simple selection for these traits is possible. The variation between the seed size of this accessions was attributed to the development process or the life cycle of the plant, and the environmental condition to which the mother plant is exposed. On the other hand, the variation in protein content among the different accessions may be due to genotype and/or seasonal influences. The relationship between protein content and 100 seeds weight in the evaluated accessions was reversible, the accession showed the lowest quantity of the total seed proteins was the accession that exhibited highest weight of 100 seeds and nearly vice versa. Each accession gave a different electrophoretic pattern except the two accessions collected from Iran, exhibited an identical one. The difference in 100 seed weight and total protein content of these accessions indicated that they are not genetically identical. The variation in the electrophoregram of the evaluated accessions located in the bands with molecular weight more than 98 kDa, the heavy subunits of alpha-lathyrin subunits and the region molecular weight around 70 kDa. The results of cluster analysis based of SDS/PAGE under reduction conditions indicated that genetic diversity between Turkish, Syrian, and Iranian and Australian accessions is pronounced, and Turkish accessions are closer to both Syrian and Iranian accessions than the relation between Syrian and Iranian. This suggested that crosses between the Iranian and Syrian accessions could create more genetic variability than crosses with Turkish accessions. The distribution of Turkish and Syrian accessions between more than one clusters revealed that genetic diversity and geographic distribution were independent of each other. PCA showed that all accessions were separated on the first principal component, indicating that the accessions showed a good association, due, probably, to parallel evolution.

Key words: 100 seeds weight, Protein analysis, multivariate analysis, germplasm characterization

IZVLEČEK

ANALIZA GENETSKE VARIABILNOSTI AKCESIJ GRAHORJA (*Lathyrus inconspicuous* (L.) S SDS POLIAKRILAMIDNO GELSKO ELEKTROFOREZO

Osemnajst akcesij grahorja (*L. inconspicuous* L.), zbranih iz različnih držav, je bilo ovrednoteno glede na variabilnost mase semen, vsebnost semenskih proteinov in elektroforetskih vzorcev celokupnih semenskih proteinov analiziranih v reducirajočih razmerah, ob prisotnosti reducenta. Ovrednotene lastnosti so pokazale pričakovano genetsko variabilnost na osnovi katere je možna preprosta selekcija. Variabilnost v velikosti semen med akcesijami je bila odvisna od razvojnih procesov v življenskem ciklu rastlin in okoljskih dejavnikov, katerim je bila izpostavljena materinska rastlina. Po drugi strain so bile razlike v vsebnosti proteinov med različnimi akcesijami odvisne od genotipa in/ali sezonskih okoljskih vplivov. Razmerje med vsebnostjo proteinov in maso 100 semen je bilo med ovrednotenimi akcesijami obratno. Akcesije, ki so imele najmanjšo vsebnost proteinov so imele največjo maso 100 semen in obratno. Vsaka akcesija, z izjemo dveh iz Irana, je imela svojski elektroforetski vzorec. Razlika med maso 100 semen in celokupno vsebnostjo proteinov analiziranih akcesij je pokazala, da akcesije genetsko niso enake. Razlike v elektroforegramih analiziranih akcesij so se pojavljale v elektroforetskih črtah z molekulsko težo večjo od 98 kDa, težjih podenot alfa latrina in v območju elektroforetskih črt z molekulsko težo okrog 70 kDa. Rezultati klasterske analize dobljeni na osnovi SDS/PAGE elektroforeze v reducirajočih razmerah so pokazali, da je genetska raznolikost med turškimi, sirijskimi, iranskimi in

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avstralskimi akcesijami izrazita in da so turške akcesije bližje sirskim in iranskim, kot pa je bližina sirskih in iranskih akcesij med seboj. To nakazuje, da bi dala križanja med sirskimi in iranskimi akcesijami večjo variabilnost kot s turškimi. Porazdelitev turških in sirskih akcesij v več kot en klaster kaže, da sta genetska raznolikost in geografska razširjenost med seboj neodvisni. Analiza glavnih component (PCA) je pokazala, da so se vse akcesije ločile že na prvi glavni komponenti, kar kaže, da so akcesije dobro povezane, verjetno zaradi paralelne evolucije.

Ključne besede: teža 100 semen, analiza proteinov, multivariatna analiza, karakterizacija genskih virov

1 INTRODUCTION

The genus *Lathyrus* is a member of the tribe *Vicieae* (*Fabaceae, Papilionoideae*); which comprises nearly 160 annual and perennial, autogamous and allogamous herbaceous creeping plants. *Lathyrus* species have a broad distribution all over the world in Europe, North America, Asia, tropical East Africa and temperate South America (Goyder, 1986). The main center of genus diversity is the eastern Mediterranean region, with smaller centers in North and South America (Kupicha, 1983).

Lathyrus has a long history as cultivated plant and it has agronomic importance as forage or fodder such as *Lathyrus inconspicuous, L. ochrus* and *L. articulatus,* as human food such as *L. cicera and L. sativus*, and as ornamental plants, such as *L. odoratus*.

Knowledge of genetic variation is a useful tool in genebank management, helping in the establishment of core collections, facilitating efficient sampling and utilization of germplasm (identifying and/or eliminating duplicates in the gene stock), and selecting of desirable genotypes to be used in breeding programs.

Characterization of germplasm using biochemical techniques (storage proteins and isozymes) has received a great attention in the last decades. This attention was attributed to the increased recognition of germplasm resources in croplants improvement. Genetic markers are useful for screening germplasm with the minimum cost in time and labour (Nakajima, 1994). The qualitative traits of the seed proteins obtained by electrophoresis have been successfully used to assess the genetic variation among the accessions of the wild species (Elham et al., 2010; Vishwanath et al., 2011). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS/PAGE) is among the biochemical technique that is widely used due to its simplicity and effectiveness for describing the genetic structure of the accessions of wild plant species. Seed storage proteins have been used as genetic markers in: (1) identifying variation among the taxa of each species; (2) screening the purity of the ever expanding number of cultivars; (3) establishing genome relationships; (4) exploiting the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield (Sammour 1991), and (5) using information on genetic diversity to make decisions regarding selection of superior genotypes for improvement yield of plants through breeding. Protein electrophoresis is considered a reliable, practical and reproducible method because seed storage proteins are the third hand copy of genomic DNA and largely independent of environmental fluctuations (Sammour, 1987;Javaid et al., 2004; Iqbal et al., 2005).

SDS/PAGE has not yet been carried out for *Lathyrus inconspicuous*, although it is an important forage or fodder in drought-stricken, rain-fed areas where soil quality is poor and extreme environmental conditions prevail (Palmer *et al*. 1989). “Despite it’s tolerance to drought it is not affected by excessive rainfall and can be grown on land subject to flooding (Kaul et al., 1986; Rathod, 1989; Campbell et al., 1994).

The present study was initiated to study genetic variation in accessions of *L. inconspicuous* on the basis of 100-seeds weight, protein content of the seed meal and SDS-PAGE markers.

2 MATERIALS AND METHODS

Plant material

The designated germplasm of *Lathyrus inconspicuous* that was used in this study included 18 different accessions. They were obtained from the International Center for Agricultural Research in The Dry Areas ICARDA, Aleppo, Syria. Accessions are listed in Table 1.

Table 1: Accession number, origin and total weight of 100 seeds of accessions of *L. inconspicuous.*

Accession number	Accession (IG)	Origin	Wt of 100 seeds (g)	Concentration of total protein in mg/ml	Number of protein bands
A	65037	TUR, Diyarbakir	1.481	130	**30**
B	65038	TUR, Siirt	1.738	123	**28**
C	65048	IRN, Lorestan	1.932	121	**29**
D	65054	IRN, East Azerbaijan	1.433	118	**29**
E	65077	AUS	1.494	125	**27**
F	65282	SYR, Homs	1.702	127	**25**
G	65436	SYR, Alepppo	1.684	139	**25**
H	65508	SYR, Idlib	1.559	140.5	**26**
I	65579	SYR, Sweida	1.345	132	**26**
J	65627	SYR, Damascus	0.885	147	**26**
K	65638	SYR, Tartous	1.828	119	**26**
L	65679	TUR, Ankara	1.296	142	**26**
M	65739	TUR, Antakya	1.995	109	**28**
N	65847	TUR, Izmir	1.896	124	**27**
O	65866	TUR, Gaziantep	1.155	134	**27**
P	65913	TUR, Urfa	0.674	130	**28**
Q	65935	TUR, K.Maras	1.225	132	**26**
R	65951	TUR, Adiyaman	1.880	124	**27**

Seed protein extraction: The seed meal was obtained from a composite sample of 18-20 dehuled seeds for each accession. Each sample was prepared by grinding cotyledons to flour; the total crude proteins were extracted using 0.125 M Tris-Borate pH 8.9 with 2% SDS (Ratio 1:10 w/v).

Protein Analysis: Total seed proteins were quantitatively estimated in each sample by the method of Bradford (1976). The final concentration was adjusted to 20 µg/µl protein in sample buffer. The extracts were denaturated in 2X sample buffer (1M Tris-HCl pH = 6.8, 2% SDS, 20% glycerol, 0.02% BPB, 5% 2-mercapto-ethanol), and heated at 100 ºC for 4 minutes. One dimensional SDS-PAGE was performed according to the method of Lammeli (1970) using 17% polyacrylamide gel. The gel was stained with Coomassie blue and visualized in white fluorescent light. Phosphorylase b (98 kDa), ova albumin (43 kDa), carbonic anhydrase (28.35 kDa) and β-lactoglobulin (18.85 kDa) were used as marker proteins.

Data Analysis: The band identification was based on electrophoretic mobility and by numerous side by side comparisons of proteins extracts. The estimation of genetic diversity within and among the samples was based on 38 reproducibly scored bands identified in the zones of highest variation of protein profile (ranging from 15 to 110 kDa). The genetic diversity among the accessions was evaluated by Jaccard similarity index (Table 2), Dendrogram was made by Euclidian distance (Figure 3). The analysis was performed using the frequencies of scored bands calculated for the accessions. A dendrogram was constructed through the Average linkage-joining rule, using the software package SYSTAT.

Table 2: Jaccard similarity coefficients between accessions of *L. inconspicuous*.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	1.000																	
B	0.933	1.000																
C	0.967	0.966	1.000															
D	0.967	0.966	1.000	1.000														
E	0.900	0.897	0.931	0.931	1.000													
F	0.833	0.893	0.862	0.862	0.926	1.000												
G	0.833	0.893	0.862	0.862	0.857	0.923	1.000											
H	0.806	0.862	0.833	0.833	0.767	0.821	0.889	1.000										
I	0.806	0.862	0.833	0.833	0.767	0.821	0.889	0.889	1.000									
J	0.697	0.742	0.719	0.719	0.656	0.700	0.759	0.793	0.793	1.000								
K	0.806	0.862	0.833	0.833	0.767	0.821	0.889	0.926	0.926	0.793	1.000							
L	0.806	0.862	0.833	0.833	0.828	0.889	0.821	0.857	0.857	0.793	0.857	1.000						
M	0.933	0.931	0.966	0.966	0.897	0.893	0.893	0.862	0.862	0.742	0.862	0.862	1.000					
N	0.900	0.964	0.931	0.931	0.862	0.926	0.926	0.893	0.893	0.767	0.893	0.893	0.964	1.000				
O	0.900	0.964	0.931	0.931	0.862	0.926	0.926	0.893	0.893	0.767	0.893	0.893	0.964	1.000	1.000			
P	0.758	0.806	0.781	0.781	0.719	0.767	0.767	0.742	0.742	0.636	0.742	0.742	0.806	0.833	0.833	1.000		
Q	0.867	0.929	0.897	0.897	0.828	0.889	0.889	0.857	0.857	0.733	0.857	0.857	0.929	0.963	0.963	0.862	1.000	
R	0.839	0.774	0.806	0.806	0.742	0.733	0.733	0.710	0.710	0.656	0.710	0.710	0.833	0.800	0.800	0.719	0.828	1.000

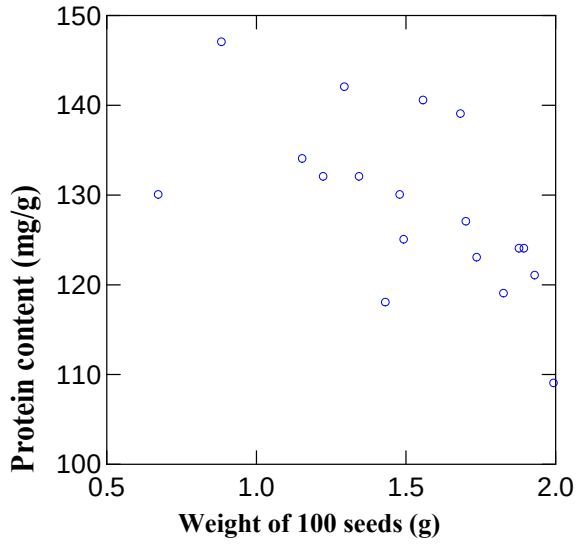

Fig.1: Scatter plot of protein contents and 100 seeds weight of 18 *L. inconspicuous* accessions.

3 RESULTS

Generally, the accessions of *L. inconspicuous* exhibited wide genetic diversity for 100 seeds weight. Moreover, the variation in 100 seeds weight was very evident for the seeds collected from the same country (Table 1). For example in Turkey, it was varied between 1.995 g in Antakya to 0.674 g in Urfa. In Syria, the variation was not such wide as in Turkey; it was ranged between 1.828 g in Tartous and 0.885 g in Damascus.

For a better characterization of the *L. inconspicuous* germplasm, relationships among protein content and 100 seeds weight were considered (Figure 1). The distribution of the points indicates clearly a reverse relationship between protein content and 100 seeds weight. Nevertheless, it may be noticed that the protein content tends to be less variable for median values of 100 seeds weight. In other words, a median range of 100 seeds weight variation corresponds to a less wide range of protein content variation.
When the total seed proteins of the studied accessions were separated by SDS/PAGE under reducing conditions, the patterns of the bands obtained were different for all the evaluated accessions, except the two accessions collected from Iran showed identical electrophoretic patterns (Figure 2). These differences were most marked amongst the proteins with molecular weights ranged between 110 kDa (the weight of the high molecular weight albumin) and 43 kDa. The electrophoretic patterns of the total seed proteins of the accessions collected from Damascus and Tartous in Syria and Ankara in Turkey were unique and very characteristic. The number of protein bands in the electrophoregram of the studied accessions ranged between 25 and 30 bands (Table 2), with a total of thirty six bands from eighteen accessions and molecular weights ranged from 10 to 110 kDa (Figure 2).

Jaccard's similarity coefficients was based on the data of SDS/PAGE profiles of the evaluated accessions (Table 2). It ranged from 100.00 (between an accession from Lorestan in Iran and East Azerbaijan in Iran) to 0.697 (between two accessions from Diyarbakir in Turkey and Damascus in Syria). It was noticed that the majority of the similarity coefficients between accessions was close to 0.7. This indicated the close relationships between the evaluated accessions, though they are collected from different country.

The dendrogram produced from electrophoretic data of the total seed protein extracts of the evaluated accessions, using Euclidean distance matrix on average linkage shows three groups, two contain each one accession (G2 and G3) and the rest of accessions in the third group (G1) (Figure 3). G1 is divide into two subgroups (G1A and G1B); G1A includes one accession and G1B divided into three clusters (C1, C2 and C3). C1 includes accessions from Turky and Iran; C2 from Syria and Australia; and C3 from Syria and Turkey.

The matrix of eigenvectors and values of the principal components (PCs) resulting from electrophoretic data of the total seed proteins (Table 3) shows that the protein data influencing 82.88% of the variability accumulated up to the first two components. All the studied accessions were separated on the first principal component.

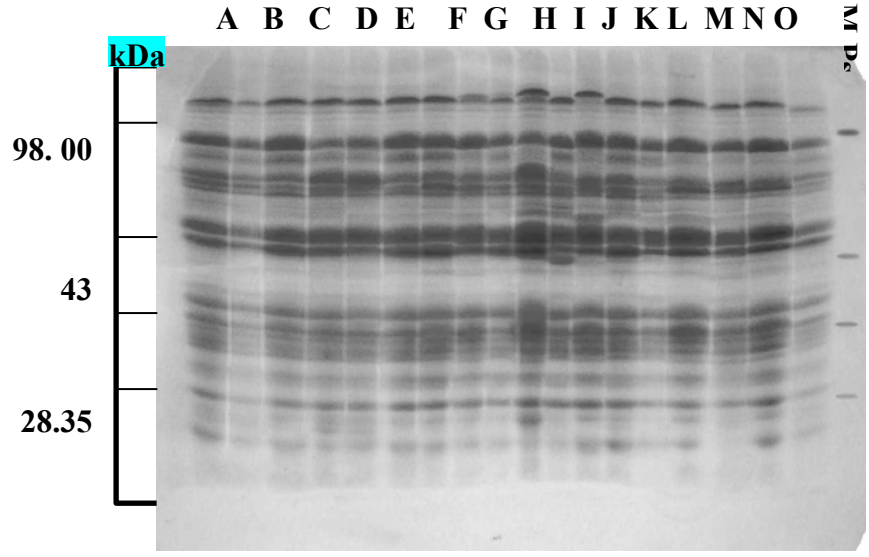

Fig.2: Electrophorogram produced by SDS/PAGE analysis of seed proteins of 18 accessions of *L. inconspicuous* L.

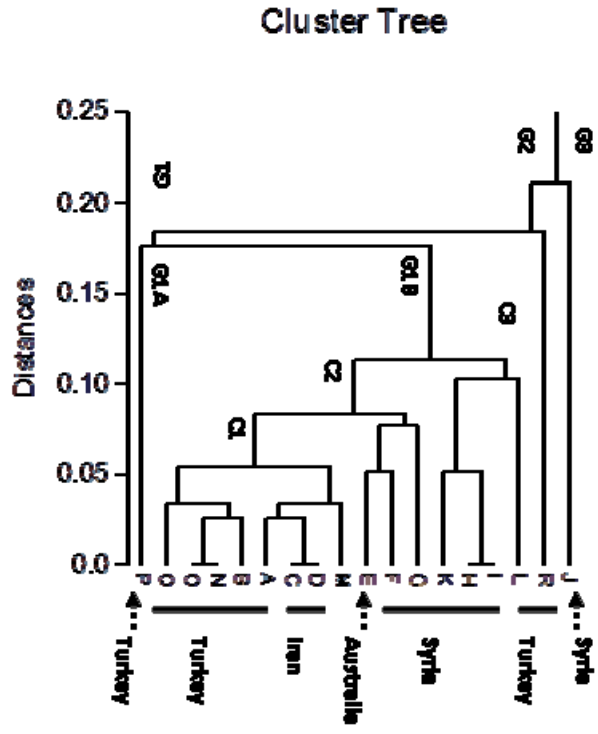

Fig.3: Dendrogram showing the genetic relationships among of 18 accessions of *L. inconspicuous* L. based on Euclidian genetic distance of SDS/PAGE.

Table 3: Origin, matrix of eigenvectors and values of the principal components for protein data of *L. inconspicuous* L. accessions

	Accession number (IG)	Origin	Principal components	
			C1	C2
A	65037	TUR, Diyarbakir	0.869	0.346
B	65038	TUR, Siirt	0.946	0.117
C	65048	IRN, Lorestan	0.924	0.279
D	65054	IRN, East Azerbaijan	0.924	0.279
E	65077	AUS	0.835	0.327
F	65282	SYR, Homs	0.902	0.053
G	65436	SYR, Alepppo	0.915	-0.119
H	65508	SYR, Idlib	0.864	-0.392
I	65579	SYR, Sweida	0.864	-0.392
J	65627	SYR, Damascus	0.618	-0.557
K	65638	SYR, Tartous	0.856	-0.354
L	65679	TUR, Ankara	0.849	-0.216
M	65739	TUR, Antakya	0.953	0.146
N	65847	TUR, Izmir	0.977	-0.008
O	65866	TUR, Gaziantep	0.977	-0.008
P	65913	TUR, Urfa	0.666	0.036
Q	65935	TUR, K.Maras	0.935	0.035
R	65951	TUR, Adiyaman	0.668	0.281
Variance Explained by Components			**13.612**	**1.305**
Percent of Total Variance Explained			**75.624**	**7.251**
Accumulated Eigenvectors			**75.624**	**82.875**

4 DISCUSSION

The pool of genetic variation within accessions of this species is the basis for selection as well as for plant improvement. A better understanding of genetic diversity and its distribution in the accessions of the studied plant is essential for its conservation and use. It will help greatly in determining what to conserve as well as where to conserve, and will enhance our knowledge and understanding of the taxonomy, origin and evolution of *L. inconspicuous.*

In the present investigation, a reasonable genetic variation was observed for 100 seeds weight, total protein content and electrophoretic pattern (SDS-PAGE) of the total protein of the seed meal of accessions of *L. inconspicuous.* The genetic variability of these traits reveals that improvement through simple selection for these traits is possible, particularly if we broaden the genetic base from diverse habitats to include most of the genetic determinants of a trait of interest (i.e. productivity, disease resistance, a biotic stress tolerance, and/or quality) (Ghafoor and Arshad, 2008).

It is well established that seed size reflects an underlying trade-off between seed size and seed number. The seedling survival increases constantly with increasing seed size (Turnbull et al., 2006). However, it is useful to consider whether a plant can vary its position in this trade-off in response to environmental conditions or /and if seed size is solely a genetic trait (Sammour et al., 2007a). The suggestion that seed size is solely a genetic trait was based on the study of Lopes et al. (2003) on genetic control of cowpea seed sizes, where they found that the mid-parental value and the additive effect were the most important genetic parameters for the determination of the seed character. However, the size of the seed is the result of three different growth programs: those of the diploid embryo, the triploid endosperm, and the diploid maternal ovule (Sundaresan, 2005). The control and coordination of these growth programs are

under genetic regulation. When the paternal genome is in excess, seed growth is promoted, and conversely, excess of the maternal genome results in smaller seeds. This is true for diploid x tetraploid crosses of plants as described in Sundaresan (2005). This confirmed the finding of Lopes et al. (2003) that the variation of the seed size among different populations of the species was attributed to the development process or the life cycle of the plant. However this variation, which is a development process, may itself enhance fitness. The variation in seed size in an individual plant may make that plant more able to adapt to a changing environment. In other context, Wulff (1986 a, b) stated that seed size as well as seed germination characteristics may vary with the environmental condition to which the mother plant is exposed. In conclusion, seed size is not solely a genetic trait, but it is affected by environment.

The seed protein content in the studied accessions varied between 109 mg/g seed meal in accession number IG65739 from Antakya in Turkey to 147 mg/g seed meal in accession number IG 65627 from Damascus in Syria. It is very interesting to notice that the accession that showed the lowest quantity of the total seed proteins was the accession that exhibited highest weight of 100 seeds and nearly vice versa. This clearly indicated the reverse relationship between protein content and 100 seeds weight. This conclusion was in agreement with that of Saxena et al. (1987) on pigeonpea, Kaushik et al. (2007) on *Jatropha curcas* and Afzal et al. (2003a, b) on mungbean. It was found that investigated accessions had significant variation in protein content. This variation was attributed to environmental factors such as geographical area, season of collecting, elevation, and annual temperature, precipitation, soil fertility and/or genotypes variation (Ries and Everson, 1973; Vollmann et al., 2000).

In general, each accession gave a different electrophoretic pattern except the two accessions collected from Iran, exhibited an identical electrophoretic pattern. The difference in 100 seed weight and total protein content of these accessions indicated that they are not genetically identical (identical duplicate). The suggestion that these two accessions may be derived from the same original population that are mixtures of lines with differing genotype frequencies, or random mating populations with the same alleles but differing allele frequencies, as reported by Van-hintum and Knüpffer (1995). However, this can not stand up, because the two accessions were collected from two different provinces far apart from each other (Lorestan and East Azerbaijan). In this study, the electrophoregam of SDS/PAGE was carried out under reducing conditions, exhibiting that variation between the different accessions located in the bands with molecular weight more than 98 kDa, the bands might include higher molecular weight albumin (Sammour,1987), the heavy subunits of alpha-lathyrin subunits (Rosa and Ferreira, 2000) and the area with molecular weight around 70 kDa. It can be noticed that the two subunits of γ-lathyrin, 24 kDa (major albumin) and 20 kDa (lectin) showed no variation between the different accessions. The genetic variability information can help the plant breeders to select the accessions to be utilized in hybridization programme or to be utilized as parents for the development of future cultivars through hybridization. It can also be used to assess its association with quantitative traits that helps in screening crop germplasm for identified markers.

The results of cluster analysis based of SDS/PAGE under reduction conditions indicated that genetic diversity between Turkish, Syrian, Iranian and Australian accessions is large. Cluster analysis showed that Turkish accessions are closer to both Syrian and Iranian accessions which they are relatively more distant from each other. On the basis of these results, it is clear that crosses between the Iranian and Syrian accessions could create more genetic variability than crosses between Turkish and those gene pools. The distribution of Turkish and Syrian accessions between more than one clusters showed that genetic diversity and geographic distribution were independent of each other and no definite relationship existed between genetic diversity and geographic diversity. SDS-PAGE under reduction conditions results revealed that the total amount of variability accounted for the first two principal components was 82.88%. All accessions were separated on the first principal component, representing 75.62% of the total variability. The variability within the investigated accessions based on SDS/PAGE, 100 seed weight, and quantitative and qualitative traits of the total seed proteins is associated with the expression of the genome.

However, to express all the variability of *L.inconspicuous* gene pools, more studies focused on detailed agronomic, biochemical and molecular traits on a wide range of accessions covering wide geographical regions are recommended and needed.

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6 REFERENCES

Afzal M.A., Bashir M. M., Luna N.K., Haque M.M., Bakr M.A. Aktar M.S. (2003a). Picking times and storage duration effects on hard seededness in mungbean (*Vigna radiata* (L.) Wilczek) seeds. Asian Journal of Plant Science.2: 566-568.

Afzal M.A., Bashir M. M., Luna N.K., Bakr M.A., Haque M.M. (2003b). Relationship between seed size, protein content and cooking time of mungbean (*Vigna radiata* (L.) Wilczek) seeds. Asian Journal of Plant Science. 2: 1008-1009.

Bradford, M.M. (1976). A rapid and sensitive for the quantification of microgram quantitie of protein utilizing the principle of protein dye binding. Anal. Biochem.72: 248–254.

Campbell C., Mehra R.B., Agrawal S.K., Chen Y.Z., Abd El Moneim A.M., Kawaja H.I.T., Yadav C.R. Tay J.U. and Araya W.A. (1994). Current status and future strategyin breeding grasspea (*Lathyrus sativus*). Euphatica 73: 167-175.

Elham A.A., El-Hady A., Atef A.A., Haiba, Nagwa R., El-Hamid A., Aida A. (2010). Phylogenetic diversity and relationships of some tomato varieties by electrophoretic protein and RAPD analysis. Journal of American Science. 6 (11): 434-441.

Ghafoor A., Arshad M. (2008). Multivariate analyses for quantitative traits to determine genetic diversity of blackgram [(*Vigna mungo* (L.) Hepper] germplasm. Pakistanian Journal of Botany. 40(6): 2307-2313.

Goyder D.J. (1986). The genus *Lathyrus*. In Lathyrus and lathyrism A. Kaul and D. Combes (eds.). Third Worled Medical Research Foundation. New York: 3-7.

Iqbal S.H., Ghafoor A., Ayub N. (2005). Relationship between SDS-PAGE markers and *Ascochyta* blight in chickpea. Pakistanian journal of Botany. 37: 87-96.

Javaid A., Ghafoor A., Anwar R. (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pakistanian Journal of Botany. 36: 25-29.

Kaul A.K., Islam M.Q., Hamid A. (1986). Screening of *Lathyrus* germplasm of Bangladesh for BOAA content and some agronomic characters. In Lathyrus and Lathyrism (A.K. Kaul and D. Combes eds.). Third World Medical Research Foundation, New York. 130-141.

Kaushik N., Kumar K., Kumar S., Kaushik N., Roy S. (2007). Genetic variability and divergence studies in seed traits and oil content of Jatropha (*Jatropha curcas* L.) accessions. 31(7): 497–502.

Kupicha F.K. (1983). The infrageneric structure of Lathyrus. Notes from the Royal Botanic Garden Edinburgh 41: 209-244.

Lammeli U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lopes F.C.C., Gomes R.L.F., Filho F.R.F. (2003). Genetic control of cowpea seed sizes. Scientia Agricola. 60(2): 315-318.

Nakajima K. (1994). Biotechnology for crop improvement and production in Japan. In: Biotechnology applications in agriculture in Asia and pacific. Published by Asian Productivity Organization: 87-107.

Palmer V.S., Kaul A.K., Spencer P.S. (1989). International Network for the improvement of *Lathyrus sativus* and the Eradication of Lathyrism (INILSEL): A TWMRF initiative. In the Grass Pea: Threat and Promise. Proc. Of the International Network for the improvement of *Lathyrus sativus* and the Eradication of Lathyrism. P. Spencer (ed.). Third World Medical Research Foundation, New York: 219-223.

Rathod K.L. (1989). Status of *Lathyrus sativus* L. in India with special reference to Madhya Pradesh. In Grass Pea: Threat and Promise. Proc. Of the International Network for the improvement of *Lathyrus sativus* and the Eradication of Lathyrism. P. Spencer (ed.). Third World Medical Research Foundation, New York: 168-174.

Ries S. K, Everson E. H. (1973**)**. Protein Content and Seed Size Relationships with Seedling Vigor of Wheat Cultivars. Agronomy Journal. 65(6): 884-886.

Rosa M. J., Ferreira R. B. (2000). Storage proteins from *Lathyrus sativus* seeds. J. Agric. Food Chem. 48: 5432-5439.

Sammour R. H. (1987). Electrophoretic and serological studies of the seed proteins of some members of the subfamily Papilionoideae. Journal of Agronomy and Crop Science. 159: 282-286.

Sammour R. H. (1991). Using electrophoretic techniques in varietal identification, biosystematics analysis,

phylogenetic relations. Journal of Islamic Academy of Sciences 4(3): 221-226.

Sammour R.H., Mustafa A., Badr S., Taher, W. (2007a). Genetic variations in accessions of *Lathyrus sativus* L. Acta Bot. Croat. 66: 1-13.

Saxena K. B., Faris D. G., Singh U., Kumar. R. V. (1987). Relationship between seed size and protein content in newly developed high protein lines of pigeonpea. Plant Foods for Human Nutrition (Formerly Qualitas Plantarum). 36(4): 335-340, DOI: 10.1007/BF01892354.

Sundaresan V. (2005). Control of seed size in plants. *Proc. Natl. Acad. Sci. USA.* 102 (50) 17887-17888.

Turnbull L.A., Santamaria L., Martorell T., Rallo J., Hector A. (2006). Seed size variability: from carob to carats. Biol Lett. 2(3): 397–400.

Van-hintum J.L., Knüpffer H. (1995). Duplication within and between germplasm collections. I Identifying duplication on the basis of passport data. Genetic Resources and Crop Evolution 42: 127-133.

Vishwanath K., Prasanna K.P.R., Pallvi H.M., Prasad S., Ramegowda D. P.J. (2011). Identification of tomato (*Lycopersicon esculentum*) varieties through total soluble seed proteins. Research Journal of Agricultural Sciences. 2(1): 8-12.

Vollmann J., Fritz C. N., Wagentristl H., Uckenbauer P. (2000).Environmental and genetic variation of soybean seed protein content under Central European growing conditions. Published online: DOI: 10.1002/1097-0010(200007)80:9<1300:AID-JSFA640>3.0.CO; 2-I:1300-1306.

Wulff R.D. (1986a). Seed size variation in *Desmodium panculatum*. I. Factors affecting seed size. Journal of Ecology.74: 87-97.

Wulff R.D. (1986b). Seed size variation in *Desmodium panculatum*.II. Effect on seedling growth and physiological performance. Journal of Ecology.74: 99-104.

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Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley

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ABSTRACT

This paper reports the effects of selenium (Se) application on some physiological characteristics of barley (*Hordeum vulgare* L. cv. Rihane-03) exposed to drought stress. Foliar application to barley at 30 g selenium ha^{-1}, as sodium selenate, increased significantly shoot dry weight and relative water content in well-watered plants. A remarkable reduction in dry weight of water-stressed plants was associated with significant decrease in maximal efficiency of PSII (F_v/F_m), stomatal conductance (g_s) and net CO_2 assimilation rate (A). Activity of antioxidant enzymes was increased by drought stress significantly. Amounts of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) remained unchanged in Se-supplemented water-deficit plants obviously because of an efficient scavenging following significant enhancement of catalase (CAT) and glutathione peroxidase (GSH-Px) activities. These results indicate that an application of selenium was favorable for biomass accumulation of barley plants under well-watered conditions. However, it did not significantly affect dry matter accumulation under drought stress, but Se-supplemented water-deficit plants exhibited better protection from oxidative damage because of higher CAT and GSH-Px activities and lower level of lipid peroxidation. These results suggest that selenium application can improve antioxidant defense system under drought stress conditions, and it may be recommended for arid and semiarid regions.

Key words: Antioxidant enzymes, barley, drought, glutathione peroxidase, selenium

IZVLEČEK

UČINEK SUŠNEGA STRESA IN ŠKROPLJENJA S SELENOM NA FOTOSINTEZO IN ANTIOKSIDATIVNO AKTIVNOST JAREGA JEČMENA

Članek poroča o učinku škropljenja s selenom na nekatere fiziološke značilnosti ječmena (*Hordeum vulgare* 'Rihane-03'), ki je bil izpostavljen sušnemu stresu. Foliarna aplikacija selena 30 g Se ha^{-1}, kot selenat je značilno povečala suho težo poganjkov in relativno vsebnost vode dobro zalivanih rastlin. Znatno zmanjšanje suhe teže rastlin v pomanjkanju vode je bilo povezano z značilnim zmanjšanjem maksimalne učinkovitosti PSII (F_v/F_m), stomatarne prevodnosti (g_s) in neto asimilacije CO_2 (A). Aktivnost antioksidativnih encimov se je v sušnem stresu značilno povečala. Količini malondialdehida (MDA) in vodikovega peroksida (H_2O_2) sta ostali nespremenjeni pri rastlinah tretiranih s Se pri sušnem stresu, kar je bila očitno posledica delovanja Se, ki se je kazala kot povečana aktivnost katalaze (CAT) in glutation peroksidaze (GSH-Px). Ti izsledki kažejo, da uporaba Se prispeva k povečanju biomase ječmena pri dobri preskrbi z vodo. Vsebnost suhe snovi se pri rastlinah tretiranih s Se ni bistveno povečala v razmerah sušnega stresa, vendar pa so se te rastline bolje zaščitile pred oksidativnimi poškodbami s povečano aktivnostjo CAT in GSH-Px in manjšo peroksidacijo lipidov. Rezultati kažejo, da uporaba Se izboljša antioksidativno obrambo rastlin pri sušnem stresu in bi njegovo uporabo v te namene priporočali v aridnih in semiaridnih območjih.

Ključne besede: antioksidativni encimi, ječmen, suša, glutation peroksidaza, selen (Se)

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1 INTRODUCTION

Although selenium (Se) is not an essential element for plants (Terry *et al*., 2000), several studies demonstrate that selenium supply may exert diverse beneficial effects, including growth-promoting activities (Turakainen *et al*., 2004; Djanaguiraman *et al*., 2005). Moreover, some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses, e.g. drought (Kuznetsov *et al*., 2003; Germ *et al*., 2007; Yao *et al*., 2009), salinity (Kong *et al*., 2005; Djanaguiraman *et al*., 2005; Hawrylak-Nowak, 2009) and heavy metals (Srivastava *et al*., 2009; Cartes *et al*., 2010) stresses. Selenium exerts beneficial effects on growth and stress tolerance of plants by enhancing their antioxidative capacity (Kong *et al*., 2005; Rios *et al*., 2009).

A stimulatory effect of foliar application of Se on growth has been reported for ryegrass (Hartikainen *et al*., 2000), lettuce (Xue *et al*., 2001), potato (Turakainen *et al*., 2004), soybean (Djanaguiraman *et al*., 2005) and green tea leaves (Hu *et al*., 2003). Selenium can also delay senescence and promote the growth of aging seedlings (Xue *et al*., 2001). Selenium supplemented water-deficit buckwheat exhibit significantly higher stomatal conductance (g_s). A significantly higher actual photochemical efficiency of PSII was obtained in Se-supplemented water-deficit plants, which was possibly due to improvement of plant water management during treatment (Tadina *et al*., 2007). Selenium supply is favorable for growth of wheat seedlings during drought condition, however, the growth and physiological responses of seedlings were different, depending on the Se concentration (Yao *et al*., 2009). Simojoki (2003) reported that small Se addition that increased Se contents in lettuce shoots tend to enhance plant growth. It was shown that Se has the ability to regulate the water status of plants under drought conditions (Kuznetsov *et al*., 2003).

Recent researches have demonstrated that Se is not only able to promote growth and development of plants, but also increases resistance and antioxidant capacity of plants subjected to various stress (Peng *et al*., 2002; Djanaguiraman *et al*., 2005). Stress factors such as drought trigger common reactions in plants and lead to cellular damages mediated by reactive oxygen species (ROS). According to Price and Hendry (1991) who studied the role of oxygen radicals in different grasses exposed to drought, water deficit stress causes an overall inhibition of protein synthesis, inactivation of several chloroplast enzymes, impairment of electron transport, increased membrane permeability, and increased activity of the H_2O_2 scavenger system. Antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) play an important role against drought stress (Apel and Hirt, 2004; Habibi and Hajiboland, 2011). However, there are few reports on the protective role of exogenous Se on drought stress in plant. Also, the plants treated with selenate induce higher increases in enzymes that detoxify H_2O_2, especially ascorbate peroxidase (APX) and glutathione peroxidase (GSH-Px), thereby improving stress resistance (Kong *et al*., 2005; Rios *et al*., 2009).

Foliar application to barley at 10 and 20 g Se ha^{-1}, as sodium selenate, increases the Se contents of barley grain (MacLeod *et al*., 1998). Morever, a stimulatory effect of foliar application of Se on nitrogen assimilation has been reported for barley (Aslam *et al*., 1990), however, there is few published work concerning the expression of selenium effects on dry matter accumulation, protection against the oxidative stress and regulation the water status of *Hordeum vulgare* L. (barley). Barley is one of the most important crops. It is well characterized by the multiplicity of its agro-industrial uses. The aim of present work was to study the influence of Se on the photosynthesis characteristics and antioxidant activity in spring barley. In addition of monitoring the growth, relative water content, chlorophyll fluorescence parameters and gas exchange pattern, we examined the effect of selenium spraying on the antioxidant defense system during drought stress in barley plants.

2 MATERIALS AND METHODS

Plant growth and treatments: Seeds of barley (*Hordeum vulgare* L. cv. Rihane-03) were grown in a field trial in sandy loam soil near Malekan, NW Iran. Seeds planted on 5 rows in each plots, the rows distance was 20 cm and the plant distance on each row was 5 cm, beginning and end of each plots closed, with regarding area of each plots. For the basal fertilization, 100 kg ha^{-1} nitrogen as NH_4NO_3 and 50 kg ha^{-1} phosphorus and potassium as KH_2PO_4 were applied before sowing. Experiments were performed in complete randomized block design with 4 replications. The replicates were separated at random into two groups; well watered group and water-stressed group. For normal irrigation (well watered group), soil was kept at approximately 70% of field capacity by watering with tap water every 7 days and water holding at the beginning of stem elongation stage in water-stressed group of plants. After 35 days of drought exposure, selenium was sprayed at 30 g ha^{-1} as sodium selenate. After 10 days of selenium exposure, the plants were harvested and parameters were determined. Thousand seed weight and seed yield were measured at the end of the experiment.

Plant harvest and analysis of water relations: Leaves were washed with distilled water, blotted dry on filter paper and after determination of fresh weight (FW) were dried for 48 h at 70 °C for determination of dry weight (DW). Relative water content (RWC) was measured and calculated according to Lara et al. (2003) all in the second youngest leaf harvested at 1 h after light on in the field.

Measurements of photosynthetic gas exchange and chlorophyll fluorescence: Before harvest gas exchange parameters were measured. Net CO_2 fixation (A, µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}) were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) either after 5 h into the light period and sealed in the leaf chamber under a photon flux density of 2000 ± 100 µmol m^{-2} s^{-1} in field conditions. Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark adapted and light adapted leaves. Leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. Initial (F_0), maximum (F_m), variable ($F_v = F_m$-F_0) fluorescence as well as maximum quantum yield of PSII (F_v/F_m) were recorded. Light adapted leaves (400 µmol m^{-2} s^{-1}) were used for measurement of "steady-state" (F_s) and maximum (F'_m) fluorescence. Calculations were made for F'_0 ($F'_0 = F_0/[(F_v/F_m)+(F_0/F'_m)]$), effective quantum yield of PSII, Φ_{PSII} $[(F'_m-F_s)/F'_m]$, photochemical quenching, qP $[(F'_m-F_s)/(F'_m-F'_0)]$ and non-photochemical quenching, qN $(1-[(F'_m-F'_0)/(F_m-F_0)])$ (Krall and Edwards, 1992).

Assay of enzymes activity and related metabolites: Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). The activity of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) was determined as described by Habibi and Hajiboland (2010). Peroxidase (POD, EC 1.11.1.7) activity was determined using the guaiacol test at 470 nm (Chance and Maehly, 1995). The enzyme was extracted by 10 mM phosphate buffer pH 7.0 and assayed in a solution contained 10 mM phosphate buffer, 5 mM H_2O_2 and 4 mM guaiacol. The glutathione peroxidase (GSH-Px, EC 1.11.1.9) activity was measured by a modification of the method of Flohé and Günzler (1984) using the H_2O_2 as substrate. Enzyme was extracted in 50 mM phosphate buffer pH 7.0 and the supernatant was added to the reaction mixture contained 0.2 mL of the supernatant, 0.4 mL GSH (0.1 mM) and 0.2 mL $KNaHPO_4$ (0.067 M). The above reagents without supernatant extract were used for the non-enzyme reaction. After preheating the mixture on water bath at 25 °C for 5 min, 0.2 mL H_2O_2 (1.3 mM) was added to initiate the reaction. The reaction was stopped by adding 1 mL 1% trichloroacetic acid and the mixture was put into an ice bath for 30 min. Then the mixture was centrifuged for 10 min at 1100 g, 0.48 mL the supernatant was placed into a cuvette and 2.2 mL of 0.32 M Na_2HPO_4 and 0.32 mL of 1.0 mM DNTB were added for colour development. The absorbance at wavelength 412 nm was measured after 5 min. The enzyme activity was calculated as a decrease in GSH within the reaction time when

compared with that in the nonenzyme reaction. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid, and the H_2O_2 concentration was determined by the potassium titanium oxalate method (Hajiboland and Hasani, 2007). Soluble protein was estimated spectrophotometrically by the Bradford method (1976). Statistical analyses were carried out using sigma stat (3.5) with Tukey test ($P < 0.05$). Correlation analysis using Spearman Rank Order Correlation in sigma stat (3.5) were conducted to determine the relationship between measurement parameters.

3 RESULTS

Both relative water content (RWC) and dry weight decreased dramatically in water-stressed plants. In contrast to drought, selenium spraying treatment increased relative water content and dry matter accumulation in well-watered plants, as compared with control plants (Table 1). Thus, the treatment with the highest dry matter accumulation (Se-supplemented well-watered treatment) showed the highest relative water content (83.5%). At the end of the experiment, Thousand seed weight and seed yield decreased by 23.4 and 49.6% under water stress, respectively, in comparison to their respective plants under well-watered conditions. Seed yield was not affected by selenium spraying treatment.

Table 1: Shoot dry weight (mg plant^{-1}), thousand seed weight (g), seed yield (kg ha^{-1}) and leaf relative water content (RWC, %) under different treatments. Each value is the mean ± SD of 20 replicates. Data of each column indicated by the same letters are not significantly different ($P < 0.05$).

Treatments	Shoot dry weight	Thousand seed weight	Seed yield	Relative water content
Control	1845 ± 131 [b]	47.7 ± 4.19 [a]	2887 ± 141 [a]	72.5 ± 2.38 [b]
Drought	1120 ± 120 [c]	36.5 ± 4.43 [b]	1455 ± 161 [b]	55.2 ± 3.11 [c]
Selenium	2100 ± 212 [a]	51.2 ± 2.38 [a]	2995 ± 174 [a]	83.5 ± 3.69 [a]
Drought+Selenium	1210 ± 143 [c]	37.2 ± 2.50 [b]	1565 ± 83 [b]	57.6 ± 2.64 [c]

The study of PSII photochemistry in the dark-adapted leaves showed that there was no significant difference in the maximal quantum yield of PSII (F_v/F_m) between control and Se-supplemented plants under well-watered conditions (Table 2). However, reduction of maximal efficiency of PSII in dark-adapted leaves (F_v/F_m) and effective quantum yield of PSII (Φ_{PSII}) were detectable in leaves of water-stressed plants. In addition, stomatal conductance to water vapor (g_s) was positively correlated with F_v/F_m ($r = 0.70$, $P < 0.05$) in water-stressed plants. Photochemical quenching (qP) and non-photochemical quenching (qN) were not influenced under selenium spraying and drought conditions.

Net assimilation rate (A) was not influenced by selenium spraying, but was reduced by drought (Table 2). Transpiration rate (E) was not affected significantly by water stress, while g_s was reduced strongly under drought conditions but increased by selenium. In this study, a remarkable reduction in shoot dry weight in drought stressed plants was associated with a significant reduction of net CO_2 assimilation rate. Water use efficiency was significantly lower in drought-stressed plants. Thus, compared with the transpiration rate, the water use efficiency showed a greater decrease during the water deficit.

Table 2: Leaf physiological traits of barley plants under different treatments. *A* net photosynthetic rate, *E* transpiration rate, g_s stomatal conductance, *WUE* (*A/E*) water use efficiency, F_v/F_m maximum quantum yield of PSII, *qP* photochemical quenching, *qN* non-photochemical quenching, Φ_{PSII} effective quantum yield of PSII. Each value is the mean ± SD of 4 replicates. Data of each row indicated by the same letters are not significantly different ($P < 0.05$).

Photochemistry	Control	Drought	Selenium	Drought+Selenium
F_v/F_m	0.84 ± 0.01 [a]	0.81 ± 0.01 [b]	0.84 ± 0.02 [a]	0.82 ± 0.01 [ab]
qP	0.96 ± 0.02 [a]	0.96 ± 0.02 [a]	0.95 ± 0.02 [a]	0.95 ± 0.01 [a]
qN	0.17 ± 0.05 [a]	0.15 ± 0.02 [a]	0.14 ± 0.09 [a]	0.16 ± 0.08 [a]
Φ_{PSII}	0.79 ± 0.01 [a]	0.76 ± 0.01 [b]	0.79 ± 0.01 [a]	0.76 ± 0.01 [b]
Gas exchange	**Control**	**Drought**	**Selenium**	**Drought+Selenium**
A (µmol m^{-2} s^{-1})	14.2 ± 3.52 [a]	5.17 ± 1.63 [b]	16.3 ± 1.46 [a]	6.93 ± 2.40 [b]
E (mmol m^{-2} s^{-1})	5.95 ± 0.67 [a]	3.61 ± 1.34 [a]	5.54 ± 0.14 [a]	4.65 ± 1.40 [a]
g_s (mol m^{-2} s^{-1})	0.41 ± 0.05 [ab]	0.27 ± 0.13 [b]	0.52 ± 0.13 [a]	0.36 ± 0.10 [ab]
WUE (µmol $mmol^{-1}$)	2.39 ± 0.59 [a]	1.44 ± 0.07 [b]	2.94 ± 0.16 [a]	1.49 ± 0.12 [b]

Activity of antioxidant enzymes were influenced by drought stress significantly (Table 3). Drought stress caused a significant increase of SOD, POD, CAT and APX activity relative to control plants. In contrast, activity of GSH-Px was only increased in Se-supplemented water-deficit plants. Activity of SOD and APX in water-stressed plants did not differ from that in Se-supplemented water-deficit plants. However, a significant rise in the activity of GSH-Px and CAT was observed in the Se-supplemented water-deficit samples relative to water-deficit treatment. Continuation of the water stress without selenium spraying caused significant accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content. In contrast to drought stressed plants, in Se-supplemented water-deficit plants, the content of MDA and H_2O_2 remained quite stable over experimental period. There was a good negative correlation ($r = -0.69$, $P < 0.05$ in selenium treatment; $r = -0.64$, $P < 0.05$ in drought+selenium treatment) between GSH-Px activity and MDA content. Thus, the malondialdehyde content of leaves was highly associated with the glutathione peroxidase activity under selenium application.

Table 3: Antioxidant index of barley plants under different treatments. SOD superoxide dismutase, CAT catalase, POD peroxidase, APX ascorbate peroxidase, GSH-Px glutathione peroxidase, MDA malondialdehyde, H_2O_2 hydrogen peroxide. Each value is the mean ± SD of 4 replicates. Data of each row indicated by the same letters are not significantly different ($P < 0.05$).

Specific activity of enzymes	Control	Drought	Selenium	Drought+Selenium
SOD (U mg^{-1} protein)	11.1 ± 0.52 [b]	21.1± 2.84 [a]	15.1 ± 0.54 [b]	26.3 ± 4.15 [a]
CAT (µmol mg^{-1} protein min^{-1})	44.2 ± 6.0 [c]	82.1 ± 16.3 [b]	52.2 ± 8.1 [c]	127 ± 10.6 [a]
POD (µmol mg^{-1} protein min^{-1})	0.27 ± 0.07 [b]	0.49 ± 0.01 [a]	0.31 ± 0.02 [b]	0.52 ± 0.01 [a]
APX (µmol mg^{-1} protein min^{-1})	0.89 ± 0.04 [b]	1.30 ± 0.14 [a]	0.82 ± 0.03 [b]	1.26 ± 0.12 [a]
GSH-Px (nmol mg^{-1} protein min^{-1})	0.10 ± 0.01 [c]	0.14 ± 0.02 [bc]	0.16 ± 0.03 [b]	0.24 ± 0.01 [a]
Metabolite content	**Control**	**Drought**	**Selenium**	**Drought+Selenium**
MDA (nmol g^{-1} FW)	14.7 ± 0.50 [b]	29.8 ± 1.20 [a]	12.9 ± 1.43 [b]	16.7 ± 0.93 [b]
H_2O_2 (µmol g^{-1} FW)	0.84 ± 0.10 [b]	1.15 ± 0.12 [a]	0.86 ± 0.04 [b]	0.93 ± 0.08 [b]

4 DISCUSSION

Most probably the first positive effect of selenium on plant growth was reported by Singh *et al.* (1980), who showed that the application of selenium stimulated growth and dry matter yield of *Brassica juncea*. In this work, similarly with that observed for lettuce, ryegrass (Hartikainen *et al.*,

1997; Hartikainen *et al*., 2000) and soybean (Djanaguiraman *et al*., 2005), selenium increased shoot dry weight in barley plants. Drought stress reduced growth activity of barley (Tables 1), as is observed by other plants species (Ramesh, 1999; Liu and Stützel, 2004; Degu *et al*., 2008). Recently, Yao *et al*. (2009) suggested that optimal Se supply is favorable for growth of wheat seedlings during drought condition. In this work, however, selenium spraying could not improve growth parameters under drought conditions.

An important role associated with the survival of the plants grown under drought conditions is played by the leaf stomata. In the present study, the stomatal density decreased significantly with water stress. Reduction of stomatal conductance (g_s) inhibits supply of CO_2 and consequently reduces CO_2 assimilation (A) is a well known phenomenon in drought stressed plants (Lawlor and Cornic, 2002). Our results confirm the great sensitivity of leaf photosynthesis to drought. Reductions in photosynthetic performance under water stress have also been observed by Tognetti *et al*. (2005), Bacelar *et al*. (2006) and Ben Ahmed *et al*. (2009). Following the drought stress, Se-supplemented plants showed a lower reduction in these photosynthetic parameters (A and g_s) in response to drought stress when compared with water-deficit plants. Thus, similarly with that observed for buckwheat (Tadina *et al*., 2007), stomatal conductance (g_s) was slightly higher in Se-supplemented water-deficit plants, but net CO_2 assimilation rate did not increase in Se-supplemented water-deficit plants relative to water-deficit plants in this study. The water status of the barley leaves has been put in evidence by water use efficiency (WUE) and relative water content (RWC) parameters, so the RWC rate and WUE decreased significantly because of unchanged transpiration rate (E) at water stress in both treatments tested. On the other hands, the comparison of the leaf water content between the two well-watered treatments showed that leaf water content of selenium treatment increased more than control. The net photosynthetic rate and stomatal conductance of higher plants leaves are known to decrease as RWC decrease (Lawlor and Cornic, 2002).

Many authors suggested application of chlorophyll a fluorescence analysis as a reliable method to assess the changes in the function of PSII under stress conditions (Price and Hendry, 1991; Broetto *et al*., 2007). Lower photosynthetic activity could be a consequence of low photochemical efficiency of PSII, as shown by its lower quantum yield (Pieters and Souki, 2005). To test the functionality of the photochemical apparatus, barley plants were treated with drought stress. We found that the significant decrease in their maximal efficiency of PSII photochemistry (F_v/F_m) were observed at water stress conditions. In agreement with the results of Angelopoulos *et al*. (1996) showing that during the development of water stress a gradual decline of the ratio F_v/F_m occurred. Recently, Boussadia *et al*. (2008) showed that F_v/F_m was reduced significantly in plants submitted to water deficit. Declining F_v/F_m values implies that photochemical conversion efficiency is altered and could indicate the possibility of photoinhibition (Ranjbarfordoei *et al*., 2006). In our study, photoinhibition was occurred in drought treatment. A reduction in maximum quantum yield of PSII (F_v/F_m) was obtained in water-deficit plants (Table 2), which was possibly due to the reduction of g_s and restriction of CO_2 for photosynthesis and indicated photoinhibition. The significant correlation between F_v/F_m and g_s confirmed the idea that limited carbon assimilation by the decrease in stomatal conductance is viewed as an important protective mechanism under drought. This positive correlation suggests that the reduction in maximal efficiency of PSII photochemistry particularly at drought stress may be due to factors affecting stomatal closure rather than to damages in the photosynthetic apparatus. In addition, increasing stomatal conductance to water vapor resulted in subsequent increase in the net photosynthetic rate under selenium spraying showed that stomata are the main limiting factor to carbon uptake (Cornic *et al*., 1992; Boussadia *et al*., 2008).

Plants protect cell systems from the cytotoxic effects of drought-accumulated active oxygen species using enzymes such as SOD, APX, glutathione peroxidase (GSH-Px) and CAT (Verhagen *et al*., 2004). Several studies have shown that a protective role of Se against the oxidative stress in higher plants coincided with enhanced GPX activity and decreased lipid peroxidation (Cartes *et al*., 2005). Selenate application at 30 g ha^{-1} caused variations in the

SOD, APX and POD activity and also increased the CAT and GSH-Px activities (Table 3). The results for GSH-Px coincide with several studies made with Se in which an increase in this trace element augmented its activity (Xue *et al*., 2001; Cartes *et al*., 2005). In this work, a significant rise in the activity of GSH-Px and CAT in the Se-supplemented water-deficit samples relative to water-deficit treatment revealed that Se exerts beneficial effects on stress tolerance of barely by enhancing their antioxidative capacity. Amounts of MDA and H_2O_2 remained unchanged under Se-supplemented water-deficit conditions obviously because of an efficient scavenging following significant enhancement of CAT and GSH-Px activity. It indicate that antioxidant defense system may protect plants under Se-supplemented water-deficit conditions, while under water stress without selenium spraying, an imbalance between production and scavenging of ROS may cause stress as could be judged by accumulation of MDA.

In summary, we investigate the changes in physiological parameters in spring barley grown under drought stress and selenium spraying. Our results show that selenium spraying (1) causes a significantly higher growth rate, (2) affects plant water relations, as expressed by a significant decrease in leaf water content and (3) causes a significant increase in antioxidative capacity under drought conditions. Physiological and molecular mechanisms that underlie the beneficial effects of Se in plants have not been fully explained yet. There are few reports on the protective role of exogenous Se on drought stress in plant. In conclusion, Se-supplemented water-deficit plants exhibited better protection from oxidative damage and this ability was associated with higher CAT and GSH-Px activities and lower level of lipid peroxidation. These data indicate that an application of selenate at low rates can be used to promote the induction in plants of the antioxidant system, thereby improving stress resistance. However, it would be necessary to confirm these results in future with studies that focus on the effect of Se during the application time and the response of the different isoenzymes that make up the antioxidant system in plants. From this conclusion we can say that Se treatment did not significantly affect dry mass under drought conditions, although it increased dry matter in well watered plants and some antioxidant index in water-deficit plants. These results suggest that selenium application can improve antioxidant defense system under drought stress conditions, and it may be recommended for soils in arid and semiarid regions.

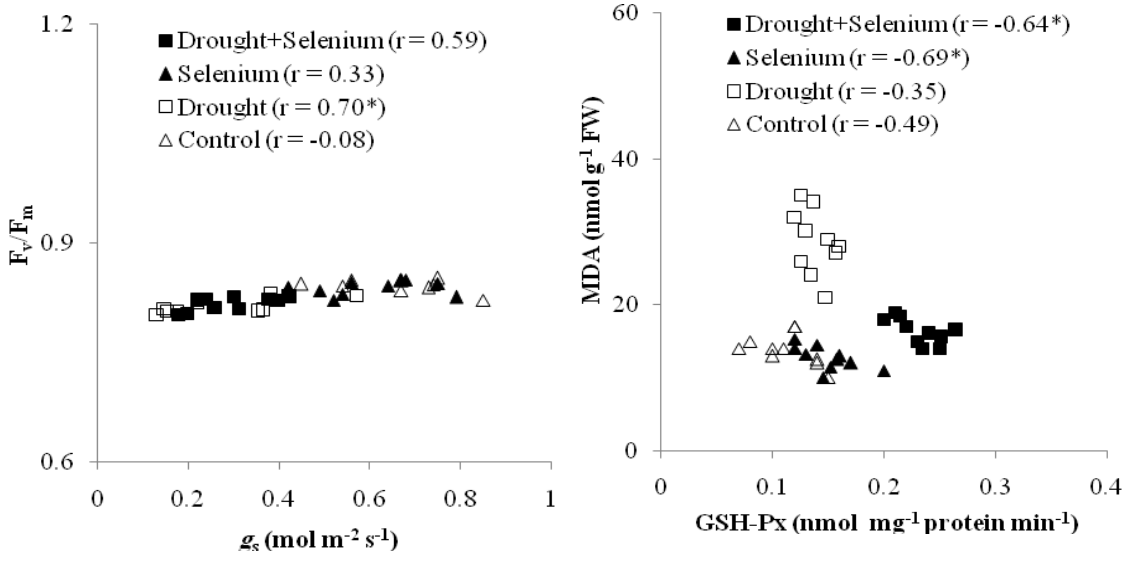

Figure 1: Correlations between maximum quantum yield of PSII (F_v/F_m) and stomatal conductance (g_s) and between glutathione peroxidase (GSH-Px) activity and malondialdehyde (MDA) content in barley plants at different treatments. ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively.

5 REFERENCES

Angelopoulos, K., Dichio, B., Xiloyannis, C. 1996. Inhibition of photosynthesis in olive trees (*Olea europaea* L.) during water stress and rewatering. J Exp Bot. 47: 1093–1100.

Apel, K., Hirt, H. 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Ann. Rev. Plant Biol. 55: 373–399.

Aslam, M., Harbit, K.B., Huffaker, R.C. 1990. Comparative effects of selenite and selenate on nitrate assimilation in barley seeding. Plant Cell. Environ. 13: 773–782.

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Gonçalves, B.C., Ferreira, H.F., Correia, C.M. 2006. Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. Plant Sci. 170: 596–605.

Ben Ahmed, C., Ben Rouinab, B., Sensoyc, S., Boukhrisa, M., Ben Abdallah, F. 2009. Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. Environ Exp Bot. 67: 345–352.

Boussadia, O., Ben Mariem, F., Mechri, B., Boussetta, W., Braham, M., Ben El Hadj, S. 2008. Response to drought of two olive tree cultivars (cv. Koroneki and Meski). Sci Hortic. 116: 388–393.

Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Broetto, F., Duarte, H.M., Lüttge, Q. 2007. Responses of chlorophyll fluorescence parameters of the facultative halophyte and C_3-CAM intermediate species *Mesembryanthemum crystallinum* to salinity and high irradiance stress. J. Plant Physiol. 164: 904–912.

Cartes, P., Gianfera, L., Mora, M.L. 2005. Uptake of selenium and its antioxidative activity in ryegrass when applied a selenate and selenite forms. Plant Soil. 276: 359–367.

Cartes, P., Jara, A.A., Pinilla, L., Rosas, A., Mora, M.L. 2010. Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. Ann. Appl. Biol. 156: 297–307.

Chance, B., Maehly, A.C. 1995. Assays of catalases and peroxidases. Methods Enzymol. 2: 764–775.

Cornic, G., Ghashghaie, J., Genty, B., Briantais, J.M. 1992. Leaf photosynthesis is resistant to a mild drought stress. Photosynthetica. 27: 295–309.

Degu, H.D., Ohta, M., Fujimura, T. 2008. Drought tolerance of Eragrostis tef and development of roots. Int J Plant Sci. 169: 768–775.

Djanaguiraman, M., Devi, D.D., Shanker, A.K., Sheeba, A., Bangarusamy, U. 2005. Selenium-an antioxidative protectant in soybean during senescence. Plant Soil. 272: 77–86.

Flohé, L., Günzler, W.A. 1984. Methods in Enzymology. In: Packer L (ed), Assays of glutathione peroxidase, pp. 114–121. Academic Press, New York.

Germ, M., Stibilj, V., Osvald, J., Kreft, I. 2007. Effect of selenium foliar application on chicory (*Cichorium intybus* L.). J. Agric. Food Chem. 55: 795–798.

Habibi, G., Hajiboland, R. 2011. Comparison of water stress and UV radiation effects on the induction of CAM and antioxidative defense in the succulent *Rosularia elymaitica* (Crassulaceae)**.** Acta Biol. Cracov. Bot. 53: 7–15.

Habibi, G., Hajiboland, R. 2010. Photosynthetic characteristics and antioxidative responses in three species of Crassulaceae following drought stress. Journal of Sciences I.R. Iran. 21: 205–212.

Hajiboland, R., Hasani, B.D. 2007. Responses of antioxidant defense capacity and photosynthesis of bean (*Phaseolus vulgaris* L.) plants to copper and manganese toxicity under different light intensities. Acta Biol. Szeged. 51: 93–106.

Hartikainen, H., Ekholm, P., Piironen. V., Xue, T., Koivu, T., Yli-Halla, M. 1997. Quality of the ryegrass and lettuce yields as affected by selenium fertilization. Agric. Food Sci. Finland. 6: 381–387.

Hartikainen, H., Xue, T., Piironen, V. 2000. Selenium as an antioxidant and pro-oxidant in ryegrass. Plant Soil. 225: 193–200.

Hawrylak-Nowak, B. 2009. Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. Biol. Trace Elem. Res. 132: 259–269.

Hu, Q.H., Xu, J., Pang, G.X. 2003. Effect of selenium on the yield and quality of green tea leaves harvested in early spring. J. Agric. Food Chem. 51: 3379–3381.

Kong, L., Wang, M., Bi, D. 2005. Selenium modulates the activities of antioxidant enzymes, osmotic

homeostasis and promotes the growth of sorrel seedlings under salt stress. Plant Growth Regul. 45: 155–163.

Krall, J.P., Edwards, G.E. 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol. Plant. 86: 180–187.

Kuznetsov, V.V., Kholodova, V.P., Kuznetsov, V.V., Yagodin, B.A. 2003. Selenium regulates the water status of plants exposed to drought. Dokl. Biol. Sci. 390: 266–268.

Lara, M.V., Disante, K.B., Podesta, F.E., Andreo, C., Drincovich, M.F. 2003. Induction of a crassulacean acid like metabolism in the C_4 succulent plant, *Portulaca oleracea* L.: physiological and morphological changes are accompanied by specific modifications in phosphoenolpyruvate carboxylase. Photosynth Res. 77: 241–254.

Lawlor, D.W., Cornic, G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 25: 275–294.

Liu, F., Stützel, H. 2004. Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. Sci Hortic. 102: 15–27.

MacLeod, J.A., Gupta, U.C., Milburn, P., Sanderson, J.B. 1998. Selenium concentration in plant material, drainage and surface water as influenced by Se applied to barley foliage in a barley red clover potato rotation. Can. J. Soil Sci. 78: 685–688.

Peng, X.L., Liu, Y.Y., Luo, S.G. 2002. Effects of selenium on lipid peroxidation and oxidizing ability of rice roots under ferrous stress. J. Northeast Agric. Univ. 19: 9–15.

Pieters, A.J., El Souki, S. 2005. Effects of drought during grain filling on PSII activity in rice. J. Plant Physiol. 62: 903–911.

Price, A.H., Hendry, G.A.F. 1991. Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. Plant Cell Environ. 14: 477–484.

Ramesh, P. 1999. Sugarcane Breeding Institute, Coimbatore, India Effect of different levels of drought during the formative phase on growth parameters and its relationship with dry matter accumulation in sugarcane. J Agr Crop Sci. 185: 83–89.

Ranjbarfordoei, A., Samson, R., Van Damme, P. 2006. Chlorophyll fluorescence performance of sweet almond [*Prunus dulcis* (Miller) D. Webb] in response to salinity stress induced by NaCl. Photosynthetica. 44: 513–522.

Rios, J.J., Blasco, B., Cervilla, L.M., Rosales, M.A., Sanchez-Rodriguez, E., Romero, L., Ruiz, J.M. 2009. Production and detoxification of H_2O_2 in lettuce plants exposed to selenium. Ann. Appl. Biol. 154: 107–116.

Simojoki, A. 2003. Allocation of added selenium in lettuce and its impact on root. Agric. Food Sci. Finland. 12: 155–164.

Singh, M., Singh, H., Bhandari, D.K. 1980. Interaction of selenium and sulphur on the growth and chemical composition of raya. Soil Sci. 129: 238–244.

Srivastava, M., Maa, L.Q., Rathinasabapathib, B., Srivastava, P. 2009. Effects of selenium on arsenic uptake in arsenic hyperaccumulator *Pteris vittata* L. Bioresour. Technol. 100: 1115–1121.

Tadina, N., Germ, M., Kreft, I., Breznik, B., Gaberščik, A. 2007. Effects of water deficit and selenium on common buckwheat (*Fagopyrum esculentum* Moench.) plants. Photosynthetica. 45: 472–476.

Terry, N., Zayed, A.M., de Souza, M.P., Tarun, A.S. 2000. Selenium in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 401–432.

Tognetti, R., d'Andria, R., Morelli, G., Alvino, A. 2005. The effect of deficit irrigation on seasonal variations of plant water use in *Olea europaea* L. Plant Soil. 273:139–155.

Turakainen, M., Hartikainen, H., Seppänen, M.M. 2004. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J. Agric. Food Chem. 52: 5378–5382.

Verhagen, J., Put, M., Zaal, F., van Keulen, H. 2004. Climate change and drought risks for agriculture. Environ. Poll. 39: 49–59.

Xue, T., Hartikainen, H., Piironen, V. 2001. Antioxidative and growth-promoting effect of selenium in senescing lettuce. Plant Soil. 27: 55–61.

Yao, X., Chu, J., Wang, G. 2009. Effects of selenium on wheat seedlings under drought stress. Biol. Trace Elem. Res. 130: 283–290.

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Agris category code: F62

Influence of seed priming on emergence and growth of coriander (*Coriandrum sativum* L.) seedlings grown under salt stress

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ABSTRACT

Salinity is one of the biggest limiting factors for agriculture in semi-arid areas of the world. For this reason, an experiment was conducted to study the effect of seed priming with NaCl and $CaCl_2$ on growth and yield responses of Tunisian coriander cultivar exposed to five levels of salinity (0, 2, 4, 6, 8 g l^{-1}). Seeds of coriander were primed with aerated solutions of 0.13 M NaCl and $CaCl_2$ for 24 h. Results indicated that with increasing salinity, emergence traits (total emergence, mean emergence time), growth parameters (plant height, shoot fresh and dry weight) and mineral contents (K^+ and Ca^{2+}) decreased, but to a less degree in primed seeds. In all of the salinity levels, primed seeds possessed higher emergence and growth rate than control. However, further studies are needed to highlight the effect of seed priming on yield and oil content of coriander under salt stress.

Key words: Coriander, salinity, seed priming, emergence, growth, mineral content

IZVLEČEK

VPLIV PRETRETIRANJA SEMEN KORIANDRA (*Coriandrum sativum* L.) S SOLNIMI RAZTOPINAMI NA VZNIK IN RAST V RAZMERAH SOLNEGA STRESA

Slanost je eden izmed največjih omejevalnih dejavnikov kmetijstva v polsušnih območjih sveta. V ta namen je bil izveden poskus za preučevanje učinkov predtretiranja semen z NaCl in $CaCl_2$ sorte tunizijskega koriandra na parametre rasti in pridelka, ki je bila izpostavljena petim stopnjam slanosti (0, 2, 4, 6, 8 g l^{-1}). Semena koriandra so bila tretirana s prezračeno raztopino 0.13 M NaCl in $CaCl_2$ za 24 h. Izsledki so pokazali, da so z naraščajočo slanostjo parametri vznika (totalni vznik, poprečni čas vznika), rasti (višina rastlin, sveža in suha masa poganjkov) in vsebnosti hranil (K^+ in Ca^{2+}) upadli, vendar manj pri predhodno tretiranih semenih. Predhodno tretirana semena so imela pri vseh stopnjah slanosti boljši vznik in večjo rast kot kontrola. Za preučitev učinka predtretiranja s solmi na pridelek in vsebnost olj koriandra v razmerah solnega stresa so potrebne še nadaljne raziskave.

Ključne besede: koriander, slanost, predtretiranje semen, vznik, rast, vsebnost hranil

1 INTRODUCTION

Salt stress is certainly one of the most serious environmental factors limiting the productivity of crop plants (Ashraf, 1999). This is due to the fact that salinity affects most aspects of plant physiology, growth and development (Borsani et al. 2003). It is expected that salinity will affect 50% of arable land in the middle of the 21st century (Wang et al. 2003). In this respect, the need to develop crops with high tolerance to salinity has increased dramatically in the last decade. Beside genetic adaptation plants can, to a certain level, acclimated to salt stress. Salt tolerance of plants can be increased by seed treatment with different osmotic solutions

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(inorganic salts, sugars, growth regulators and polyethylene glycol) known as seed priming. Seed priming is a pre-sowing treatment that involves exposure of seeds to low external water potential that limits hydration. This hydration is sufficient to permit pre-germinative metabolic events but insufficient to allow radicle protrusion through the seed coat. This technique has become a common seed treatment that can increase emergence, growth, yield and salt tolerance mainly under unfavorable environmental conditions. Seeds with rapid germination under salt stress are expected to have a high percentage of germination and early establishment of culture and a better yield (Rogers et al. 1995). In general, coriander is known as a species moderately tolerant to salinity, the salinity effect appears mainly during germination and plant growth. In this context, the objective of this work is to improve the germination, growth and mineral contents of coriander using NaCl and $CaCl_2$ as osmotic solutions under salt stress.

2 MATERIALS AND METHODS

The experiment was carried in the experimental field research of High Institute of Agriculture, Chott Mariem, Tunisia. Coriander (*Coriandrum sativum* 'Sandra') seeds were primed with 0.13 M aerated solutions of NaCl and $CaCl_2$ for 24 hours, at 22 °C. After priming, primed and non-primed seeds (control seeds) were sown directly in the soil in March 2011 in the field experiment of High Institute of Agronomy, Chott-Mariem, Tunisia. The climate of the region is described as semi-arid, with an average annual precipitation of 230 mm and an approximate daily evaporation of 6 mm day^{-1}. In winter, the average minimum temperature is 6 °C and the average maximum is 18 °C, while in summer average minimum is 23 °C and average maximum is 38 °C. The soil is sandy clay with an organic matter of 1%. Throughout their vegetative cycles, plants from primed and control seeds were irrigated with saline water at five levels of NaCl concentrations (0, 2, 4, 6 and 8 g l^{-1}). The experiment was arranged factorial in a completely randomized design with two factors which are priming treatment (NaCl primed seeds, $CaCl_2$ primed seeds and control seeds) and salinity levels (0, 2, 4, 6 and 8 g l^{-1} NaCl) with three replications and 20 plants per replicate.

Plants were harvested at the flowering stage for both salt treated and non-treated plants. Data on total emergence (%), mean emergence time (days), plant height (cm), shoot fresh and dry weight (g $plant^{-1}$). Coriander shoot mineral contents (Na^+, K^+, Ca^{2+}, Na^+/K^+ and Na^+/Ca^{2+}) was determined according to the method of Taleisnik and Grunberg (1994). Dried matters of leaves and stems of coriander were digested with nitric acid 0.1 N. Cations concentrations in the extracts such as Na^+, K^+ and Ca^{2+} were determined by flame spectrophotometer.

Emergence, growth and mineral parameters of coriander were evaluated with analysis of variance (ANOVA) and Duncan multiple range test ($p < 0.05$) using SPSS software version (13.0). Differences were considered significant at the 5% level (means followed by different letters).

3 RESULTS

3.1 Total emergence and mean emergence time

Total emergence of coriander seedlings from both primed (P) and non-primed seeds (NP) decreased significantly with increasing NaCl salinity. However, this reduction in total emergence was higher for NP seeds, compared to P seeds. NaCl primed seeds have the highest total emergence. At 4 g l^{-1} NaCl, it was about 3% and 18% higher, in comparison with $CaCl_2$ and control seeds, respectively. This difference becomes more pronounced with increasing NaCl level.

Mean emergence time (MET) increased with rising of salinity levels in both primed and un-primed seeds (Table 1). Rising salinity increases MET from 7.3 days at 0 g L^{-1} to 20.3 days at 8 g L^{-1} for control seeds. Meanwhile, MET in primed seeds in all salinity levels was less than that of un-primed seeds. In fact, at 4 g l^{-1}, MET is about 16.5 days for

control seeds, 14.3 days for $CaCl_2$ primed seeds and 12.6 days for NaCl primed seeds.

3.2 Plant height

Salinity levels and seed priming had a significant ($p < 0.05$) effect on plant height. Its reduction by salinity was more severe in control seeds when compared with primed seeds. Similarly, the effect of seed priming was more profound on plant height at high salinity level (8 g l^{-1}). Plant height decreased significantly ($p < 0.05$) with increasing salinity for both plants derived from primed and control seeds; however, this decrease was less pronounced in plants originated from primed seeds. Increasing salinity from 0 g l^{-1} to 8 g l^{-1} drastically decreases coriander plant height of about 80% in plant derived from control seeds; nevertheless, this decrease was less marked in plants derived from NaCl primed seeds (62%) and plants derived from CaCl2 primed seeds (65%).

3.3 Plant fresh and dry weight

Fresh and dry weight of coriander plant significantly decreased due to an increase in NaCl salinity in both primed and control seed (Table 1). Under saline conditions, plants of primed group had a higher fresh weight than non primed group. At 6 g l^{-1} NaCl, plant fresh weight of NaCl and $CaCl_2$ primed group is 32% and 12% higher, respectively, than of control group. In general, increased NaCl salinity significantly decreased plant dry weight in both primed and control groups. However, dry weight in plant of primed group was significantly higher in each salinity level than in the non primed group. In fact, at 4 g l^{-1} NaCl, dry weight of primed group (NaCl and $CaCl_2$ seed priming) is 1.02 and 0.82 g $plant^{-1}$ respectively and 0.73 g $plant^{-1}$ for plants derived from control seeds.

3.4 The content of minerals

Mineral elements (sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), Na^+/K^+ and Na^+/Ca^{2+} ratios), were significantly affected by increasing salinity level (Table 2). Na^+, K^+ and Ca^{2+} concentrations of coriander shoot were significantly ($p < 0.05$) affected by increasing salinity levels and seed priming treatments. Increasing salinity levels increased the accumulation of Na^+ and decreased K^+ and Ca^{2+} content of coriander shoot. Plants derived from control seeds accumulated more Na^+ and less K^+ and Ca^{2+} than plants derived from primed seeds when exposed to different salinity levels. Plants from NaCl primed seeds accumulated 35% less Na^+, 32% more K^+ and 28% more Ca^{2+} at 6 g l^{-1} when compared with non-primed treatment. Na^+/K^+ and Na^+/Ca^{2+} ratios significantly increased with increasing salinity in both primed and non-primed seeds.

Table 1: Effect of seed priming and salinity on growth characteristics of coriander under NaCl stress. Means ± standard errors are presented. Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Treatments		Total Emergence (%)	Mean Emergence Time (days)	Plant Height (cm)	Shoot Fresh Weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
NaCl (g l^{-1})	**Seed priming**					
0	NaCl priming	98.6 ± 2.01^{a}	7.3 ± 0.12^{g}	51.2 ± 1.33^{a}	4.21 ± 0.12^{a}	1.62 ± 0.06^{a}
	$CaCl_2$ priming	98.4 ± 1.89^{a}	10.2 ± 0.18^{f}	48.2 ± 1.35^{b}	3.42 ± 0.16^{b}	1.45 ± 0.05^{b}
	Control seed	97.8 ± 1.96^{a}	12.4 ± 0.16^{e}	45.3 ± 1.31^{c}	3.18 ± 0.17^{b}	1.31 ± 0.02^{c}
2	NaCl priming	86.7 ± 1.65^{b}	9.4 ± 0.11^{f}	40.8 ± 1.22^{d}	2.84 ± 0.09^{c}	1.18 ± 0.03^{d}
	CaCl2 priming	81.8± 1.54^{c}	12.8 ± 0.17^{e}	37.2 ± 1.18^{e}	2.44 ± 0.08^{c}	1.06 ± 0.02^{de}
	Control seed	78.3± 1.43^{cd}	14.6 ± 0.19^{d}	31.6 ± 1.19^{f}	2.12 ± 0.07^{d}	0.91 ± 0.01^{e}
4	NaCl priming	77.4 ± 1.45^{cd}	12.6 ± 0.21^{e}	32.6 ± 1.11^{f}	2.58 ± 0.09^{c}	1.02 ± 0.02^{de}
	CaCl2 priming	75.1 ± 1.44^{d}	14.3 ± 0.22^{d}	29.3 ± 1.08^{g}	1.76 ± 0.04^{e}	0.82 ± 0.01^{ef}
	Control seed	63.7 ± 1.38^{e}	16.5 ± 0.26^{c}	20.8 ± 1.02^{h}	1.55 ± 0.02^{f}	0.73 ± 0.02^{f}
6	NaCl priming	56.4 ± 1.23^{f}	14.1 ± 0.31^{d}	28.2 ± 1.19^{g}	2.09 ± 0.06^{d}	0.89 ± 0.01^{ef}
	CaCl2 priming	51.8 ± 1.21^{g}	16.7 ± 0.29^{c}	26.4 ± 1.21^{g}	1.66 ± 0.02^{ef}	0.72 ± 0.02^{f}
	Control seed	37.1 ± 1.11^{h}	18.4 ± 0.28^{b}	17.8 ± 0.93^{i}	1.41 ± 0.01^{g}	0.61 ± 0.01^{g}
8	NaCl priming	38.1 ± 1.02^{h}	16.8 ± 0.28^{c}	19.2 ± 0.82^{h}	1.91 ± 0.03^{de}	0.76 ± 0.02^{f}
	CaCl2 priming	31.2 ± 0.92^{i}	18.3 ± 0.25^{b}	17.3 ± 0.91^{i}	1.49 ± 0.02^{fg}	0.65 ± 0.01^{g}
	Control seed	16.8 ± 0.86^{j}	20.3 ± 0.35^{a}	9.4 ± 0.12^{j}	1.27 ± 0.01^{h}	0.52 ± 0.03^{h}

Table 2: Effect of seed priming and salinity on growth characteristics of coriander under NaCl stress. Means ± standard errors are presented. Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Treatments		Na^+	K^+	Ca^{2+}	Na^+/K^+	Na^+/Ca^{2+}
NaCl (g l^{-1})	**Seed priming**	(meq mg^{-1} dw)	(meq mg^{-1} dw)	(meq mg^{-1} dw)		
0	NaCl priming	0.61 ± 0.01^{j}	0.63 ± 0.01^{a}	0.71 ± 0.04^{a}	0.96 ± 0.06^{j}	0.85 ± 0.04^{g}
	$CaCl_2$ priming	0.69 ± 0.01^{ij}	0.51 ± 0.02^{b}	0.59 ± 0.03^{b}	1.35 ± 0.08^{i}	1.16 ± 0.09^{fg}
	Control seed	0.82 ± 0.02^{h}	0.42 ± 0.03^{d}	0.51 ± 0.02^{c}	1.95 ± 0.09^{g}	1.57 ± 0.11^{f}
2	NaCl priming	0.72 ± 0.03^{i}	0.53 ± 0.02^{b}	0.58 ± 0.02^{b}	1.35 ± 0.08^{i}	1.24 ± 0.06^{fg}
	CaCl2 priming	0.78 ± 0.02^{h}	0.47 ± 0.02^{c}	0.52 ± 0.03^{c}	1.65 ± 0.06^{h}	1.51 ± 0.05^{f}
	Control seed	1.15 ± 0.03^{g}	0.36 ± 0.01^{e}	0.43 ± 0.01^{e}	3.19 ± 0.15^{e}	2.67 ± 0.08^{e}
4	NaCl priming	1.14 ± 0.04^{g}	0.46 ± 0.02^{c}	0.51 ± 0.03^{c}	2.47 ± 0.23^{f}	2.23 ± 0.07^{ef}
	CaCl2 priming	1.24 ± 0.05^{fg}	0.41 ± 0.01^{d}	0.46 ± 0.04^{d}	3.02 ± 0.21^{e}	2.69 ± 0.09^{e}
	Control seed	1.41 ± 0.06^{de}	0.31 ± 0.02^{f}	0.36 ± 0.02^{f}	4.53 ± 0.27^{d}	3.91 ± 0.11^{d}
6	NaCl priming	1.27 ± 0.05^{f}	0.39 ± 0.04^{d}	0.46 ± 0.02^{d}	3.25 ± 0.26^{e}	2.76 ± 0.08^{e}
	CaCl2 priming	1.34 ± 0.07^{e}	0.30 ± 0.03^{f}	0.38 ± 0.02^{f}	4.46 ± 0.31^{d}	3.52 ± 0.09^{d}
	Control seed	1.72 ± 0.09^{b}	0.22 ± 0.01^{g}	0.29 ± 0.01^{h}	7.81 ± 0.42^{b}	5.93 ± 0.17^{b}
8	NaCl priming	1.48 ± 0.05^{d}	0.28 ± 0.01^{fg}	0.34 ± 0.02^{g}	5.28 ± 0.36^{c}	4.35 ± 0.12^{c}
	CaCl2 priming	1.58 ± 0.07^{c}	0.14 ± 0.02^{h}	0.27 ± 0.03^{h}	13.36 ± 0.34^{ab}	5.85 ± 0.13^{b}
	Control seed	1.88 ± 0.10^{a}	0.10 ± 0.01^{i}	0.21 ± 0.01^{i}	15.42 ± 0.53^{a}	8.95 ± 0.21^{a}

4 DISCUSSION

Primed seeds had better emergence percentage in salt stress in comparison with un-primed seeds. It is obvious that metabolic activities in primed seeds during germination process commenced much earlier than radicle and plumule appearance, so primed seeds emerged earlier than non-primed ones (Hopper et al., 1979). Like emergence percentage, primed seeds had lower mean emergence time (MET) compared with un-primed seeds. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Szabolcs, 1994; Sivriteps et al., 2003). There are several reports that seed priming can homogenize seed germination in a short period of time (Zhu, 2002; Khajeh-Hosseini et al., 2003). The present study also demonstrated that plant height recorded in plants derived from primed seeds were significantly different from plants derived from non-primed treatments when exposed to different salinity levels. Similar results are also reported by Sivritepe et al (2003) in melon. It was observed that boosting levels of salinity has gradually decreased plant height which might be due to decreased physiological activities resulting from water and nutrients stress occurring under salinity stress. The adverse effect of salinity on plants may lead to disturbances in plant metabolism, which consequently led to reduction of plant growth and productivity (Shafi. et al. 2009). Seed priming and salinity levels extensively affected shoot fresh and dry weight (g $plant^{-1}$) of coriander. Shoot weight decreased progressively with the rise of stress level compared with control. Fortmeier and Schubert (1995) also reported similar results in barley.

The toxic effect of sodium at high salt levels and physical damage to roots decreased their ability to absorb water and nutrient which caused marked

reduction in photosynthesis, enzymatic process and protein synthesis (Tester and Davenport, 2003), which resulted in stunted growth and poor leaf area development. The decrease in the rate of photosynthesis due to leaf area might be responsible to decrease shoot fresh and in turn dry weight. It is evident from results that primed seeds in comparison with control seeds resulted in more crop growth rate (Basra et al., 2003). Therefore, it is concluded that seed priming improve coriander growth under salt stress. These results agree with the finding of Harris et al. (2001) and Basra et al. (2003). They reported greater plant weight following seed priming.

Similarly, the effect of priming with $CaCl_2$ improved the quantity and quality of germination. These results are consistent with those found by Iqbal et al. (2006); Ashraf and Rauf, (2001) working on wheat (*Triticum aestivum* L.).

NaCl salinity affects ion transport processes in plants, which may change the nutritional status and ion balance (Läuchli and Epstein, 1990). Under salt stress, plants have evolved complex mechanisms allowing for adaptation to osmotic and ionic stress caused by high salinity. These mechanisms include the lowering of the toxic ions concentration in the cytoplasm by restriction of Na^+ influx or its sequestration into the vacuole and/or its extrusion (Hajibagheri et al. 1987). The results of the present study showed that NaCl treatments caused an increase in Na^+ concentration and Na^+/K^+; Na^+/Ca^{2+} ratios, and a decrease in K^+ and Ca^{2+} concentrations of coriander shoot derived from both primed and non-primed seeds (Table 2). Previous studies showed similar effects of salinity in melon (Botia et al. 1998) as well as in celery (Pardossi et al. 1999), pepper (Chartzoulakis and Klapaki, 2000) and tomato (Romero et al. 2001). However, seed priming induced avoidance of coriander shoot from toxic and nutrient deficiency effects of salinity on growth because of less Na^+ but more K^+ and especially Ca^{2+} accumulation. In fact, numerous studies indicated that an increase in the concentration of Ca^{2+} in plants challenged with salinity stress could ameliorate the inhibitory effects on growth (Navarro et al. 2000; Kaya et al. 2002). Na^+/K^+ and Na^+/Ca^{2+} balances of seedlings derived from the primed seeds were significantly lower than those of the non-primed seeds under similar salinity levels. These results suggested that seed priming of coriander seeds increased salt tolerance by promoting K^+ and Ca^{2+} accumulation.

5 CONCLUSIONS

The results of this experiment showed that NaCl and $CaCl_2$ seed priming improves emergence, growth and mineral parameters of coriander. This method is simple, cheap and it does not require any special equipment, so farmers can use it to increase percent and homogeneity of emergence of plants under environmental stresses. Further, this study needs to investigate the effects of seed priming on later growth and yield stages of this plant. In addition, coriander is appreciated for its essential oils biochemical analysis of different plant organs (stems, roots, seeds) would be recommended to evaluate the effect of seed priming on qualitative and quantitative parameters of these oils.

6 REFERENCES

Ashraf, M. 1999. Breeding for salinity tolerance proteins in plants. Crit. Rev. Plant Sci., 13: 17-42.

Ashraf M., Rauf H. 2001. Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts: Growth and ion transport at early growth stages.Acta Physiol. Plant 23, 407 – 414.

Basra, S.M.A., E. Ehsanullah, A. Warraich, M.A. Cheema and Afzal, I. 2003. Effect of storage on growth and yield of primed canola seed. Int. J. Agric. Bio., 117-120.

Borsani, O., Valpuesta, V. and Botella, M. A. 2003. Developing salt tolerant plants in a new century: A molecular biology approach. Plant Cell, Tiss. Org. Cult. 73: 101-115.

Botia, P., Carvajal, M., Cerda, A., Martinez, V. 1998. Response of eight *Cucumis melo* cultivars to salinity during germination and early vegetative growth. Agronomie.18, 503–513.

Chartzoulakis, K., Klapaki, G. 2000. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages.Sci. Hortic. 86, 247–260.

Fortmeier, R. and Schubert. S. 1995. Salt tolerance of maize (*Zea mays* L.) The role of sodium exclusion. Plant cell and Environ. 18(9): 1041-1047.

Hajibagheri, M.A., Harvey, D.M.R., Flowers, T.J. 1987. Quantitative ion distribution with in root cells of salt-sensitive and salt-tolerant maize varieties. New Phytol. 105, 367- 379.

Harris, D., Pathan, A.K., Gothkar, P., Joshi, A., Chivasa W., and Nyamudeza. P. 2001. On farm seed priming: using participatory methods to revive and refine a key technology. Agric. Sys. 69(1-2): 151-164.

Hopper, N.W., Overholt, J.R., Martin, J.R. 1979. Effect of cultivar, temperature and seed size on the germination and emergence of soy beans (*Glycine max* (L.) Merr.). Ann. Bot. 44: 301-308.

Iqbal, M., Ashraf, M., Jamil, M., Rehman, S.U. 2006. Does seed priming induce changes in the levels of some endogenous plant hormones in hexaploid wheat plants under salt stress? Journal of integrative plant biology. 48 (2): 181 - 189.

Kaya, C., Kirnak, H., Higgs, D., Saltali, K. 2002. Supplementary calcium enhances plantgrowth and yield in strawberry cultivars grown at high (NaCl) salinity. Sci. Hortic. 93: 65–74.

Khajeh-Hosseini, M., Powell, A.A., Bimgham, I.J. 2003. The interaction between salinity stress and seed vigor during germination of soybean seeds. Seed Sci. Technol. 31: 715-725.

Ingram, J., Bartels, D. 1996. The molecular basis of dehydration tolerance in plants.Annu Rev. Plant Mol. Biol. 47: 377– 403.

Läuchli, A., Epstein, E. 1990. Plant responses to saline and sodic conditions. In: Tanji, K.K. (Ed.), Agricultural Salinity Assessment and Management.

Levitt, J. 1980. Responses of plants to environmental stresses.Academic press. New York.

Navarro, J.M., Botella, M.A., Cerda, A., Martinez, V. 2000. Effect of salinity and calcium interaction on cation balance in melon plants grown under two regimes of orthophosphate. J.Plant Nutr. 23, 991-1006.

Pardossi, A., Bagnoli, F., Malorgio, C.A., Campiotti, C.A., Tognoni, F. 1999. NaCl effects on celery (*Apium graveolens* L.) grown in NFT. Sci. Hortic. 81, 229–242.

Rogers, M.E., Noble, C.L., Halloran, G.M., Nicolas, M.E. 1995. The effect of NaCl on the germination and early seedling growth of white clover (*Trifolium repens* L.) populations selected for high and low salinity tolerance. Seed Sci. Technol. 23: 277 –278.

Romero-Aranda, R., Soria, T., Cuartero, J. 2001. Tomato plant–water uptake and plant water relationships under saline growth conditions. Plant Sci. 160: 265–272.

Shafi, M., Bakht, J., Raziuddin, and Zhang. G. 2009. Effect of Cadmium and Salinity stresses on growth and antioxidant enzymes activity of wheat genotypes. Bull. Environ. contaim.Toxicol., 82(6): 772-776.

Sivritepe N., Sivritepe H.O., Eris, A. 2003. The effect of NaCl priming on salt tolerance in melon seedling grown under saline condition. Sci. Hort. 97: 229-237.

Szabolcs, I. 1994. Soils and salination. In Pessarakli M (ed.). Handbook of Plant and Crop Stress. Marcel Dekker, New York, p. 311.

Taleisnik, E., Grunberg K. 1994. Ion balance in tomato cultivars differing in salt tolerance. I. sodium and potassium accumulation and fluxes under moderate salinity. Physiol. Plant. 92: 528-534.

Tester, M., and Davenport, R. 2003. Na+ tolerance and Na+ transport in higher plants. Ann. Bot., 9: 503-527.

Wang, W., Vinocur B., Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance, Planta 218: 1–14.

Zhu, J.K. 2002. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53: 247-273.

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Effect of intermittent irrigation with saline water on rice yield in Rasht, Iran

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ABSTRACT

Guilan, a well-known province in rice production in Iran, has been facing water shortage and water degradation. In order to study the effects of salinity stress as well as water stress on rice a pot experiment was conducted at Rice Research Institute of Iran. Five water salinity levels: fresh water (EC = 1 dS m^{-1}), 2, 4, 6 and 8 dS m^{-1} and five irrigation regimes: continues flooding, Alternative Wetting and Drying (AWD), intermittent irrigation at 100, 90 and 80 percent of field capacity (FC) were considered as irrigation treatments. The results showed severe effects of water and salinity stresses on rice yield and yield components. Fresh water produced the highest yield, 18.57 gr pot^{-1}, whereas, the yield in salinity levels of 2, 4, 6 and 8 dS m^{-1} were 13.78, 5.78, 3.61 and 0.74 gr pot^{-1}, respectively, with the yield losses of 25, 70, 80 and 97%, respectively. Intermittent irrigation at FC produced the highest yield. The yield increased 8 and 13% in AWD and intermittent irrigation at FC treatments respectively, while it decreased 8 and 27% in intermittent irrigation at 80 and 90% of FC treatments as compared with continues flooding treatment. The highest yield with application of intermittent irrigation at FC was valid only in water salinity less than 4 dS m^{-1}. When water salinity was higher than 4 dS m^{-1} all irrigation methods gave the same yield. This study showed that the best method to use saline water was intermittent irrigation at FC with EC = 2 dS m^{-1}. In case of more salinity, mixing fresh and saline water and intermittent irrigation can mitigate the severe effects of salinity on rice.

Key words: rice, irrigation, saline water, Iran

IZVLEČEK

UČINEK PERIODIČNEGA NAMAKANJA S SLANO VODO NA PRIDELEK RIŽA V PROVINCI GUILAN, RASHT, IRAN

Provinca Guilan v Iranu, ki je poznana po pridelavi riža se sooča s pomanjkanjem vode in slapšanjem njene kakovosti. Z raziskovanje učinka slanosti in vodnega stres na riž je bil izveden lončni poskus na Inštitutu za preučevanje riža v Iranu (Rice Research Institute of Iran). Uporabljeno je bilo pet slanostnih stopenj vode: sladka voda (EC = 1 dS m^{-1}), 2, 4, 6 in 8 dS m^{-1} in pet režimov namakanja: stalna poplavljenost, izmenično namakanje in osuševanje (AWD), in periodično namakanje pri 100, 90 in 80 procentni poljski kapaciteti (FC). Izsledki so pokazali močne učinke solnega in vodnega stresa na pridelek riža in njegove komponente. Pridelek je bil največji v sladki vodi, 18.57 g/lonec, medtem ko so bili pridelki pri slanostih 2, 4, 6 in 8 dS m^{-1} 13.78, 5.78, 3.61 in 0.74 g/lonec, z izgubo pridelka 25, 70, 80 in 97 %. Periodično namakanje pri poljski kapaciteti je dalo največji pridelek. Pri izmeničnem namakanju in osuševanju se je pridelek povečal za 8 in periodičnem namakanju za 13 %, vendar se je v primerjavi s postopkom stalne poplavljenosti zmanšal za 8 in 27 % pri izvedbi tretmajev pri 80 in 90 % poljski kapaciteti. Največji pridelek pri periodičnem namakanju pri poljski kapaciteti je bil dosežen samo pri slanosti vode manj kot 4 dS m^{-1}. Če je bila slanost vode večja, so dali vsi postopki namakanja enak pridelek. Raziskava je pokazala, da je najboljši način periodičnega namakanja s slano vodo pri poljski kapacite s prevodnostjo vode za namakanje 2 dS m^{-1}. V primeru večje slanosti je potrebno izmenično namakati s sladko in slano vodo, da se izognemu velikemu učinku slanosti na pridelek riža.

Ključne besede: riž, namakanje, slana voda, Iran

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1 INTRODUCTION

With 230 thousands hectares of rice cultivated land, Guilan province, in the north of Iran, is one of the most important rice production region. Sepidrood dam and its vast irrigation network provide required water for this region and the agricultural activities. Recently, a dramatically reduction in fresh water resources has been causing concerns about rice production sustainability in Guilan. Climate change, water scarcity and consequently drought as well as anticipation of increasing the trend speed (Abbaspour et al., 2009), have led us to be more worried about the future of agriculture and farmers' income. Previous studies by the authors proved intermittent irrigation as an applicable strategy to overcome the consequences of new circumstance. This method can reduce water consumption and increase water productivity, while no yield loss (Rezaei and Nahvi, 2007; Rezaei et al., 2010a). On the other side, reports anticipated water scarcity, quality changes and its degradation (Abbaspour et al., 2009). To turn the circumstance even worse, construction of numerous dams upstream to Sefidroud dam will result in reduction of inlet water and disposal of drainage water to the river. In this situation further increase of quality changing trend and related salinity stress are predictable (Rezaei et al., 2010b). Rice is a very sensitive crop to salinity (Doberman and Fairhurst, 2000; Zeng and Shannon, 2000). Some researches proved EC threshold of local varieties yield loss to be 1-2 dS m^{-1} (Homaee, 2002; Yousefi, 2006). In this situation, increasing tendency has been arisen to use saline and brackish water in rice production (Ghadiri et al., 2006). But the capability of intermittent irrigation methodology with saline water is questionable.

Several studies have been carried out to better understanding of rice reaction to drought stress and finding new solution for mitigating the effects of the new condition (Bouman and Tuong, 2001; Belder et al., 2005). Water stress prevents transferring nutrients to plants (Wopereis et al., 1999) which results in decrease of tillerings numbers, leaf area, dry matter, filled grains, number of panicle, kernel weight and yield, so it is recommend avoiding long drought period for decreasing water use (Belder et al., 2005; Rezaei and Nahvi, 2007). Reports confirmed rice tolerance to a mild soil water potential decline in root zone resulting from intermittent irrigation up to -30 kPa (Belder et al., 2005). Those studies led to finding different approaches such as raised beds and alternative wetting and drying (AWD). The role of AWD in reducing water consumption and increasing water productivity has been proved. Even some evidences of increasing rice yield were also presented in case of adequate soil moisture control (Tabbal et al., 2002; Belder et al., 2004, 2005, 2007; Tuong et al., 2005; Yang et al., 2007; Zhang et al., 2008, 2009).

Local studies showed the effectiveness of AWD method in decreasing water consumption and increasing water productivity in Iran. A procedure of 8 days irrigation interval for local and 5 days irrigation interval for hybrid and improved varieties were recommended in Guilan province. The studies suggest that local rice varieties are resistant to non-flooding condition. Water stress up to of 80% of saturation or irrigation 3 days after disappearing of water from field surface does not cut crop yield but lower moisture has negative effect on yield (Amiri, 2006; Rezaei and Nahvi, 2007; Rezaei et al., 2010a). In spite of promising achievements, it is still necessary to have more studies for better understanding of rice reaction to drought stress.

In addition to water scarcity, salinity problem in coastal line, changes of water resources quality due to decrease of water input into network and entering low quality waters from upstream have been also under consideration (Rezaei et al., 2010b). Reports indicated that salinity stress caused reduction in leaf water potential, evapotranspiration, stomatal conductance, leaf area and yield of plants (Asch et al., 2000; Casanova et al., 2000; Zeng and Shannon, 2000; Zeng et al., 2003; Castillo et al., 2007). Although some investigations were devoted on salinity effects on rice, the number of studies performed in Iran is still few.

Despite of all mentioned researches, not enough attention was paid to synchronise of drought and salinity stress on rice yield. The change in rice reaction to salinity stress with drought stress has been proved in only Iran experiment carried out in

Fars province by Yousefi (2006). She reported that in AWD, the effects of saline water will be alleviated. She attributed the phenomena to decrease in evapotranspiration leading to less water absorption and consequently low accumulation of salt in plant tissues. Plant will usually have low yield in unsuitable conditions due to less photosynthesis. This natural rice reaction could be considered as a strategy for using saline water in rice cultivation. There has been no special study in Guilan, being the largest rice cultivation area. This research has been carried out to study the effects of synchronization of drought and salinity stress on rice in Rasht.

2 MATERIALS AND METHODS

A pot experiment was performed in a randomized complete block design (RCBD, and three replications) with Hashemi, a local variety at Rice Research Institute of Iran under a five-meter high shelter with plastic sheet coverage surrounded by paddy field. To avoid temperature rising, the sides of the shelter were not covered to let the air flow. Five levels of salinity, S0 = fresh water (EC = 1 dS m^{-1}) S1, S2, S3 and S4: saline water with 2, 4, 6 and 8 dS m^{-1}, respectively were used along with five Irrigation methods including: Permanent irrigation (PI), Alternative wetting and drying (AWD), Irrigation at field capacity (FC), 90% of FC and 80% of FC. About 9 kg of rice farm soil was put into each plastic pot. After flooding the soil; transplantation began with three 25-day old seedlings. The pots were irrigated by fresh water for a week as a recovery period then treatments were applied. All phosphorus (100 kg ha^{-1}) and potassium (150 kg ha^{-1}) and half of nitrogen (75 kg ha^{-1}) fertilizers from triple super phosphate, potassium and urea were mixed with soil in paddy preparation operation time. Those amounts of fertilizers are common fertilizer doses in the region, recommended by legal organizations. The remaining nitrogen was applied at the maximum tillering. Saline water was prepared based on canal water using NaCl and $CaSO_4$ (2:1). In order to prevent salt accumulation in pots, leaching and washing with fresh water in several stages was applied. Irrigation was set at specified time as high as 5 cm from the soil surface. All cultivation practices were performed as local practices. Finally yield, straw yield, tillering numbers, fertile and non-fertile panicle were measured. Mean comparison was done after analysis of variance using the Duncan multiple range test (DMRT).

3 RESULTS AND DISCUSSION

The results of soil chemical and physical analysis and Rasht meteorological station data were shown in Table 1, Table 2, and Table 3, respectively.

Table 1: Soil chemical analysis

Potassium ppm	Phosphorus ppm	Total Nitrogen %	pH
290	17	0.155	7.4

Table 2: Soil physical analysis

Soil texture	80% FC	90% FC	FC*	saturation	
Silty-Clay	40	45	50	65	Water content (volumetric, %)

*FC at -33 kPa

Table 3: Rasht meteorological station data in 2010

ETo	Sunshine hours	Rainfall	Relative humidity (%)		Temperature ºC		
mm		mm	Max	Min	Max	Min	Month
47	114	67	98.8	68.3	16.2	8.3	Apr
72	123	149	98.8	71.5	21	14	May
149	277	2	95.2	59.5	29.8	20.4	Jun
168	371	22	95.1	55.1	32	22.7	July
184	217	23	93.8	51.2	33.9	21.5	Aug
103	200	55	98	57.6	29.9	19.5	Sep

ETo = reference evapotranspiration

The result (Table 4) showed that salinity of irrigation water had statistically significant effects on all traits except of unfilled panicles, but water stress showed significant effects only on yield, biomass and total panicles. It seems that salinity had more severe effects on rice in comparison with water stress. No interaction between water and salinity stress was observed. Some reports proved that rice in general and Iranian local variety, Hashemi, particularly to be resistant to intermittent irrigation and non-submerged irrigation (Belder et al., 2005; Amiri, 2006; Rezaei et al., 2010a).

3.1 Salinity stress

The analysis of mean comparison of the yield (Table 5) showed that rice is sensitive to salinity of irrigation water. Among treatments, control (EC = 1 dS m^{-1}) with 18.57 gr pot^{-1} had the highest yield. Increasing in salinity to 2 dS m^{-1} resulted in yield loss to 13.78 gr pot^{-1}, a considerable yield loss of about 25%. The same trend observed with increasing in salinity to 4 dS m^{-1}, which showed a 70% yield loss with 5.78 gr pot^{-1}. The yield loss with the salinity of 6 and 8 dS m^{-1} were remarkable amount of 80 and 97%, respectively. Some reports proved the high sensitivity of rice to salinity of irrigation water (Kavoosi, 1995; Sultana et al., 1999; Yousefi, 2006). It seems relatively high temperature of the cropping season, being of 22.4 °C (being normally of 18.9 °C) intensified the effects of salinity of irrigation water on rice (Asch et al., 2000).

As stated, while yield loss was about 97%, the maximum decline in straw was about 20% (Figure 2). This conclusion showed that in salinity stress, yield loss in contrast with decrease in production of straw suffers from a faster rate. Reviewing yield in different salinities (Figure 1), showed clearly that quadratic equation presents yield loss more accurately (R^2 = 0.98) in comparison with linear equation (R^2 = 0.91) presented by Mass and Huffman (1997).

Table 4: Analysis of variance

Error	Salinity× Irrigation	Irrigation	Salinity	Rep.	Source of variance
48	16	4	4	2	Degree of freedom
43.7	36.2 ^{ns}	88.9 ^{ns}	350.2 **	78	Straw weight (gr pot^{-1})
9	16.3 ^{ns}	29.1 **	828.7 **	60.6	Yield (gr pot^{-1})
76.5	135.6 ^{ns}	124.5 ^{ns}	3810.3 **	443	Yield / Straw
48.4	30.7 ^{ns}	141.6*	1604 **	33.1	Biomass (gr pot^{-1})
28.3	43.4 ^{ns}	42.1 ^{ns}	1716.7 **	196.6	Harvest Index
39.4	25.7 ^{ns}	7.4 ^{ns}	308.7 **	18.5	No. of tillering
15.7	14.3 ^{ns}	12.3 ^{ns}	228.2 **	9.6	No. of filled panicle
13.1	23 ^{ns}	22.9 ^{ns}	23.4 ^{ns}	2.8	Unfilled panicle
20.5	20.8 ^{ns}	30 **	846.6 **	19.5	Total panicle
4081	3153.8 ^{ns}	3697.7 ^{ns}	9990.4 **	3589.3	Filled panicle (%)
4.2	1.8 ^{ns}	2 ^{ns}	17.3 **	12.2	Unfilled /filled panicle

*, **: represent statistically significant differences at 95 and 99 respectively
ns: represent not statistically significant differences

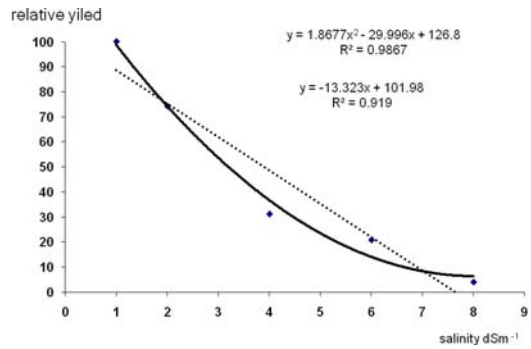

Figure 1: Relative yield in different salinity levels

Since statistically significant effects of salinity on yield and straw dry weight, it could be expected that harvest index would be completely influenced by this tension. As expected, salinity of irrigation water had a high influence on harvest index, so that the index is declined from 28.5% when irrigated with fresh water to 1.99% when irrigated with saline water of 8 dS m^{-1} (Table 6). In this case water salinity adversely influenced the number of rice tillerings. While decreasing in number of tillering due to salinity stress, number of filed panicle and ratio of filed panicle to tillering highly declined too. Effect of salinity on percent of filled panicles has also been reported by other researchers (Clermont-Dauphina et al., 2010). In fact these traits are the most important_factors to reach the maximum yield of rice (Casanova et al., 2000). Therefore any kind of reduction in these traits highly affects the yield.

Table 5: Analysis of mean comparison of rice yield (gr pot^{-1})

	Salinity (dS m^{-1})					
Irrigation	1	2	4	6	8	mean
FI	22.2 A a	11.9 B b	5.9 A bc	2.9 A c	0.8 A c	8.7 AB
AWD	21.9 A a	15.6 AB a	5.7 A b	2.9 A b	1.2 A b	9.5 AB
FC	19.4 A a	18.8 A a	6.4 A b	4.2 A b	0.8 A b	9.9 A
90FC	18 A a	11.9 B ab	5.7 A bc	4.3 A c	0.3 A c	8 AB
80FC	11.3 B a	10.7 B a	5.3 A ab	3.7 A b	0.7 A b	6.4 B
mean	18.6 a	13.8 b	5.8 c	3.6 c	0.7 d	

Table 6: Analysis of mean comparison for different water salinity levels

Salinity dS m^{-1}	Straw weight gr pot^{-1}	No. of tillering	No. of filled panicle	Filled panicle (%)	Harvest index (%)
1	39.8 ab	34.4 a	29.2 a	67.3 a	28.5 a
2	40.9 a	31.4 a	26.0 a	64.4 a	22.7 b
4	41.8 a	30.2 a	19 b	50.1 ab	10.5 c
6	33.9 bc	25.6 b	16.6 b	51.6 ab	10.2 c
8	30.7 c	23.1 b	10.3 c	16.6 b	2 d

Same letter means no diffrence at 99 % by DMRT (Duncan multiple range test)

Salinity decreased number of tillerings per pot. Number of tillerings in fresh water and saline water of 8 dS m^{-1} were 34.4 and 23.1, the maximum and minimum amount, respectively. This trend of reduction due to salinity stress was observed for total number of panicles and numbers of filled panicles and the ratio of the number of filled panicles to the number of tillerings. It would be interesting to know that although majority of measured traits were affected by salinity, regardless to water salinity, the number of unfilled panicles remained unchanged. Contrasting to straw dry weight production, increasing salinity to 4 dS m^{-1} had no adverse effect on rice vegetative growth (Figure 2 and Table 6) but increasing water salinity to 8 dS m^{-1} decreased rice growth and biomass accumulation of the plant by 15 and 23% comparing with fresh water, respectively.

Table 7: Analysis of mean comparison for different irrigation treatments

Irrigation	Straw weight gr pot^{-1}	No. of tillering	No. of filled panicle	Filled panicle (%)	Harvest index (%)
FI	39.3 a	29.6 a	21.8 a	50 a	14.2 a
AWD	40.8 a	28.3 a	21.7 a	51 a	15.1 a
FC	39.6 a	29.7 a	19.3 a	51.8 a	17.2 a
90FC	35.9 a	29 a	19.2 a	49.5 a	14.7 a
80FC	36 a	28.2 a	19.2 a	48.1 a	12.6 a

Same letter means no diffrence at 99% by DMRT (Duncan multiple range test)

Figure 2: Rice relative yield and dry matter in different salinity levels

3.2 Water stress

The table of mean comparison (Table 7) showed that applied irrigation treatments had no statistically significant effects on measured yield components such as number of tillerings, filled and unfilled panicles, ratio of unfilled/filled panicles and straw dry weight and all placed in the same class but biomass and water tension decreased biomass production. Due to ignorable change in straw dry matter, this phenomenon could be attributed to the change in yield. Reviewing yield in different irrigation methods (Table 5) showed that performing intermittent irrigation not only did not decrease yield but also water tension up to FC caused a yield increase, a finding which had been proved by the authors (Rezaei and Nahvai, 2007). Irrigation treatment of 90% FC and 80% of FC had the minimum yield. Comparing with PI which had a yield of 8.74 gr pot^{-1}, applying intermittent irrigation at FC and AWD with 9.89 and 9.46 gr pot^{-1}showed an increase in yield as much as 13 and 8%, respectively. Two treatments of 80 and 90% of FC with 27 and 8% decrease in yield (comparing with PI) had the least amount of yield, respectively. The roles of intermittent irrigation on increasing rice production have been reported by other researches too. Belder et al. (2004) also reported that water tension up to 33 kPa did not cause yield reduction. According to the mentioned report, increasing water tension more that FC decreased yield. Using intermittent irrigation to reduce water consumption has been applying in North farms of Iran for a while. The method is based on wide studies by authors in the Rice Research Institute of Iran (RRII) and was accepted as an applicable method to mitigate water scarcity.

3.3 Salinity and water stress interactions

Rice response to salinity stress remained unchanged in all applied irrigation methods in this research; yield decreased when salinity increased (Figures 1 and 2). The reduction trend in low water stress including PI, AWD and FC was quadratic equation but in other two sever water tension treatments i.e. 80 and 90% of FC, linear equation. According to the figures 1 and 2, it is concluded that in quadratic equation, yield reduction slope with salinity to 4 dSm^{-1} is very high and after that the reduction continues with fewer slopes and in harmony with slope of first class linear equations. On the other side with fresh water although applying intermittent irrigation treatments i.e. FI, AWD, FC and 90% of FC did not cause yield differences, using saline water of 2 dS m^{-1} showed a significant difference. In this circumstance posing water stress up to FC resulted in a trend of yield rise which followed by a falling trend with more severe water stress. Yield in severe salinity stress, salinity more than 4 dS m^{-1}, all irrigation treatments yielded the same, suggesting that in excessive salinity, irrigation management did not have any effect on yield. Yousefi (2006) also reported that alternative irrigation reduced effect of salinity tension and attributed it to less absorption of water and saline solvable in water and as a result to less accumulation of salt in plant tissue.

4 CONCLUSIONS

According to Figure 2 and Table 5, it is concluded that if irrigation water salinity is about 1 dS m^{-1}, the best irrigation methods are permanent flooding, alternative irrigation or irrigation at FC, and 90% of FC, but as applying intermittent irrigation (non-submerged) reduces water use, non-submerged is suggested. In this case, in contrast with other treatment, more yields will produce. When water salinity is 2 dS m^{-1} irrigation at FC is suggested, since alternative irrigation decreases salinity effects. When salinity is more than that amount, all methods of irrigation has the same result; in this case irrigation at 90% of FC has a little more yield. In any case in this condition, yield reduction is so high that rice cultivation is not recommended. Generally, we concluded that in some cases, mixing fresh water and saline water to decrease water salinity to an acceptable level of 2 dS m^{-1} and using alternative irrigation at FC, prevents yield losses.

5 REFERENCES

Abbaspour K., Faramarzi M., Seyed-Ghasemi S., Yang H. 2009. Assessing the impact of climate change on water resources in Iran. Water resources research, 45, 1-15

Amiri E. 2006. Study of water balance and rice yield in irrigation managements in paddies using model (model and experimental field). Ph.D. dissertation, Science and Research Branch of Islamic Azad University, Tehran. (In Persian)

Asch F., Dingkuhn M., Dorffling K. 2000. Salinity increases CO_2 assimilation but reduces growth in field-grown irrigated rice. Plant and Soil, 218, 1-2: 1-10

Belder P., Bouman B.A.M., Cabangon R., Guoan L., Quilang E.J.P., Li Y., Spiertz J.H.J., Tuong T.P. 2004. Effect of watersaving irrigation on rice yield and water use in typical lowland conditions in Asia. Agricultural Water Management, 65, 193–210

Belder P., Spiertz J.H.J., Bouman B.A.M., Lu G., Tuong T.P. 2005. Nitrogen economy and water productivity of lowland rice under water-saving irrigation. Field Crops Research, 93, 2-3: 169-185

Belder P., Bouman B.A.M., Spiertz J.H.J. 2007. Exploring options for water saving in lowland rice using a modeling approach. Agricultural Systems, 92: 91–114

Bouman BAM., Tuong T.P. 2001. Field water management to save water and increase its productivity in irrigated lowland rice. Agricultural Water Management, 49, 1: 11-30

Casanova D., Goudriaan J., Catala-Forner M.M., Withagen J.C.M. 2000. Rice yield prediction from yield components and limiting factors. European Journal of Agronomy, 17, 1: 41-61

Castillo E.G., Phuc T., Abdelbaghi M.A., Kazuyuki I. 2007. Response to salinity in rice: comparative effects of osmotic and Ionic stress. Plant Production Science, 10, 2: 159-170

Clermont-Dauphina C., Suwannang N., Grünberger O., Hammecker C., Maeght J.L. 2010. Yield of rice under water and soil salinity risks in farmers' fields in northeast Thailand. Field Crops Research, 118: 289-296

Doberman A., Fairhurst H.T. 2000. Rice nutrient disorder and nutrient management. Potash and phosphate institute, International rice research institute, Makati city, Singapore

Ghadiri H., Dordipour I., Bybordi M., Malakouti M.J. 2006. Potential use of Caspian Sea water for supplementary irrigation in Northern Iran. Agricultural Water Management, 79, 3: 209-224

Homaee M. 2002. Responses of plants to salinity. Tehran: Iranian National Committee on Irrigation and Drainage Press. (In Persian)

Kavoosi M. 1995. Choosing an appropriate model for rice yield estimation at different salinities for Sepidroud, Hasan Saraee and Khazar cultivars. M. Sc. Thesis, University of Tabriz, Tabriz. (In Persian)

Mass E.V., Hoffman G.J. 1997. Crop salt tolerance-current assessment. Journal of Irrigation and Drainage engineering. 103: 115-134

Rezaei M., Nahvi M. 2007. Effect of different irrigation management in clay soils on water efficiency and some features of local rice cultivars at Guilan. Agricultural Sciences Research, 9: 15-25. (In Persian)

Rezaei M., Motamed M.K., Yousefi U., Amiri E. 2010a. Water use variations in different irrigation

managements and its effect on different rice cultivars. Water and Soil, 24, 3: 569-577. (In Persian)

Rezaei M., Davatgar N., Tajdari K., Abolpour B. 2010b. Investigation the spatial variability of some important groundwater quality factors in Guilan, Iran. Water and Soil, 24, 5: 932-941. (In Persian)

Sultana N., Ikeda T., Itoh R. 1999. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environmental and Experimental Botany, 42: 211-220

Tabbal D.F., Bouman B.A.M., Bhuiyan S.I., Sibayan E.B., Sattar M.A. 2002. On-farm strategies for reducing water input in irrigated rice: Case studies in the Philippines. Agricultural Water Management, 56: 93–112

Tuong T.P., Bouman B.A.M., Mortimer M. 2005. More rice, less water-Integrated approaches for increasing water productivity in irrigated rice-based systems in Asia. Plant Production Science, 8, 3: 231-241

Wopereis M.C.S., Donovan C., Nebié B., Guindo D., N'Diaye M.K. 1999. Soil fertility management in irrigated rice systems in the Sahel and Savanna regions of West Africa: Part I. Agronomic analysis. Field Crops Research, 61, 2: 125-145

Yang J., Liu K., Wang Z., Du Y., Zhang J. 2007. Water saving and high-yielding irrigation for lowland rice by controlling limiting values of soil water potential. Journal of Integrative Plant Biology, 49, 10: 1445-1454

Yousefi U. 2006. Effect of interaction among water deficit and salinity stress on rice yield. M. Sc. Thesis, Shiraz University, Shiraz. (In Persian)

Zeng L., Shannon M.C. 2000. Effects of salinity on grain yield and yield components of rice at different seeding densities. Agronomy Journal, 92, 3: 418-423

Zeng L., Lesch S.M., Grieve C.M. 2003. Rice growth and yield respond to changes in water depth and salinity stress. Agricultural Water Management, 59: 67-75

Zhang H., Zhang S., Zhang J., Yang J., Wang Z. 2008. Postanthesis moderate wetting drying improves both quality and quantity of rice yield. Agron Journal, 100: 726–734

Zhang H., Xue Y., Wang Z., Yang J., Zhang J. 2009. An alternate wetting and moderate soil drying regime improves root and shoot growth in rice. Crop Science, 49, 6: 2246-2260

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Production of rocket (*Eruca sativa* Mill.) on plug trays and on a floating system in relation to reduced nitrate content

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ABSTRACT

The effect of the growth system on the yield and nitrate content of rocket (*Eruca sativa* Mill.) was evaluated in two experiments. In the first experiment, different sizes of cells in plug trays and different fertilization treatments were tested. In the second, a floating system was used with different substrates and the nitrate content in rocket leaves was analysed. The size of the cells did not affect the yield of rocket significantly but the yield was notably higher (2.02 - 2.21 kg m^{-2}) when plugs were fertigated once per week with 6:12:36 + ME + natural biostimulant in comparison with plugs fertigated only with a water soluble fertilizer (1.53 - 1.74 kg m^{-2}). The best yield was obtained in vermiculite and perlite in 20 ml cells (2.13 and 1.89 kg m^{-2}). The different substrates used in the floating system had no effect on the dry matter content, which was on average 13.7 % and was significantly lower than the dry matter content of leaves grown in peat (19.1 %). The nitrate content in leaves measured a day before and 10 days after the replacement of the nutrient solution with tap water, fell greatly from 4,288-6,764 mg NO_3 kg^{-1} FW to 52-634 mg NO_3 kg^{-1} FW.

Key words: fertigation; biostimulant; rock-wool flocks; vermiculite

IZVLEČEK

PRIDELAVA NAVADNE RUKVICE (*Eruca sativa* Mill.) V GOJITVENIH PLOŠČAH IN NA PLAVAJOČEM SISTEMU IN MOŽNOSTI REDUKCIJE VSEBNOSTI NITRATA

Učinek tehnologije gojenja na pridelek in vsebnost nitratov pri navadni rukvici (*Eruca sativa* Mill.) je bil izvrednoten v dveh poskusih. V prvem poskusu smo primerjali različne velikosti vdolbin v gojitvenih ploščah in različna gnojila. V drugem poskusu pa smo gojili rukvico na plavajočem sistemu v različnih substratih in analizirali vsebnost nitratov v listih. Velikost vdolbin ni vplivala statistično značilno na pridelek. Pridelek je bil največji (2,02-2,21 kg m^{-2}), ko smo gojitvene plošče fertigirali enkrat tedensko z vodotopnim gnojilom 6:12:36 + ME in dodatkom naravnega biostimulanta v primerjavi z ploščami, ki smo jih fertigirali samo z vodotopnim gnojilom (1,53-1,74 kg m^{-2}). Najboljši pridelek smo dobili v vermikulitu in perlitu v 20 ml vdolbinah (2,13 and 1,89 kg m^{-2}). Različni substrati niso imeli vpliva na % sušine v listih, ki je bila v plavajočem sistemu povprečno 13,7 % in statistično značilno manjša od sušine v listih navadne rukvice, gojene v šoti (19,1 %). Vsebnost nitrata v listih smo merili 10 dni pred pobiranjem, ko so rastline še rastle v hranilni raztopini in na dan pobiranja, ko smo hranilno raztopino nadomestili z navadno vodo. Vsebnost nitratov se je zelo zmanjšala, iz 4,288-6,764 mg NO_3 kg^{-1} sveže snovi na 52-634 mg NO_3 kg^{-1} sveže snovi.

Ključne besede: fertigacija; biostimulant; kosmiči kamene volne; vermikulit

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1 INTRODUCTION

Rocket (*Eruca sativa* Mill.) is a fast growing, cool-season crop. The leaves can be cut after 20 or more days and sequentially harvested from re-growth. The main obstacle to sequential production of this crop appears to be early bolting during increasing day-length period (Morales and Janick, 2002). It is a leafy vegetable suitable for growing in plug trays as a fresh-cut vegetable (Nicola et al., 2004). Interest in rocket has been increasing in recent years because of the spicy taste of its leaves. It is mainly used to garnish and flavour salads and a large variety of meals. According to D'Antuono et al. (2009), rocket is a new potential health-promoting vegetable owing to the glucosinolates content.

Production technologies are being investigated in order to simplify harvest, improve the quality of the product and to reduce production costs and environmental impact. A floating system is one of the simplest soilless culture systems, with a number of advantages compared to cultivation in soil; the produced material is clean at harvest, consequently reducing the number of washing treatments; soil disinfection is not necessary; there is full control of the inputs, since substrates are mainly inert (Pasotti et al., 2003; Lazzarin and Giordano, 2007).

Rocket has a short production cycle and can accumulate large amounts of nitrate in leaves (up to 10 g kg^{-1} FW), forming compounds believed to be potentially toxic to human health (Santamaria et al., 2001; D'Anna et al., 2003; Ferrante et al., 2003). Nitrate *per se* is relatively non-toxic but its metabolites, nitrite, nitric oxide and nitroso compounds, make nitrate of regulatory importance because of their potentially adverse health implications, such as methaemoglobinaemia and carcinogenesis (Nitrate..., 2008; Ferrante et al., 2003). On the other hand, some research has shown that its conversion to nitrite plays an important antimicrobial role in the stomach (McKnight et al., 1999), and other nitrate metabolites also have important physiological/pharmacological roles (Lundberg et al., 2004 and 2006; Bryan, 2006).

A large number of leafy vegetables can accumulate high levels of nitrate. The concentrations depend on a range of factors, including season, light, temperature, growing conditions, fertilizer use, and storage of the crop (Premuzic et al., 2001; Magnani et al., 2007; Frezza et al., 2005; Kim and Ishii, 2007)

Nitrate predominately enters the human body exogenously from vegetables, water and other foods but is also formed to a limited extent endogenously (Lundberg et al., 2004 and 2006). In plants, nitrate is mainly found in cell vacuoles and is transported in the xylem from the roots to the leaves, from where it is then translocated to the growing points and to the storage organs, such as seeds or tubers. This means that leaf crops such as cabbage, lettuce, spinach and rocket may have fairly large nitrate concentrations, whereas storage organs such as potato tubers, carrots, leeks, onions, seeds and the pods of pea and bean plants have relatively small concentrations (Bottex et al., 2008). Another consequence of the transport system is that young leaves have lower nitrate concentrations than older leaves. Such a relationship has been shown for cabbage, with the highest nitrate concentrations in the outer leaves and much smaller nitrate concentrations in the innermost leaves (Greenwood and Hunt, 1986).

The Regulation on nitrate (Nitrate..., 2008) applies only to 2 vegetable crops: fresh spinach and fresh lettuce. Because of the widely varying climatic conditions, production methods and eating habits in different parts of the European Union, maximum levels for fresh spinach and fresh lettuce are fixed depending on the season. All maximum levels are expressed as mg nitrate/kg fresh weight. The maximum levels for nitrate in those foodstuffs are generally higher for plants that are harvested between 1 October and 31 March than they are for plants harvested between 1 April and 30 September. Moreover, with respect to fresh lettuce, the Regulation differentiates between lettuce grown under cover and lettuce grown in the open air, with lower levels for the latter. Since rocket is becoming more and more popular, its production has been extended over the whole year.

The aim of our study was first to test the plug tray system for growing rocket with a biostimulant and

second, to produce rocket with a low nitrate content on a floating system.
This paper summarizes the results of the two experiments.

In the first experiment, rocket was grown in peat substrate in cells of different sizes, with three fertigations (no fertigation; fertigation with a water soluble fertilizer (WSF); fertigation with WSF and a biostimulant). In the second experiment, we compared plug trays with various substrates (perlite; rock-wool flocks; vermiculite; expanded clay pellets and peat substrate), using 2 techniques (floating system and growing in a peat substrate in plug trays with fertigation). The nitrate content in leaves was analyzed twice: 10 days before harvest, i.e., just before the nutrient solution was replaced by tap water and at harvest, after the plants had been growing on tap water for 10 days.

2 MATERIALS AND METHODS

2.1 Plant growth conditions and media

Both experiments were conducted in a non-heated greenhouse covered with glass in Ljubljana – central part of Slovenia (46 °N, 300 m asl, mean annual T 10 °C). In the first experiment, seeds of rocket were sown on 16th March in polystyrene plug trays with 40 cells (cell volume 60 ml) or with 84 cells (cell volume 35 ml) filled with a peat substrate. In the 60 ml cells 10 seeds were sown per cell and in the 35 ml cells 5 seeds were sown per cell, so the density was 2,400-2,520 plants per m^2. Three rates of fertilization were used: no fertigation; fertigation with WSF 10:5:26 + ME and fertigation with WSF 6:12:36 + ME + natural biostimulant, based on lyophilized cattle manure, marine algae and sugar beet pulp, containing 8 % of N, 1 % of P_2O_5 and 1 % of K_2O. Fertigation was performed once a week – 8 times during the trial. Both treatments resulted in a similar amount of added elements (Table 1). There were four repetitions and 1-3 harvests. Plants were cut (harvested) for the first time 37 days after sowing, when 3-4 leaves had formed (22th April). The height and weight of plants grown in the middle of the tray (9 cells per tray) were measured.

Table 1: The amount of nutrients added during fertigation in different treatments of rocket growth

Treatment	Fertilization	Concentrations	Amount of added elements/ m^2
Non-fertigate	-	-	-
NPK	2 g/l of 10-5-26	200 ppm N 100 ppm P_2O_5 520 ppm K_2O	10 g N 5 g P_2O_5 26 g K_2O
NPK + bio stimulant	1.1 g/l of 6-12-36 + 1.7 ml of bio stimulant	200 ppm N 128 ppm P_2O_5 380 ppm K_2O	3.3 g + 6.8 g = 10.1 g N 6.6 g + 0.85 g = 7.5 g P_2O_5 19.8 g + 0.85 g = 20.7 g K_2O

The second (59 days after sowing - 14th May) and third (77 days after sowing - 1st June) harvests were performed only on fertilized treatments, because non-fertilized plants began to flower. The same measurements were done and part of the yield was dried at 60 ºC for 2 days, to determine the % of dry matter.

The second experiment was performed in the same glasshouse. Seeds from the same seed company (Semenarna Ljubljana) were sown on 20th January in plug trays with 84 (35 ml; 3 seeds per cell) and 160 cells (20 ml; 2 seeds per cell), which gave us 2 densities – 1,500 and 2,000 seeds m^{-2}. Cells were filled with 5 different substrates: rock-wool flocks, perlite (3-5 mm), expanded clay with 6-8 mm pellets, vermiculite (3-4 mm) and peat substrate. There were 3 repetitions of each plug tray, so there were 2 densities × 5 substrates × 3 repetitions = 30 trays. Trays with peat were placed on raised benches, with a local heating system under the bench and other trays were placed in an improvised pool – 10 m long, 1.5 m large and 0.05 m deep, filled with water to a depth of 0.03 m. The whole "pool" contained 0.45 m^3 of aerated water in which 250 g of WSF 18:18:18 + ME (B (0.05 %), Cu

(0.02 %), Fe (0.14 %), Mn (0.08%), Mo (0.008 %) and Zn (0.05 %)) was dissolved a few days after sowing, when the seeds began to germinate. During the experiment, the water was aerated and topped up several times because of evapotranspiration. When the EC (electro-conductivity) dropped under 1 mS cm^{-1}, the appropriate amount of fertilizer was added to the pool. Altogether, 750 g of 18:18:18 + ME was used on a surface of 15 m^2, which is equivalent to 90 kg N, 90 kg P_2O_5 and 90 kg K_2O per ha. The control trays with peat were irrigated as necessary and fertigated each week with 100 ppm N, P_2O_5 and K_2O – the same concentration as the nutrient solution of the floating system. Plants were harvested twice - for the first time when 3-4 leaves had formed, 39 days after sowing (23th February) and 57 days after sowing (23th March). The yield of rocket leaves was measured each time and plants from 10 cells per tray were randomly selected and measured in detail – height of plants, number of leaves and their weight (data not shown). At the end of the second experiment, leaves were dried at 60 °C for 2 days and dry matter content was determined. Twice per week, the pH, T and EC of the nutrient solution were also measured. Ten days before harvest, the nutrient solution was replaced with water, to reduce the nitrate content in the rocket leaves.

The temperature in the greenhouse was at least 10 °C during nights and air humidity was mainly more than 90 %. The temperature of the nutrient solution was between 17 and 19 °C, pH 5.6-8 and EC 0.8-1.6 mS cm^{-1}. When the solution was replaced with water, the EC was 0.48 mS cm^{-1}.

2.2 Determination of nitrate content in leaves

The concentration of nitrate in leaves was analyzed twice - before and after the change of nutrient solution with tap water. For each sample, about 2.5 g of well homogenized fresh leaves were put in a 50 ml test tube with 20 ml of distilled water and disintegrated with Ultrathurax T25. The solution was then heated in a water bath (60-70 °C) for 20 min, cooled and filtered through Whatman No 41 filter paper into a 50 ml polypropylene centrifuge tube with a screw cap (ISOLAB, Germany) filled to 50 ml with DI water and stored at -20 °C until analysis. Three replicate extractions per treatment were performed.

The content of nitrate in defrosted samples was determined according to ISO13395:1996 using a continuous-flow analyzer (Flowsys, Alliance Instruments, Salzburg, Austria). No nitrite was detected in any of the samples.

The collected data were subjected to analysis of variance and Duncan's Multiple Range Test at 95 % confidence level.

3 RESULTS AND DISCUSSION

3.1 First experiment

Rocket plants were harvested three times, except for the control plants, which were not fertilized and began to flower after the first cut. Plants were cut when they had 3-4 leaves and were around 10 cm high. Where a biostimulant was added, the plants grew faster and were significantly higher than plants that had received almost the same amount of nutrients but no biostimulant. The weight of leaves was understandably much higher in 60 ml cells in which 10 seeds were sown than in 35 ml cells with five seeds. Again, the treatment with biostimulant was significantly better than the treatment without the addition of biostimulant. Measurements are presented in Table 2.

Table 2: Average height and weight of rocket plants grown in 35 and 60 ml cells and cut once or several times. The data represent means (± SD of 36 plants in 4 replicates).

Fertilization	Cell volume	Plant height (cm)			Weight of leaves per cell (g)		
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
None	60 ml	8.1±1.17	-	-	2.2±0.80	-	-
	35 ml	8.1±0.87	-	-	1.5±0.42	-	-
	Average	**8.1**	**-**	**-**	**1.8**	**-**	**-**
NPK	60 ml	10.5±1.52	11.6±1.15	10.1±0.99	3.5±0.81	1.9±0.62	1.8±0.60
	35 ml	9.3±0.84	10.7±1.02	8.9±1.46	1.3±0.42	1.0±0.33	0.9±0.30
	Average	**9.9**	**11.2**	**9.5**	**2.4**	**1.4**	**1.3**
NPK+bio-st.	60 ml	12.2±1.54	14.4±1.42	11.7±1.35	3.1±0.96	3.2±1.01	2.1±0.64
	35 ml	11.0±0.78	12.9±1.45	10.9±1.57	1.6±0.52	1.5±0.61	1.3±0.48
	Average	**11.6**	**13.7**	**11.3**	**2.4**	**2.3**	**1.7**

Biostimulants appear to work best when the plants are under some kind of stress, either environmental, such as poor growing conditions, or due to disease or lack of nutrients (Blake, 2002). This was definitely the case in our experiment, since plants were growing in plug trays with limited space and a limited amount of substrate (20-60 ml).

Figure 1 shows that the biostimulant added to the fertilizer had a positive influence on the growth of rocket in all 3 cuts. The cumulative yield of rocket was 2.1 kg m^{-2} and was significantly higher than in the treatment fertilized only with WSF, in which the cumulative yield was only 1.6 kg m^{-2} (Fig. 2). The size of the cells did not significantly influence the yield (Fig. 3). The addition of a natural biostimulant in our study resulted in 29 % higher yield, even though the amount of added nutrients was approximately the same as in fertigation alone. Plants without fertigation bolted very quickly (after the first cut).

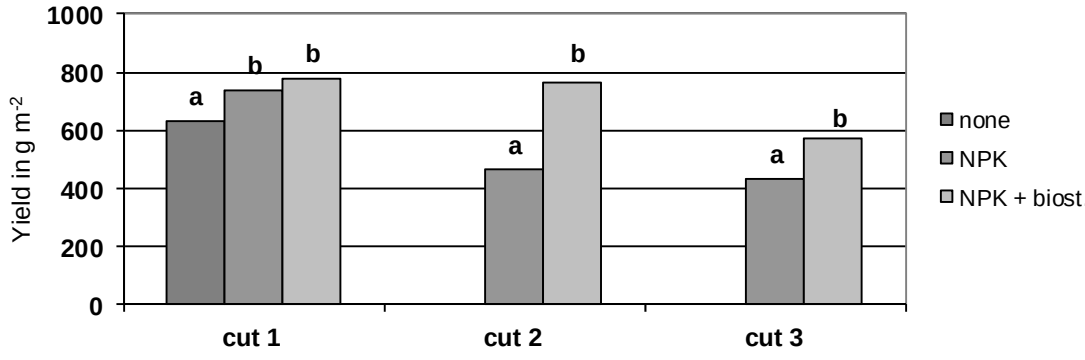

Fig. 1: Average yield of rocket from the 1st experiment in g m^{-2}. Mean values of each cut followed by the same letter are not significantly different according to Duncan's Multiple Range test at $P<0.05$

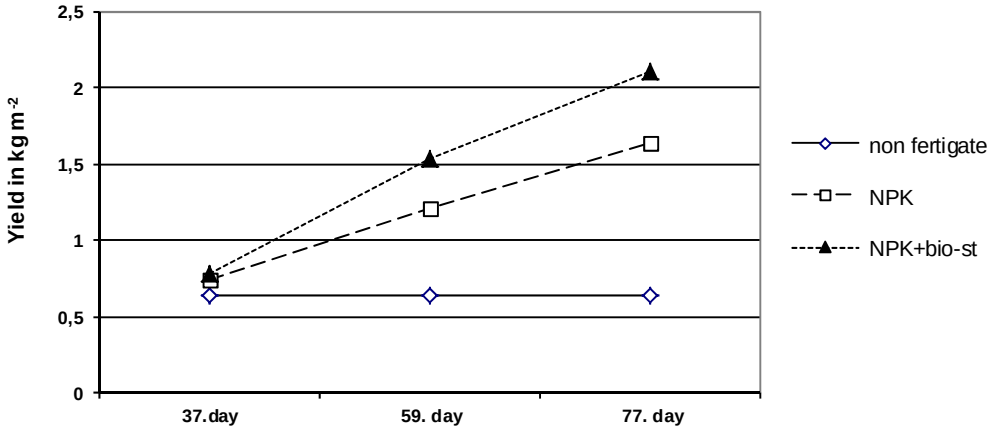

Fig. 2: Average cumulative yield of rocket (kg/m^2) from all 3 cuts (exp. 1)

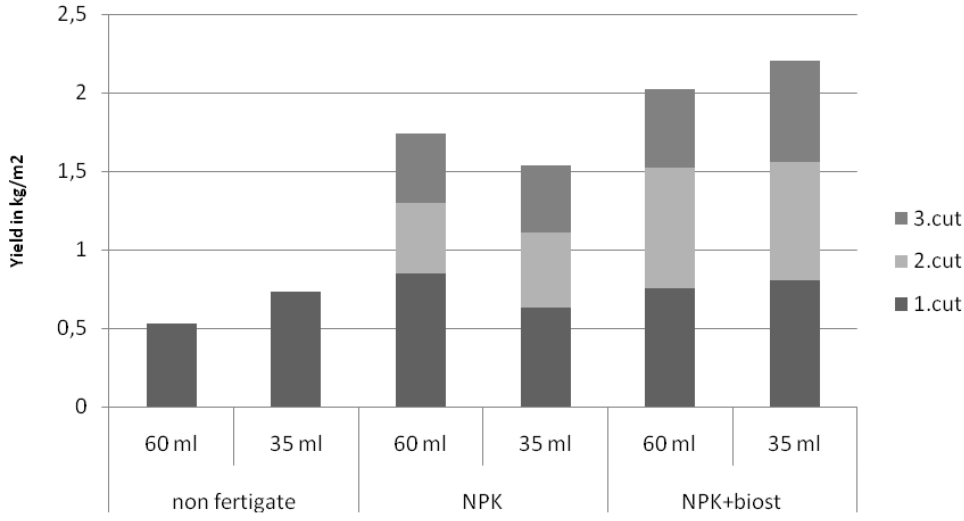

Fig. 3: Yield of rocket (kg m^{-2}) with regard to cell volume and fertigation treatment (exp. 1).

The biostimulant may enhance the metabolism, increase chlorophyll efficiency and production, increase antioxidants and enhance nutrient availability. However, the nature and the effects of biostimulants may vary widely. After an extensive series of experiments with different biostimulants in a nursery, Thompson (2004) concluded that biostimulants do not improve growth when all the factors are optimal, but act more as an insurance policy to protect crops against the vagaries of nature.

3.2 Second experiment

The second experiment was conducted on a floating system, and the germination of seeds was also recorded. Statistical analysis showed no significant differences between the substrates except for the expanded clay, which turned out to be an inappropriate substrate for raising seedlings. Germination was very poor, probably due to a low water holding capacity of the substrate. The impact of cell volume on the germination of seeds was insignificant in all tested substrates (Fig. 4).

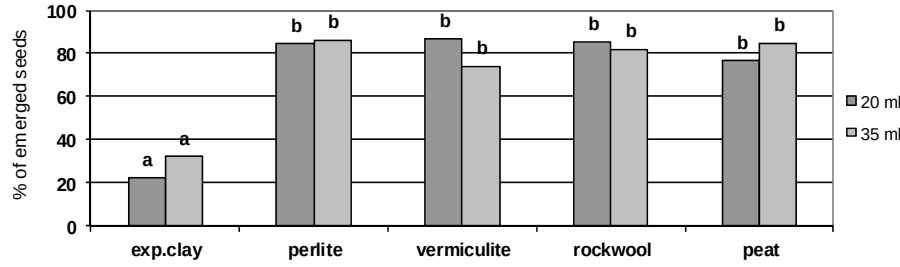

Fig. 4: Percentage of emerged seeds of rocket in different substrates and cell volumes (exp. 2).

The rocket was cut twice, when the plants had reached a height of 12-14 cm and had 3-4 leaves. Because of poor germination, the yield was lowest in expanded clay. The highest yield was recorded in vermiculite and perlite when smaller cells were used, but the yield in rockwool flocks was not significantly lower. Smaller cells and a higher plant density (2.000 seeds per m^2) gave better results and did not affect plant quality. The yield in peat was significantly lower than in vermiculite, regardless of the cell volume (Fig. 5).

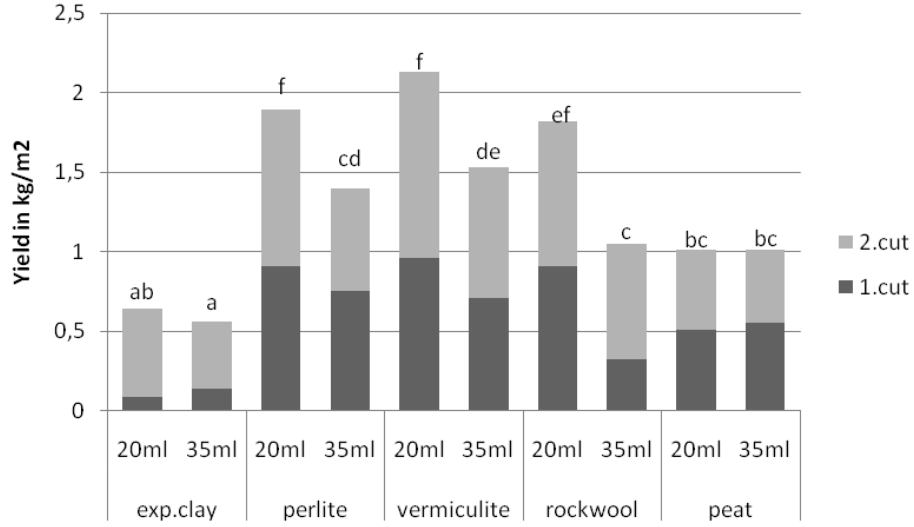

Fig. 5: Cumulative yield of rocket (kg m^{-2}) in different substrates and volumes of cells (exp.2).
Mean values followed by the same letter are not significantly different at $P<0.05$ according to Duncan's Multiple Range test

Nicola et al. (2004) reported that rocket plants produced more leaf area and fresh weight when growing in a mixture of peat and perlite (3:1) than in rockwool. In their experiment, rockwool media gave in general the worst results. Growing rocket in vermiculite has not previously been reported.

Growing rocket in a plug tray has proven successful. The technology is not new and has already been described by several authors (Fontana and Nicola, 2009). Nicola et al. (2005) compared a traditional and soilless culture system with overhead irrigation to produce rocket. The soilless culture had about 75 % higher yield than rocket grown in local soil and peat. The highest yield (around 2 kg m^{-2}) was obtained with the highest plant density (2.134 plants m^2), which is similar to our results.

Rocket leaves grown in peat substrate had significantly higher dry matter content (19.1 %) compared to those grown in a floating system (the

average in different substrates was 13.7 %) in which the influence of the substrate on dry matter content was insignificant (Fig. 6). One of the disadvantages of plants grown in a floating system is the higher content of water in the products. Nicola et al. 2005 reported that dry matter in rocket leaves grown in a traditional way was 15.9 %, and those grown in a soilless culture was 11.2 %.

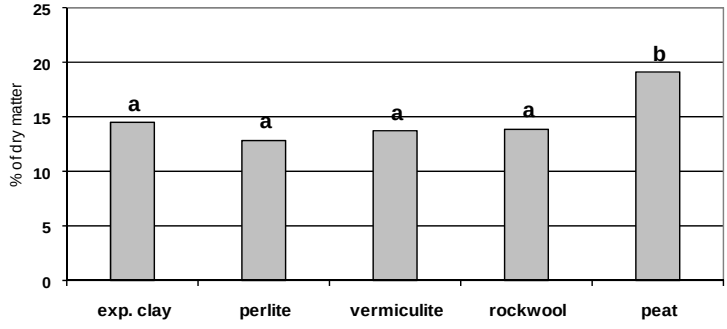

Fig. 6: Dry matter content (%) of rocket grown in different substrates.
Values followed by the same letter are not significantly different at $P<0.05$ according to Duncan's Multiple Range test

The nitrate concentration in rocket leaves was reduced drastically after the plants were grown for 10 days in tap water. The nitrate content in rocket leaves grown on a floating system in a nutrient solution was between 4288.4 and 6763.9 mg kg^{-1} FW, which was significantly higher than that of plants grown in peat (2067.7-2356.3 mg kg^{-1} FW). After 10 days of "water treatment", the nitrate concentrations in leaves dropped significantly and were between 51.9 and 633.8 mg kg^{-1} FW in different substrates. No nitrate was detected in leaves grown in peat (Table 3). Ferrante et al. (2003) reported that soil grown rocket usually contains a high level of nitrate - about 7.000-8.000 mg kg^{-1} and, during the winter period, this value can easily surpass 9000 ppm. Our results thus demonstrate that the floating system offers an excellent means of reducing the nitrate content in rocket leaves.

Table 3: The mean nitrate concentration ± SE (mg kg^{-1} FW) in the leaves of rocket grown in different substrates in plant nutrient solution until 10 days before harvest and in tap water until the day of harvest.

Substrate	No. of cells	NO_3-N in mg/ kg FW.		NO_3 in mg/kg FW	
		before harvest	on harvest	before harvest	on harvest
perlite	84	1422.6±70.81	93.6±19.89	6299.9±313.61	414.7±88.18
	160	1290.2±85.09	11.7±85.09	5713.9±376.79	51.9±22.51
Exp. clay	84	1423.3±151.36	82.5±41.85	6303.1±670.36	365.2±185.4
	160	1527.3±118.86	108.5±17.90	6763.9±526.37	480.5±79.22
peat	84	532.1±64.95	0.0±0.0	2356.3±287.63	0.0±0.0
	160	466.9±102.77	0.0±0.0	2067.7±455.09	0.0±0.0
vermiculite	84	1068.1±69.39	85.9±14.30	4730.2±307.29	380.3±63.38
	160	1283.8±45.81	55.6±15.96	5685.3±202.88	246.3±129.22
rockwool	84	968.3±67.93	143.1±21.45	4288.4±300.88	633.8±94.95
	160	1478.7±67.55	122.3±21.52	6548.4±299.25	541.7±95.29

Santamaria et al. (2002) suggested that vegetables constitute the major dietary source of nitrate. Though rocket is mainly used to flavour salads, the ingestion of only 100 g of raw vegetables with a nitrate concentration of 2.500 mg kg^{-1} FW would already lead to an intake of 250 mg NO_3. Consuming this item alone, the amount of nitrate would exceed the ADI (acceptable daily intake) for a person of 60 kg, by 13 %. Assuming the partial conversion of nitrate to nitrite (5 %) after ingestion, the current SCF (Scientific Committee on Food) ADI for nitrite (0.06 mg kg^{-1} body weight) would be exceeded by 247 %.

4 CONCLUSIONS

The plug tray system is suitable for growing rocket and the addition of a biostimulant can be beneficial, since plants are growing in a small amount of peat substrate. Smaller cells and a higher plant density (2.000 seeds per m^2) gave better results and did not affect plant quality. Statistical analysis showed no significant differences between used substrates (rock-wool flocks, perlite (3-5 mm), vermiculite (3-4 mm)) except for the expanded clay, which turned out to be an inappropriate substrate for raising seedlings.

Since the yield of rocket leaves grown in a floating system was more than 80 % higher than the yield in plug trays filled with peat (1.01 kg m^{-2} in peat; 1.83 kg m^{-2} in vermiculite), farmers could be expected to prefer to use a floating system for the production of rocket, in spite of the high nitrate content. So the suggestion of replacing the nutrient solution with water for the last 10 days before harvest can be useful for the consumer and for the producer, since it would allow higher and safer consumption of rocket leaves.

5 REFERENCES

Blake T. J. 2002. Antioxidant and natural biostimulant enhancement of seedling growth and stress tolerance in conifer seedlings. Journal of the Ministry of Forests, British Columbia *14* (1, 2): 10-17

Bottex B., Dorne J.L.C.M., Carlander D., Benford D., Przyrembel H., Heppner C., Kleiner J., Cockburn A. 2008. Risk – benefit health assessment of food – food fortification and nitrate in vegetables. Trends in food science & technology, 19: 113-119

Bryan N. S. 2006. Nitrite in nitric oxide biology: Cause or consequence? A systems -based review. Free Radical Biology & Medicine, 41: 691-701

D'Anna F., Miceli A., Vetrano F. 2003. First results of floating system cultivation of Eruca sativa L. In Pardossi E. et al. (ed.), Proceeding on greenhouse salinity, Acta Horticulturae, 609:361-364

D'Antuono L.F., Elementi S., Neri R. 2009. Exploring new potential health-promoting vegetables: glucosinolates and sensory attributes of rocket salads and related Diplotaxis and Eruca species. Journal of the Science of Food and Agriculture, 89: 713-722

Fontana E., Nicola S. 2009. Traditional and soilless culture systems to produce corn salad (*Valerianella olitoria* L.) and rocket (*Eruca sativa* Mill.) with low nitrate content. Journal of Food, Agriculture & Environment, 7 (2): 405-410

Ferrante A., Incrocci L., Maggini R., Tognoni F., Serra G. 2003. Preharvet and postharvest strategies for reducing nitrate content in rocket (*Eruca sativa*). Acta Horticulturae, 628: 153-159

Frezza D., León A., Logegaray V., Chiesa A., Desimone M., Diaz L. 2005. Soilless culture technology for high quality lettuce. In: Urrestarazu G.M. (ed.), International symposium on soilless culture and hydroponics, Acta Horticulturae 697: 43-48

Greenwood D.J., Hunt J. 1986. Effect of nitrogen fertilizer on the nitrate contents of field vegetables grown in Britain. Journal of the Science of Food and Agriculture, 37: 373-383.

Kim S-J., Ishii G. 2007. Effect of storage temperature and duration on glucosinolate, total vitamin C and nitrate contents in rocket salad (*Eruca sativa* Mill.) Journal of the Science of Food and Agriculture, 87:966–973

Lazzarin R, Giordano A. 2007. Usare il floating per ottenere ortive da foglia da taglio (The use of floating for growing fresh cut vegetables). Colture protette, 11: 55-62

Lundberg J.O., Weitzberg E., Cole J.A., Benjamin N. 2004. Nitrate, bacteria and human health. Nature Reviews Microbiology, 2: 593-602

Lundberg J.O., Feeliscbh M., Bjorne H., Jansson E.A., Weitzberg E. 2006. Cardioprotective effects of vegetables: is nitrate the answer? Nitric Oxide, 15 (4): 359-362

Magnani G., Filippi F., Borghesi E., Vitale M. 2007. Impact of sunlight spectrum modification on yield and quality of ready-to-use lettuce and rocket salad grown on floating system. In: De Pascal et al. (ed.) International symposium on high technology for greenhouse system management. Acta Horticulturae, 801: 163-169

McKnight G.M., Duncan C.W., Leifert C., Golden M.H. 1999. Dietary nitrate in man: friend or foe? British Journal of Nutrition, 81: 349-358

Morales M., Janick J. 2002. Arugula: A promising specialty leaf vegetable. In: J. Janick, A. Whipkey

(ed.), Trends in new crops and uses. ASHS Press, Alexandria, VA. 418-423

Nicola S, Hoeberechts J., Fontana E. 2004. Rocket (*Eruca sativa* Mill.) and corn salad (*Valerianella olitoria* L.): Production and shelf-life of two leafy vegetables grown in a soilless culture systems. Acta Horticulturae, 633: 509-516

Nicola S, Hoeberechts J., Fontana E. 2005. Comparison between traditional and soilless culture systems to produce rocket (*Eruca sativa*) with low nitrate content. In Urrestarazu G. M. (ed.), International symposium on soilless culture and hydroponics. Acta Horticulturae, 697: 549-555

Nitrate in vegetables, 2008. Scientific Opinion of the Panel on Contaminants in the Food chain1. The EFSA Journal, 689: 1-79

Pasotti P.P., Cavicchi L., Trentini L. 2003. Il floating system in Emilia-Romagna (The floating system in Emilia-Romagna). Colture protette, 8: 15-18

Premuzic Z., Gárate A., Bomilla I. 2001. Yield and quality of greenhouse lettuce as affected by form of N fertiliser and light supply. In: Horst Walter W.J., Sattelmacher B., Schmidhalter U., Schubert S., Wirén N. Von: Plant nutrition – Food security and sustainability of agro-ecosystem, 300-301

Santamaria P., Elia A., Serio F. 2002. Effect of solution nitrogen concentration on yield, leaf element content, and water and nitrogen use efficiency of three hydroponically grown rocket salad genotypes. Journal of plant nutrition, 25(2): 245-258

Santamaria P., Gonnella M., Elia A., Parente A., Serio F. 2001. Ways of reducing rocket salad nitrate content. In: Maloupa E., Gerasopoulos D. (ed.), International symposium on growing media and hydroponics. Acta Horticulturae, 548: 529-536

Thompson B. 2004. Five years of Irish trials on biostimulants: the conversion of a skeptic. In: Riley L.E., Dumroese, R. K., Landis T. D., National proceedings: Forest and Conservation Nursery Associations RMRS-P-33, 72-79

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Wheat straw decomposition, N-mineralization and microbial biomass after 5 years of conservation tillage in Gleysol field

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ABSTRACT

Long-term field experiment to follow various effects of conservation soil tillage is conducted at Biotechnical faculty in Ljubljana. The soil is Eutric Gleysol. Conventional treatment with plowing, 22 cm deep P, and conservation treatment by rotary harrow to a depth of 10 cm N, and subplots with compost amendment (-c) and without fertilization (-n) were observed. After 5 years nutrient contents and organic matter were higher in N than P. Compost addition had a positive effect on microbial biomass, but the tillage system did not affect it. However, nitrogen mineralization and decomposition of straw were higher in P as in N, and in the soil depth of 15 – 20 cm than in the 5 – 10 cm.

Key words: soil, sustainable agriculture, conservation tillage, minimum tillage, mouldboard plowing, soil fertility, residue decomposition

IZVLEČEK

RAZGRADNJA PŠENIČNE SLAME, MINERALIZACIJA-N IN MIKROBNA BIOMASA PO PETIH LETIH OHRANITVENE OBDELAVE OGLEJENIH TAL

V dolgoletnem poljskem poskusu na BF v Ljubljani, na evtričnih oglejenih tleh, merimo različne učinke ohranitvene obdelave tal. Obravnavamo konvencionalno obdelavo z oranjem 22 cm globoko P in ohranitveno obdelavo z vrtavkasto brano do 10 cm globine N, in podploskvi z dodatkom komposta (-c), in brez gnojenja (-n). Po 5 letih so bile višje vsebnosti hranil in organskih snovi v N v primerjavi P. Kompost je imel pozitiven učinek na mikrobno biomaso, obdelovani sistem pa nanjo značilno ni vplival. Mineralizacija dušika in razgradnja slame sta bila večja v P kot v N, ter v globini tal od 15 – 20 cm kot v 5-10 cm.

Ključne besede: tla, trajnostno kmetijstvo, ohranitvena obdelava, konzervacijska obdelava, minimalna obdelava, rodovitnost tal, razgradnja ostankov

1 INTRODUCTION

Modern conventional tillage includes a large number of serial passages in the preparation of soil for planting. After planting the soil surface remain uncovered until development of the crop and as such exposed to precipitation and wind. The result of such a situation is erosion, damage to soil structure and nutrient losses (run-off and leaching to deeper layers) (Wells et al., 2000). Decomposition is faster in areas with higher mean annual temperatures and higher annual total precipitation (Franzluebbers, 2002, Balota et al., 2003). In such conditions, soil cultivation additionally speeds up the decomposition; the more intense, the faster is the decomposition of organic matter. Deep intensive tillage incorporates plant residues into the soil, and also physically damages soil aggregates and exposes humus which was before protected in the structural aggregates, which facilitates decomposition of soil organic matter by soil organisms (Wright et al., 2005). By leaving

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substantial amounts of crop residue (at least 30%) on the soil surface, conservation tillage reduces soil erosion from wind and water, increases water retention, and reduces soil decomposition as well as water and chemical runoff. In addition, conservation tillage reduces the carbon footprint of agriculture (Ilan and Lal, 2013). It has been established that, after prolonged period of more than 10 years practising conservation tillage, soil organic matter can be increased to new higher equilibrium, with accompanying increases in microbial activity (Ilan and Lal, 2013). One of the main agronomical challenges is achieving or maintaining a high level of soil organic matter, while keeping the concentration of inorganic nitrogen low during periods subject to leaching losses. Compared with conventional tillage, conservation tillage creates more compact and cooler soil. The result is slower mineralization and the release of plant nutrients from soil organic matter (Doran and Werner, 1990).

Conservation tillage should be developed within the context of specific climates and soils (Abadalla et al., 2013). In Slovenia, there is a lack of experimental evidence on conservation tillage effects on the soil organic matter decomposition and mineralization. To fill the gap and to get the knowledge about conservation tillage effects we established in year 2000 a long-term field experiment of conservation vs. conventional tillage in Ljubljana, Slovenia. Relatively warm and humid climate (in Ljubljana: 10.9 °C average year temperature, 1400 mm precipitation), and Eutric Gleysol present a specific agro-ecological conditions. In this work we are testing following hypotheses:

- Conservational tilled soil N consecutively for 5 years inhabit higher soil microbial biomass and has greater ability to decompose fresh organic residue (wheat straw) in the top 10 cm compared to conventional ploughing system (P).
- Mineralization of soil nitrogen is slower in the top soil at conservation tillage (N) was compared to conventional plowing system (P).

2 MATERIALS AND METHODS

Long-term field experiment comparing conservation and conventional plowing soil tillage systems are conducted at Biotechnical faculty; Agronomy experimental field in Ljubljana from year 2000.

Field conditions and experiment design

The soil is Eutric Gleysol, with silty-clay-loam texture, with good nutritional status (Tab. 1; soil P and K supply are in desired, C-level of according to fertilization guidelines; Mihelič et al., 2010). The climate is relatively warm and humid (10.9 °C average year temperature, 1400 mm precipitation).

The experiment was divided into the block of conventional treatment with plowing 20 – 25 cm deep (P) and the block of conservation treatment by rotary harrow to a depth of 10 cm (N). Within each block, we considered two variants: unfertilised and fertilized with compost. The plots were without fertilization from year 2000 (-n); at plots (-c) compost made of source separated municipal biogenic waste from Komunalno podjetje Vrhnika d.d. composting plant was surface applied in the end of March, after oat seeding. With it we supplied 105 kg N/ha, 30 kg P_2O_5/ha and 28 kg K_2O/ha. Average yearly N input from the compost in the period 2000 to 2005 was 113 kg/ha. The compost was applied every second year. Each treatment is designed with three replications.

Soil sampling

Soil samples were collected 18th April 2005 from two depths (0-10 cm and 10-20 cm) and stored until analyses in a refrigerator at 4 °C up to one three days or frozen for long term storage.

Straw decomposition test in litter bags (Schinner, 1996)

Nylon litter bags were filled with wheat straw (straw particles ca. 1 – 2 cm long; Pict. 1) and exposed to field conditions by burying them into the soil at depths of 5 - 10 and 15 – 20 cm 1st June,

2005 in 6 repetitions per soil depth and treatment. 10 g of straw was put into each bag. Filled bags were first dried at a temperature of 95 °C 24 hours, and then cooled for two hours in a desiccator. Cold bags were weighed, and then buried into the soil. After incubation period bags were dug out the soil the 2nd August, 2005. The bags were dried 2-3 hours at room temperature, and then we carefully removed the soil stuck to the outer walls of the bags with soft brush for clothes. Caution was necessary to remove roots that grew into bags. After cleaning bags were dried at a temperature of 95 °C for 24 hours, followed by cooling in desiccator and weighing it. Straw decomposition was calculated by mass differences.

Picture 1: Litter bag with straw before field installation

Crop management at the experimental field

In the August 2004 we planted oil rape (*Brassica napus var. oleifera*) as a green manure. The oil rape was desiccated with total herbicide (glyphosate: Boom Effect) in the late autumn. In early March 2005 the soil was either plowed 22 cm deep + rotary harrowed 10 cm deep: treatment P or just rotary harrowed: treatment N. Oat (*Avena Sativa* L.) cv. Expander was then seeded in to the field 24th March 2005.

Laboratory analyses

Kjeldahl total nitrogen analysis was done using titanium dioxide as catalyst (ISO 11261).

Determination of soluble nitrogen forms (NO_3-N, NH_4-N) was done in 0.01 M $CaCl_2$ extraction following SIST ISO 14255 and measured by flow injection analyses (FIAS Perkin-Elmer 3000).

Determination of total carbon was done following the protocol ISO/DIS 10694 (1994): a method for the determination of the total carbon content in soil after dry combustion. Organic matter was calculated by multiplying C with a factor of 1.72.

Fumigation incubation method was done according to Schinner (1996). During the fumigation chloroform kills soil micro-organisms, so they become susceptible to mineralization. After fumigation we added inoculum (1% mass of non-fumigated soil), followed by incubation for 10 days in the dark at 25 °C. During this time, killed biomass partially mineralized (microorganisms from non-fumigated inoculum made the mineralization), resulting in an increased release of carbon dioxide. Extracted carbon dioxide binds to sodium hydroxide. From the consumption of acid for titration of the residual sodium hydroxide the amount of soil microbial biomass is calculated. Each treatment was measured in triplicates.

Determination of net nitrogen mineralization by incubation under controlled aerobic conditions (Drinkwater et al., 1996): samples in triplicates were incubated in 100 ml containers, at 60% of field capacity and at 20 °C for twenty-eight days. After incubation the extraction of samples with 0.01 M $CaCl_2$ to determine the quantity of extractable mineral nitrogen (NO_3-N and NH_4-N) was done. Extraction of the first batch of samples was performed on the first day, the second batch on the 14th and the third at 28th day after the start of the incubation.

3 RESULTS AND DISCUSSION

Nutrients and microbial biomass carbon in the soil

Microorganisms are the active component of soil organic matter. They rapidly respond to the changes in the soil temperature, moisture content, crop residues (Rice et al., 1996). Due to the quick response of microorganisms to the soil conditions, microbial biomass-C content could also be a good indicator of soil tillage induced changes (Alverez and Alverez, 2000).

Five years after the start of the experiment, nutrient content and organic matter were higher in conservation N vs. conventional tillage P, although the differences were not significant (Tab. 1).

Table1: Soil analysis (0 – 20 cm) after 5 years of the experiment

Treatment	pH	P_2O_5 AL-method (mg/100 g)	K_2O AL-method (mg/100 g)	Org. matter %	N %	C:N
Conservation tillage **N**	6,8	24	40	4,3	0,25	10,1
Confidence interval (±)	0,1	2	5	0,3	0,01	0,3
Plowing tillage **P**	6,7	21	32	4,0	0,23	10,0
Confidence interval (±)	0,1	2	3	0,2	0,01	0,4

The measurements of microbial biomass-C showed higher values at a depth of 0 – 10 cm in all treatments (Fig 2), however no significant differences were found between treatments, thus our first hypothesis was denied, which is contrary to established theory. For conservation vs. conventional tillage literature reports the increase of the content of microorganisms in the topsoil (0 – 10 cm), while the differences in the deeper layers are smaller (Mühlbachová and Růžek, 2002; Alvarez and Alvarez, 2000; Franzluebbers , 2002; Gonzales-Chavez et al., 2010).

Compost addition had a positive effect on microbial biomass in the plowed soil where microbial biomass was relatively high and consistent in the both soil depths. The differences between treatments were not significant, because a high variability found.

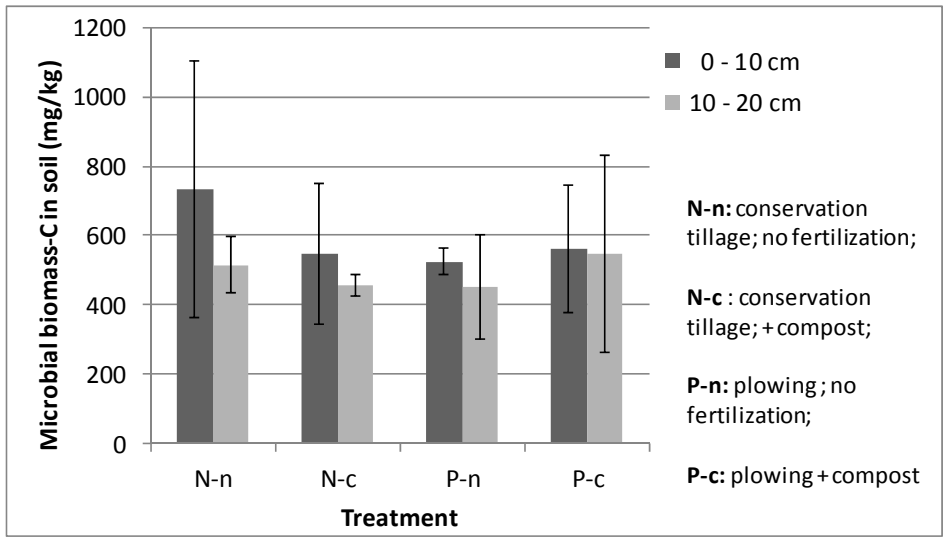

Figure 2: Microbial biomass in soil dependent on soil tillage and soil depth

Net nitrogen mineralization

N mineralization was higher in the plowed soils (P-n, P-c) in the both soil depths (Fig. 3). N-mineralization started faster in the plowed soils, especially in the upper 10 cm. This could be explained by more plant residue concentrated in the upper 10 cm at N treatment. Plant residue (fresh and from previous years) can cause temporary immobilization of N released from soil by mineralization. Immobilization is significant if the C/N ratio of plant residue is >20 – 25 (Nicolardot et al., 2001). Oil rape which was incorporated in a flowering stage in early autumn 2004 can have a C/N ratio around 30 or even higher. Kriauciuniene et al. (2008) report that oil rape plant residues contain a substantial amount of lignified matter especially roots and stems, which are very much resistant to decomposition.

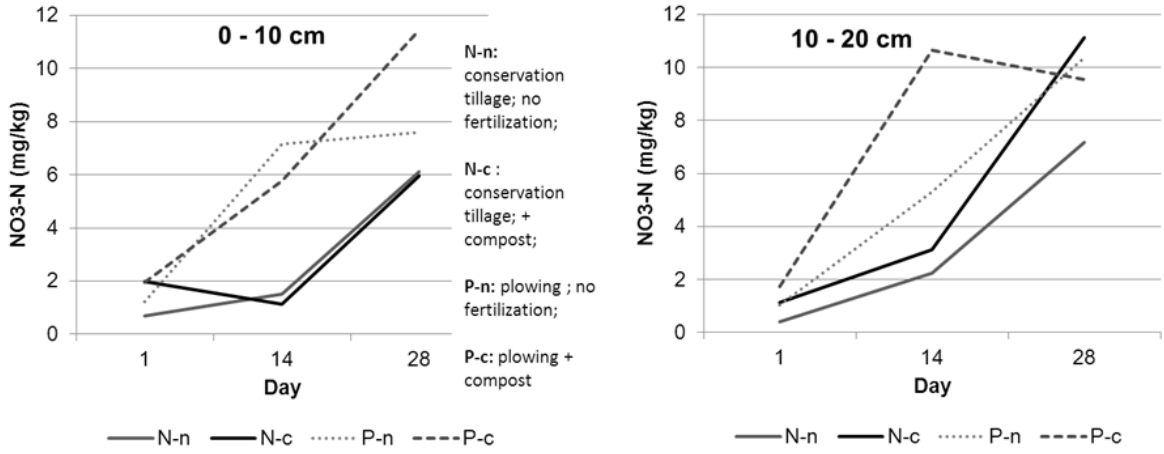

Figure 3: Nitrate-N accumulation during aerobic soil incubation

On the contrary to plant residues, the compost we used had a C/N ratio 17.0 and thus immediate net N-mineralization was expected with compost addition. Positive effect of the compost on N-mineralization was pronounced at P, but not at N where the mineralization had a lag period of 14 days with or without compost addition. Later, in the days 14 to 28, the concentration of NO_3-N in the upper 10 cm was still significantly higher in P than N at the end of incubation. Similar trend was in the layer 10 – 20 cm, although the N-c produced the same amount of NO_3-N as P-c at the end of incubation.

This confirms that conventional tillage promotes mineralization, reduces the level of organic matter in the soil, what can lead in a long term to decreases of soil fertility and can consequently deteriorate the economics of field crop production (Balota et al., 2003; Alvarez and Alvarez 2000; Wright et al., 2004). On the other hand, less mineralization at conservation treatment N could mean a need for higher doses of nitrogen fertilizer for crops, especially for good vegetative growing of young plants (Javůrek, 1998). Conservation tillage practices can thus lead to enhanced nitrous oxide emissions (Abadala et al., 2013).

Wheat straw decomposition

Litter bags were buried in the soil for 62 days, throughout June and July 2005. The average temperature in June was 19.5 °C and in July 21.1 °C, precipitation being 83.9 mm and 141.9 mm, respectively (Meteorology yearbook, 2005), so the environmental conditions for straw decomposition were good.

More intense straw decomposition was found in P, 46% then in N, 42% (Fig. 4). In practice this would mean that wheat straw would almost completely decompose in just more than four months after the harvest. However, we must realize that the process takes longer, as autumn comes cooler weather.

Higher decomposition in P is in line with the N-mineralization rate and microbial biomass measurement. Similarly, as for the higher N-mineralization in conventional tillage can also be

higher decomposition of straw favoured by less compact soil in this treatment P, which in turn means more air to soil organisms and hence their greater activity compared to conservation tillage N. Soil depth had no significant influence on straw decomposition. The addition of compost slightly enhanced straw decomposition only in 15 – 20 cm, in spite that we spread the compost on the soil top after seeding of oat. This could also be an effect of compost additions from previous years.

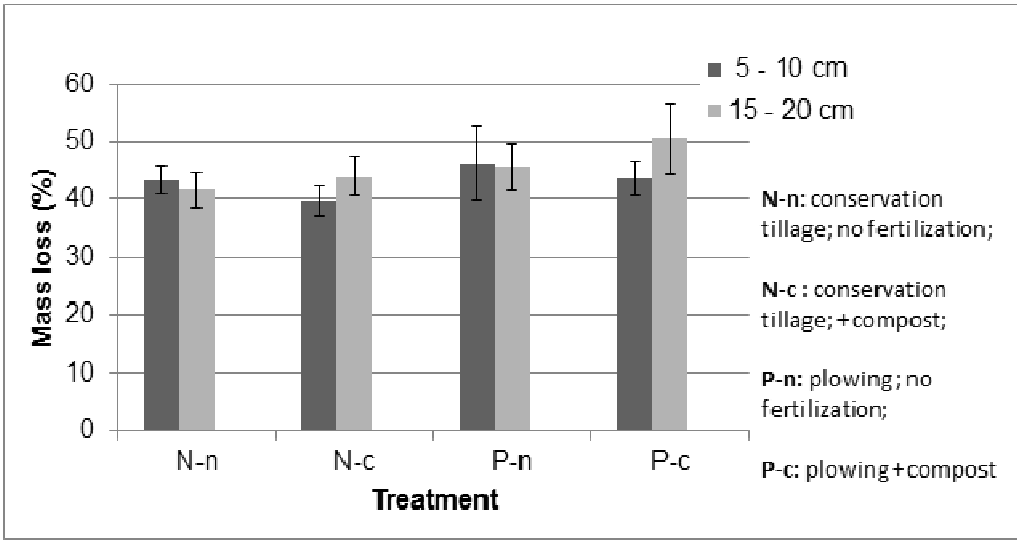

Figure 4: Wheat straw mass loss (% of initial) after 62 days of decomposition experiment (means and SD of 6 replicates are shown)

4 CONCLUSIONS

After five years of field experiment comparing conservation tillage N with the conventional plow tillage P the content of nutrients and organic matter increased in N, however biomass-C in soil was not significantly affected, although it was slightly higher at the plowed treatment P. Straw incorporated into the soil degraded relatively fast. On average 44% of straw decomposed in just two months. Soil conservation treatment using only rotary harrow N had slightly lower capacity for decomposition of wheat straw compared to P, but the differences were not significant. Such result is in line with the N-mineralization rate and microbial biomass measurement. Slower decomposition of organic matter results in a slower release of nutrients as shown by N-mineralization incubation test. This may indicate the need for more substantial fertilization with mineral nitrogen at conservation tillage system used in this experiment. We continue with the experiment to observe further long-term changes in the soil induced by conservation tillage.

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6 REFERENCES

Abdalla M, Osborne B., Lanigan G., Forristal D., Williams M, Smith P., Jones M. B. 2013. Conservation tillage systems: a review of its consequences for greenhouse gas emissions. Soil Use and Management. 4 Feb 2013. doi: 10.1111/sum.12030.

Alvarez C.R., Alvarez R. 2000. Short term effects of tillage systems on active soil microbial biomass. Biol Fertil Soils: 31: 157-161.

Balota E.L.; Colozzi-Filho A.; Andrade D. S.; Richard P. D. 2003. Microbial biomass in soils under different tillage and crop rotation systems. Biol Fertil Soils, 38: 15-20.

Doran W.J. in Werner J.W. 1990. Managment and soil biology. V: Sustainable Agriculture in Temperate Zones. Francis A. C.; Butler Flora C.; King L. D. (ur.). Wiley Interscience Publication: 205-223.

Drinkwater L.E.; Cambardella C.A.; Reeder J.D.; Rice C.W. 1996. Potentially Mineralizable Nitrogen as an Indicator of Biologicaly Active Soil Nitrogen. V: Methods for Assessing Soil Quality. Doran W. in Jones A. J. (ur.). Madison, Wisconsion, USA, Soil science Society of America: 217-231.

Franzluebbers A.J. 2002. Soil organic matter stratification ratio as an indicator of soil quality. Soil &Tillage Reserch, 66: 95-106.

González-Chávez MCA, Aitkenhead-Peterson JA, Gentry TJ, Zuberer D, Hons F, Loeppert R. 2010. Soil microbial community, C, N, and P responses to long-term tillage and crop rotation. Soil Tillage Res 106:285–293

Ilan S., Lal, R. 2013. Agriculture and greenhouse gases, a common tragedy. A review. Agronomy for Sustainable Development. Springer-Verlag. 275-289. http://dx.doi.org/10.1007/s13593-012-0110-0

Javůrek M. 1998. Conservation tillage and stand establishment systems in winter wheat and soybean crop rotation. V: Tajnšek, A. (ur.), Šantavec, I. (ur.). Novi izzivi v poljedelstvu '98 : zbornik simpozija, [Dobrna, 3. in 4. december 1998]. Ljubljana: Slovensko agronomsko društvo. 210-213

Kriauciuniene Z, Velicka R, Raudonius S., Rimkeviciene M. 2008. Changes of lignin concentration and C:N in oilseed rape, wheat and clover residues during their decomposition in the soil. Agronomy Research 6(2), 489–498, 2008

Meteorološki letopis 2005. Republika Slovenija. Ministrstvo za kmetijstvo in okolje. http://www.arso.gov.si/vreme/podnebje/meteorolo%C5%A1ki%20letopis/meteoroloski_letopisi.htm

Mihelič, R, Čop, J, Jakše, M, Štampar, F, Majer, D, Tojnko, S, Vršič, S. 2010. Smernice za strokovno utemeljeno gnojenje. Ljubljana: Ministrstvo za kmetijstvo, gozdarstvo in prehrano, 2010. 182 str., ilustr. ISBN 978-961-6761-09-3.

Mühlbachová G. in Růžek P. 2002. The effeects of conventional, reduced and no-tillage practices on some biological and physical properties of soils. V: Novi izzivi v poljedelstvu 2002. Tajnšek, A. (ur.), Šantavec, I. (ur.).: zbornik simpozija : proceedings of symposium, [Zreče, 5. in 6. december 2002]. Ljubljana: Slovensko agronomsko društvo, str 23-27.

Nicolardot, B., Recous, S., Mary, B.. 2001. Simulation of C and N mineralisation during crop residue decomposition: A simple dynamic model based on the C:N ratio of the residues. Plant and Soil, 228, 83-103

Rice C. W.; Moorman B.T.; Beare M. 1996. Role of Microbial Biomass Carbon and Nitrogen in Soil Quality. V: Methods for Assessing Soil Quality. Doran W.J.in Jones A.L. (ur.) Madison, Wisconsion USA, Soil Science Society of America: 144-173

Schinner F., Öhlinger R., Kandeler E. and Margesin R. (eds) (1996) Methods in Soil Biology. Springer Verlag, Berlin Heidelberg New York, pp 1-426

SIST ISO 11261. 1995. Soil quality- Determination of total nitrogen: 4 p

SIST ISO 14255. 1999. Soil quality- Determination of soluble nitrogen fractions: 5 p

Wells A.T.; Chan K.Y.; Cornish P. S. 2000. Comparison of conventional and alternative vegetable farming systems on the properties of Yellow earth in New South Wales. Agriculture, Ecosystems and Environment, 80: 47-60.

Wright A.L.; Hons F.M.; Matocha J.E.Jr. 2005. Tillage impacts on microbial biomass and soil carbon and nitrogen dynamics of corn and cotton rotations. Applied Soil Ecology, 29: 85-92.

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Contribution of agricultural policy measures to maintain grassland areas (the case of Radensko Polje Landscape Park)

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ABSTRACT

Within this research paper, the enforcement of agricultural policy measures for farms that have their agricultural lands within the Radensko Polje Landscape Park (RPLP) were studied. The purpose of the study was to evaluate the effectiveness of the additional payment for the extensive rearing of female bovine animals (ERB) and agri-environmental measures (AE measures) in terms of maintaining extensive agricultural systems or transitions from intensive systems to sustainable ones. This is especially desirable in the protected areas that also include landscape parks. The results of the survey of farmers of RPLP showed that both the ERB and AE measures are inefficient and fail to encourage farmers to implement more extensive farming. The main reasons for the poor enforcement of claims for ERB are intensive livestock production (milk production or bovine animals fattening), and the lack of information about the possibility of claim enforcement for ERB. Regarding AE measures, the main reasons for the failure are burdensome conditions and low financial compensation. Inventories of the composition of plant species on sample grasslands showed that the conditions of the habitats are still relatively good, because a relatively large number of species of high conservation value is present.

Key words: agricultural policy, grasslands, landscape parks, sustainable development, nature conservation/subvention

IZVLEČEK

PRISPEVEK UKREPOV KMETIJSKE POLITIKE K OHRANJANJU TRAVIŠČ (PRIMER KRAJINSKEGA PARKA RADENSKO POLJE)

V raziskavi smo na območju Krajinskega parka Radensko polje (KPRP) proučevali uveljavljanje ukrepov kmetijske politike za kmetije, ki imajo kmetijska zemljišča znotraj parka. Namen naloge je bil ugotoviti učinkovitost dodatnega plačila za ekstenzivno rejo ženskih govedi (ERG) in ukrepov kmetijsko okoljskega programa (KOP) v smislu ohranjanja ekstenzivnih kmetijskih sistemov oziroma prehodov iz intenzivnih sistemov v trajnostno naravnane. To je še posebej zaželeno na zavarovanih območjih, med katere sodijo tudi krajinski parki. Rezultati ankete pri kmetovalcih so pokazali, da so tako ukrep ERG kot ukrepi KOP na območju KPRP neučinkoviti in ne stimulirajo kmetovalcev k izvajanju bolj ekstenzivnega kmetijstva. Glavni razlogi za slabo uveljavljanje zahtevkov za ukrep ERG so v usmerjenosti v bolj intenzivno živinorejo (prireja mleka oziroma reja pitancev) in v premajhni informiranosti o možnosti uveljavljanja tega ukrepa. Pri ukrepih KOP so glavni razlogi za neuveljavljanje prezahtevni pogoji in premajhna finančna nadomestila. S popisi vegetacije vzorčnih travišč na območju parka smo ugotovili, da je kljub neuveljavljanju zahtevkov stanje habitatov še zmeraj relativno dobro, saj je še prisotno relativno veliko število naravovarstveno pomembnih vrst.

Ključne besede: kmetijska politika/travišča/krajinski parki/trajnostni razvoj/varstvo narave/ subvencije

Prispevek je del magistrskega dela: Prispevek ukrepov kmetijske politike k ohranjanju travišč (primer Krajinskega parka Radensko polje), mentor: prof.dr. Franc Batič.

This paper is a part of the master's thesis: *Contribution of agricultural policy measures to maintain grasslands (the case of Radensko Polje Landscape park)*, mentor: Prof, Franc Batič, Ph.D.

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1 INTRODUCTION

Grasslands comprise a great part of agricultural land and are an important part of the environment; therefore, their sustainable management is important. They cover 52.5 million km^2 (i.e. 40.5% of the Earth's land surface), excluding Greenland and Antarctica (Suttie et al., 2005). Grasslands are also significant in Slovenia, comprising 58% of all agricultural land. (Pomembnejši podatki popisa kmetijstva …, 2010). Grasslands in Slovenia are endangered similarly as in other parts of the world, specifically by the change in land use as a consequence of urbanisation, the building of infrastructure and changes in the soil water regime, the intensification of agriculture (ploughing of meadows, increased use of mineral fertilisers and seeds of cultivated grassland species) and the abandonment of agricultural use (Pregled stanja …, 2001).

Of all economic activities, agriculture has influenced nature for the longest period of time (Batič et al., 2002). It has changed natural ecosystems and reduced their cover; at the same time, agriculture has made new habitats and increased overall landscape biodiversity. In the 1990s, the concept of agricultural areas with high natural value was developed as a consequence of the recognition that the conservation of biodiversity and cultural landscapes in Europe depends on the existence of low intensity agricultural systems (Beaufoy et al., 1994; Bignal in McCracken, 1996). At the same time, the Common Agricultural Policy (CAP) of the EU changed and started to move from market-oriented production support towards environment-protection support in order to promote efficient and sustainable agriculture. Among other direct payments, an extensification payment scheme emerged in order to conserve and promote extensive livestock farming, which should support biodiversity in the rural agricultural landscape. These payments were carried out also in Slovenia from 2003 to 2006. In 2007, these payments were halted with CAP reform, but on the basis of 69th article of the European Community Council Act (No. 1782, 2003) a new measure, called ERB (extra payments for extensive rearing of female bovine cattle) was introduced, as part of a set of specific supports, which also supports extensive agricultural practices.

AE measures of the Agri-environmental Programme are designed to implement environmentally friendly farming practices. They reflect the multi-functionality of agricultural production, expressed in the public services of maintenance of landscape, biotic diversity and the rural population, by taking into account ecological, social and site dependent aspects of rural areas (Program razvoja podeželja …, 2007). For the conservation of grasslands, the measures of the second pillar of the Slovenian Rural Development Programme are of great importance. These are devoted either to the conservation of nature, biotic diversity, soil fertility or the maintenance of the traditional cultural landscape (Group II measures), or the conservation of protected areas (Group III measures).

In this research, it was our intention to determine to what extent the ERB and AE measures are implemented in the Radensko Polje Landscape Park (RPLP) from 2007 onward. Additionally, we analysed reasons for the rejection of the ERB measure by farmers during that time, and estimated the potential number of farmers who could apply for this measure. In the second part of our research, we investigated the effectiveness of the ERB measure, i.e. the extent to which it contributed to the conservation of grasslands in the RPLP. Critically, we evaluated the definition of extensive farming and the use of the term “extensive”, and compared how many criteria of extensive farming are covered by the obligations of the ERB measure. Using these analyses, we estimated the intensity of agricultural systems on the RPLP area, which may serve as a good contribution to the search for new, more suitable and more efficient measures for achieving RPLP objectives. On the basis of an overview of national and foreign studies, we can assume the validity of the generally accepted thesis that less intensive agricultural systems have positive effects on the conservation of permanent grasslands and their ecosystem services (Kramberger, 1994; Nösberger and Rodriguez; 1996; Nösberger et al., 1994; Bignal et McCracken, 1996; Nielsen in Debosz, 1994; Zechmeister et al., 2002; Miles, 1981; Brak et al., 2004; Critchley et al., 2007; Hayes et al., 2007; Dunn et al., 2007; Gulliver et al., 2007;

Buckingham and Peach, 2007, Marriot et al., 2009, Ketiš, 2010).

2 MATERIAL AND METHODS

The research was carried out on the RPLP, on which there are two protection zones (Uredba o Krajinskem parku Radensko Polje, 2011). The first zone covers the areas of the most valuable habitats for nature conservation and is primarily devoted for protection and conservation of natural values and the favourable conservation status of specific plant and animal species and their habitats. The second zone is primarily devoted to the protection and conservation of natural values and biodiversity of a landscape. Areas of the park outside of both zones (delimited as a third zone) are dedicated for the protection of the landscape diversity and for the promotion of sustainable development. According to habitat-type mapping, the RPLP is divided into four main subareas: part of Grosupeljsko Polje west of the village Veliko Mlačevo, and the northern, central and southern parts of Radensko Polje (Inventarizacija flore in favne na Radenskem polju, 2000). Considering habitat types, the most preserved and valuable part is the central part of the park where the continuous mosaic pattern of wetland plant communities, mostly wet meadows, prevails. Among the meadows, *Molinia caerulea* (*Molinietum caeruleae* W. Koch 26) or *Deschampsia cespitosa* (grasslands from the *Deschampsion littoralis* Oberd. et Dierss in Dierss. 75 alliance) prevail. Regarding the flora of these grasslands and other wetland areas, the following plant species relevant for nature conservation thrive here: *Carex pulicaris* L., *Fritillaria meleagris* L., *Gentiana pneumonanthe* L., *Gratiola officinalis* L., *Iris sibirica* L., *Ludwigia palustris* (L.) Elliot, *Menyanthes trifoliata* L., *Pedicularis palustris* L., *Potentilla palustris* (L.) Scop., *Schoenoplectus mucronatus* (L.) Palla, *Teucrium scordium* L. and *Utricularia australis* R.BR. (Inventarizacija flore in favne na Radenskem polju, 2000).

The research was divided into three sections. In the first section, we performed an analysis of measures of agricultural policy in the study area, which was based on data obtained from the Agency of the Republic of Slovenia for Agricultural Markets and Rural Development (ARSKTRP), the Geodetic Institute of the Republic of Slovenia (GURS), the Register of Agricultural Holdings (RKG), the Surveying and Mapping Authority of the Republic of Slovenia (SMA) and the Institute of RS for Nature Conservation (ZRSVN). For the analyses of the ERB measure and AE measures for the period from 2007 to 2011, the necessary data were obtained via special enquiry submitted to ARSKTRP. The administrative borders of the Grosuplje municipality were derived from GURS. The official borders of the Radensko Polje Landscape Park, protected areas and subareas were obtained from ZRSVN. The spatial data (land use and graphical units of agricultural use, Natura 2000 areas) needed for the analyses in the investigated area were obtained by special request from Ministry of Agriculture and the Environment (MKO).

In the second section, socio-economic analyses were carried out with an inquiry among 15 farmers in which the socio-economic and production characteristics of farms were obtained (property and size of farms, types and history of land use, grassland management practices, use of agricultural mechanisation), attitudes of farmers towards the formation of RPLP (acquaintance with reasons for park formation and mode of providing this information, knowledge of Natura 2000 areas) and reasons for not applying for the ERB and AE measures. Farms used in the sampling inquiry were selected randomly from all the farms with land within the RPLP. In all, 25 farms were chosen but only 15 of them expressed a willingness to participate in the inquiry. Fourteen selected farms out of the 75 that had land within the first conservation zone in 2011 comprised 54.66 ha of the park area, which represents 47.2% of agricultural land of the first conservation zone of the RPLP. One of the farms had agricultural land only in the second and the third conservation zones, representing 8.65 ha.

In the botanical section of the research, the vegetation composition of 12 grasslands scattered in all three conservation zones of RPLP was assessed. Seven of these grasslands were in agricultural use in 2011, belonging to seven

different farms. The owners of one of these farms did not want to participate in the inquiry. Five of the grasslands in agricultural use extended to all three conservation zones; two grasslands extended only to either the second and third conservation zone. Vegetation composition was assessed twice in 2011; first, shortly before the first mowing in the April-May period; second, in September. To estimate the cover and abundance of plant species, the modified method of Braun-Blanquet (1964) was used in vegetation surveys.

Spatial analysis was carried out using the GIS software ArcGIS 9.3. The majority of inquiry data was processed using MS Excel. Data were analysed by means of descriptive statistics and were presented in tables and figures. The similarity of grasslands, based on plant species composition was determined using correspondence analysis with a removed trend (DCA).

3 RESULTS AND DISCUSSION

Implementation of ERB of AE measures in the areas of RPLP

ERB should promote extensive bovine rearing and in this way contribute to grassland conservation. From the measures promoting rural development, some of the AE measures were analysed (measures important for conservation of grassland habitats included in the Natura 2000 site, important for the conservation of birds and butterflies on extensive wet meadows).

In the analysis of how many of indicators of low intensity systems (after Beaufoy et al. (1994)) are included in requirements to apply for the ERB measure, we can conclude that only two are included: stocking rates and cattle breeds. The breeds are not restricted to being native to the area but must be adapted to low-intensity use. Limited fodder use is regulated indirectly by the livestock unit (LU) load, which is calculated as LU/ha with the condition that permanent grasslands have to account for 50% of all fodder areas. The input of fertilisers could be partly controlled by the control of the cross compliance of measures. We can conclude that the essential elements of extensive bovine rearing are included in the ERB measure; the remaining problem is that too much LU has been permitted. One potential solution in this area is the requirement for the farmers to have at least 30% of permanent grasslands from all lands in agricultural land use from 2010 onward, which means better contribution for the conservation of grasslands than the former requirement of 50% of permanent grasslands from all fodder areas.

Table 1: Number of claims for ERB by areas and years (ARSKTRP..., 2012)
Preglednica 1: Število zahtevkov za ERG po območjih in letih (ARSKTRP …, 2012)

	2007		2008		2009	
Območje Area	Št. kmetij Number. of farms	Št. živali Number of animals	Št. kmetij Number. of farms	Št. živali Number of animals	Št. kmetij Number. of farms	Št. živali Number of animals
RS	16146	48531	14927	44971	14981	45672
KPRP RPLP	28	82	24	73	24	72
Prvo in drugo VO* KPRP First and second PA* RPLP	22	70	20	66	19	63

VO*=Varstveno območje
PA*= Protection area

From 2007 to 2009, 15,351 farms applied for the ERB measure for 46,391 animals throughout the Republic of Slovenia. On the RPLP, 25 farms applied for the ERB with 76 animals in the first conservation zone, and 20 farms with 66 animals in the second conservation zone. A decrease in the application for the ERB measure was observed in Slovenia from 2007 to 2008 regarding the number of farms and animals. A slight increase was observed in 2009, but number of application did not reach the 2007 levels. The overall steady decrease of applications for ERB measure has been observed in the RPLP. In 2007, the ERB measure was applied by 29% of farms of RPLP, and only 24% in 2008 and 2009.

Analysis of mistakes in the application for the ERB measure in the Republic of Slovenia in the period of 2007–2010 showed that the proportion of mistakes is decreasing, indicating better familiarity of farmers with the requirements for the measure. The remaining problems, related to the extensive agriculture, are the following: proportion of permanent grasslands, stocking rate of fodder areas, and affiliation of the cattle in the area to the herds of calves breeding for meat production. This problem also occurs in the RPLP. Exceeding the permitted LU/ha is even greater in the RPLP than in Slovenia as a whole.

The average proportion of permanent grasslands regarding the fodder areas in farms that applied for the ERB measure was bigger in Slovenia as a whole than in the RPLP. The proportion of permanent grasslands in the Republic of Slovenia is around 94%, and this share was stable during the 2007–2009 period. On the first and second conservation zones of the RPLP, these proportions are 7 to 10 percentage points smaller and are fluctuating over time. The average LU in the the first and second conservation zone of the RPLP during the 2007–2009 period was 1.22 LU/ha, which is slightly higher than at the country level (1.14 LU/ha).

Using the data obtained by ARSKTRP, it was calculated that ERB measure could be applied by nine additional farms for 16 cows within the whole RPLP, and seven 7 farms for 14 cows within the first and second conservation zones. This indicates that some farmers are still not sufficiently familiar with possibilities of applying for agricultural subsidies or that the procedure to obtain these subsidies is overly complicated.

Table 2: Number and percentage of farms involved in AE measures, which have land in the first and second PA RPLP in the 2007–2011 period (ARSKTRP..., 2012)
Preglednica 2: Število in delež kmetij, vključenih v ukrepe KOP, ki imajo zemljišča na območju prvega in drugega VO KPRP v obdobju od leta 2007 do 2011 (ARSKTRP …, 2012)

Leto Year	Skupno število kmetij Total number of farms	Število kmetij KOP Number of AE farms	Delež (%) Share (%)
2007	96	24	25.0
2008	98	23	23.5
2009	98	19	19.4
2010	97	12	12.4
2011	96	11	11.5

Regarding the farms with agricultural land within the first and second conservation zones of the RPLP, the proportion of farms applying for the AE measures is small. From 2007, when it accounted 25% of farms, it decreased to 11.5% in 2011. Only three such farms applied for specific measures intended for Natura 2000. That is very little, having in mind that entire first and second conservation zones of RPLP are within Natura 2000.

Data on the application of the ERB measure and AE measures of RPLP farms show little interest among farmers for these measures. Using our

inquiry information, we determined that the majority of farms do not implement the ERB measure simply because they do not breed cows (53%). A total of 33% of farms claim that they do not fulfil the requirements, either due to milk production (4 farms) or exceeding LU (1 farm). As a main reason for not applying for the AE measures, farmers mention overly demanding requirements making their farming economically unprofitable under these conditions. Similar findings were also reported by other investigators (Udovč and Čemažar 2002, Pust Vučajnk and Udovč 2008, Žvikart 2010).

Analysis of agriculture in the RPLP and attitude of farmers towards the formation of the landscape park

The majority of the studied farms are oriented towards animal husbandry, mostly cattle breeding, and horse breeding to a lesser degree. Breeding of Black-White, Brown and Simmental cattle breeds prevail; in horse breeding, the Cold-blooded Slovenian horse (*Slovenski hladnokrvni konj*) prevails. The average LU for all RPLP farms was 0.75 LU/ha in 2010, meaning that the intensity of animal husbandry within the park area is not very high. In accordance with the requirements of cross-compliance, the annual input of nitrogen should not exceed 170 kg/ha of the agricultural land in use on the farm level. Regarding data of the ARSKTRP, the average annual input of nitrogen on farms having land within the first and the second conservation zones of RPLP accounted 50.5 kg N/ha/year in 2010, and no farm exceeded the allowed yearly input. In most cases, fertilization was carried several times during the vegetation period (67% of farms within the first and second conservation zone of RPLP, 73% of farms in the third conservation zone of the park and 75% of farms outside the park). Fertilizer is applied in February, March, April, June and October. This is in accordance with the regulation claiming that slurry application is prohibited in areas without green cover from 15th of November to 15th of February and on lands with green cover from 1st of December to 15th of January.

The attitude of farmers towards the establishment of the RPLP is not very encouraging. The study showed that 53% of the studied farmers stated that park formation brought a limitation to the agricultural development to the RPLP area. The results of the inquiry are much worse than those obtained by the park management authorities in 2008 in which 94% of inquired farmers were in agreement with formation of the park. The reason for this discrepancy might be the smaller number of people included in our investigation or a shift of attitude in recent years.

Analysis of the grasslands management in the RPLP.

Results of vegetation mapping and evaluation of habitats on the RPLP showed (Inventarizacija flore in favne na Radenskem polju, 2000) that approximately 40% of the park was rated as the highest nature conservation grade (4, 5) (IUCN, 2012). The majority of this area is wet meadows and lowland pedunculate oak-hornbeam forests. A roughly equal area is covered by cultivated meadows, which are not of high value from a nature conservation point of view. On the RPLP, 450 vascular plant species were registered, of which 44 are endangered and 18 protected (Strokovne podlage za zavarovanje Radenskega polja, 2008).

The largest part of permanent grasslands was assessed by the inquiry within the first and the second conservation zones of the RPLP (87% of farms declared that their grasslands are within the first and second conservation zones). The average height of the grassland turf before the first mowing was 37 cm in the first and second conservation zones of RPLP, 44 cm in the third zone, and 46 cm outside of the RPLP, indicating smaller yields of the grassland in first and second conservation zones. For the areas of Natura 2000, the first mowing is recommended after the first of August, and at least one mowing and harvesting per year. During field work, we noticed that the first mowing had already been performed in the middle of May. We also observed areas where mowing was abandoned. In spite of a relatively earlier vegetation period in 2011, such an early mowing time is not suitable for nature conservation, even more so when taking into account the modern harvesting technology (wrapping mowed plants into plastic bales which does not enable natural seeding of meadow plants). All analysed farms in the RPLP, regardless the conservation zones, perform only mowing. The majority of the farms

perform three cuts per year, and the proportion of three-cut meadows increases with the distance from the first and second conservation zones of the park. Only one cut per year is the most common in the first and second conservation zones of the RPLP due to low quality of the fodder and poor regeneration after the first cut. Two cuts per year are performed on 31% of farms within the park and on 21% of farms outside the park.

With multivariate processing of data (Fig. 1.), it was determined that there was no distinct group of grasslands according to plant surveys, but plant species could be linked with the gradient of soil moisture and nitrogen content of the soils. For the entire area investigated, wet meadows are characteristic, sorted by analysis into moist, moderately fertilised meadows with fritillary, extensive wet meadows with purple moor-grass, extensive wet meadows with purple moor-grass in the first stage of heather encroachment, and grasslands belonging to alliance *Magnocaricion elatae* W. Koch 26. Altogether, we found evidence of 211 plant species, of which 21 are important for nature conservation, and 17 of which are in the red list of vascular plants of Slovenia (Wraber and Skoberne, 1989).

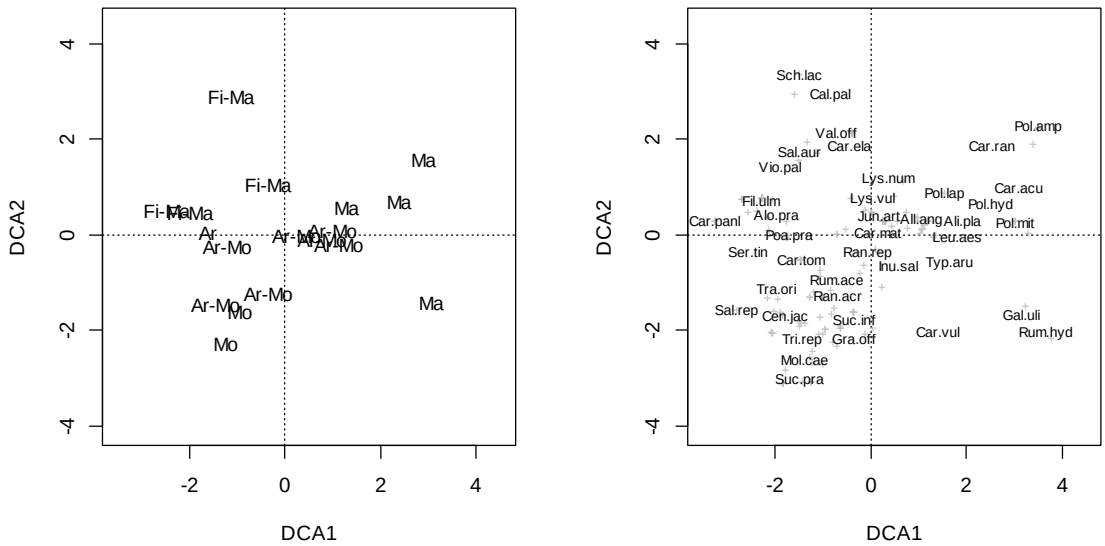

Figure 1: Ordination of vegetation relevees and characteristic plant species of the Radensko polje Landscape Park according to detrended correspondence analysis. Relevees are labeled by their affiliation to main grassland alliances or their transitions (Ar: *Arrhenatherion*, Ar-Mo: *Arrhenatherion-Molinion*, Ma: *Magnocaricion*, Mo: *Molinion*, Fi-Ma: *Filipendulion-Magnocaricion*).

Slika 1: Ordinacija popisov vegetacije in značilnih rastlin po korespondečni analizi z odstranjenim trendom na Krajinskem parku Radensko polje. Popisi so označeni glede na njihovo pripadnost glavnim zvezam združb na traviščih ali prehodov med njimi (Ar: *Arrhenatherion*, Ar-Mo: *Arrhenatherion-Molinion*, Ma: *Magnocaricion*, Mo: *Molinion*, Fi-Ma: *Filipendulion-Magnocaricion*).

4 DISCUSSIONS

Analysis of application of the ERB measure and AE measures on the RPLP area enabled us to estimate the effectiveness of payments devoted for the conservation of extensive agricultural systems, or for the transition from intensive systems to more sustainable ones.

The majority of farms in the RPLP are oriented to animal husbandry, mainly cattle breeding and to a lesser degree to horse breeding. The average LU for the first and second conservation zones of the RPLP was 0.75 LU/ha for agricultural lands in use in 2010. Farmers do not use exceedingly high amounts of mineral fertilisers. Within the first and

second conservation zones, lower use of stable manure is also observed in comparison to the areas that the same farmers cultivate outside the park. Data on annual input of nitrogen in the RPLP show that the Nitrate Directive is respected (Poročilo Slovenije na podlagi 10. člena Direktive sveta 91/676/EEC..., 1991). Cumulatively, these results show that on average the intensity of agriculture in the area is low presently.

Surveys of plant species indicate relatively good state of habitats in the RPLP, proved by the relatively high number of species important for nature conservation.

On the basis of the data gathered regarding the implementation of the ERB and AE measures on the RPLP (analysis of the requirement for the ERB measure, state of implementation of the ERB and AE measures, analysis of mistakes in the implementation of ERB measure, average proportion of permanent grassland to all fodder areas at the implementation of the ERB, reasons for not applying for the measures), we confirmed the assumption that current measures of the agricultural policy do not stimulate farmers to perform more extensive agriculture and are inefficient for the conservation of favourable status of grassland habitats. Park management has done much to inform the farmers about the formation of the RPLP and about the changes the formation would bring to inhabitants. In spite of this, the majority of farmers (53% according to the inquiry) still share the opinion that the park limits the agricultural development in the area.

For the future conservation of the grasslands in the RPLP, further intensification of agriculture should be prevented. More attention should be paid to late mowing and to the encouragement of farmers to recultivate the abandoned areas in an extensive way. For implementation of these plans for the transition from intensively cultivated meadows to more extensive ones, new agri-environmental measures should be considered, being more adapted to the specifics of this area, simpler to implement, and (above all else) providing additional financial means.

Considering the biodiversity of the RPLP, we can conclude that the conservation of habitats in the area is more supported by the traditional use of grasslands (only mowing) than the ERP and AE measures, but the establishment of the landscape park also helped to keep the traditional land use. Too short a time has elapsed since the establishment of the RPLP and start of the implementation of agro-environmental measures to detect significant changes or effects on grasslands. Certain signs of intensification (overly early moving, use of stable manure) represent threats to the existing good condition of the protected habitats and species; therefore, the started measures should continue and should be improved in both sides, e.g. to keep the existing biodiversity and enable farmers sustainable management of their land.

5 REFERENCES

ARSKTRP-SBV-OBR-PROD 2007 do 2011. 2012. Ljubljana, Agencija RS za kmetijske trge in razvoj podeželja (osebni vir, izpis iz baze podatkov, jan. 2012).

Batič F., Eler K., Šircelj H., Vidrih M. 2002. Biotska pestrost in kmetijstvo – stanje v Sloveniji, razvoj kazalnikov in monitoring. V: Novi izzivi v poljedelstvu. Zbornik simpozija. Zreče, 5. in 6. december 2002. Ljubljana: 206–210.

Beaufoy G., Baldock D., Clark J. 1994. The Nature of farming: Low intensity farming system in nine European countries. London, Institute for European Environmental policy: 66 str.

Bignal E. M., McCracken D. I. 1996. Low intensity farming system in the conservation of the countryside. Journal of Applied Ecology 33: 413–424.

Brak B. H., Hilarides L., Elbersen B. S., van Wingerden W. K. R. E. 2004. Extensive livestock systems and biodiversity: The case of Islay. Wageningen, Alterra, Alterra raport 1100: 85 str.

Braun-Blanquet, J. 1964. Pflanzensoziologie, Grunzüge der Vegetations kunde. 3. Neu bearb. Aufl. – Springer. Berlin, Wien, New York, 865str.

Buckingam D. L., Peach W. J. 2007. Grassland habitat requirement for bird conservation in relation to the needs of other interest Groups. V: High value

grassland: providing bidiversity, a clean environment and premium products. Occasional Symposium No. 38, British Grassland Society, Keele University, Staffordshire, UK, 17–19 April 2007. Hopkins J. J., Duncan A. J., McCracken D. I., Peel S., Tallowin J. R. B. (ed.). British Grassland Society: 193–196.

Critchley C. N. R., Martn D., Fowbert J. A., Wright B. 2007. Providing the evidence base to improve the efficacy of management guidelines for upland hay meadows. V: High value grassland: providing bidiversity, a clean environment and premium products. Occasional Symposium No. 38, British Grassland Society, Keele University, Staffordshire, UK, 17–19 April 2007. Hopkins J. J., Duncan A. J., McCracken D. I., Peel S., Tallowin J. R. B. (ed.). British Grassland Society: 129–134.

Dunn R. M., Hopkins A., Buller H., Jones O., Morris C., Wood J. D., Whittington F., Kirwan J. 2007. Can biological diversity act as input into sustainable rural development? A Case-study using salt marsh raised lamb. V: High value grassland: providing bidiversity, a clean environment and premium products. Occasional Symposium No. 38, British Grassland Society, Keele University, Staffordshire, UK, 17–19 April 2007. Hopkins J. J., Duncan A. J., McCracken D. I., Peel S., Tallowin J. R. B. (ed.). British Grassland Society: 158–163.

EEA. Extensive agriculture-definition. 2010. Evropska agencija za okolje. http://glossary.eea.europa.eu/terminology/concept_html?term=extensive%20agriculture (25. avg. 2010).

Gulliver R., Gulliver M., Sydes C., Long D. 2007. The Use of exclosures to produce a favourable grazing regim for the United Kingdom biodiversity action plan (BAP) Orchid, *Spiranthes romanzoffiana*, on Colonsay, Inner Hebrides, Scotland. V: High value grassland: providing bidiversity, a clean environment and premium products. Occasional Symposium No. 38, British Grassland Society, Keele University, Staffordshire, UK, 17–19 April 2007. Hopkins J. J., Duncan A. J., McCracken D. I., Peel S., Tallowin J. R. B. (ed.). British Grassland Society: 229–232.

Hayes M. J., Tallowin J. R. B. 2007. Recreating biodiverse grasslands: long term evaluation of practical management options for farmers. V: High value grassland: providing bidiversity, a clean environment and premium products. Occasional Symposium No. 38, British Grassland Society, Keele University, Staffordshire, UK, 17–19 April 2007. Hopkins J. J., Duncan A. J., McCracken D. I., Peel S., Tallowin J. R. B. (ed.). British Grassland Society: 135–140.

Inventarizacija flore in favne na Radenskem polju. 2000. Miklavž na Dravskem polju, Center za kartografijo favne in flore: 1–50.

IUCN, 2012. IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp. http://www.radenskopolje.si/images/PDF/porocilo.pdf (5. apr. 2011).

Ketiš K. 2010. Vpliv gospodarjenja s travišči na naravovarstveno vrednost teh habitatov v upravni enoti Radlje ob Dravi. Magistrsko delo. Ljubljana, Biotehniška fakulteta, Oddelek za agronomijo: 176 str.

Kramberger B. 1994. Vpliv izkoriščanja na botanično sestavo ruše trajnega travinja. Novi izzivi v poljedelstvu. Kočevje. 7.–8. september 1994. Kotnik T. (ur.), Ljubljana, Biotehniška fakulteta, Oddelek za agronomijo: 209–216.

Marriot C. A., Hood K., Fisher J. M., Pakeman R.J. 2009. Log-term impacts of extensive grazing ad abandonment on the species composition, richness, diversity and productivity of agricultural grassland. Agriculture, Ecosystems ad Environment, 13: 190–200.

Miles J. 1981. Problems in heathland and grass land dynamics. Vegetatio, 46: 61–74.

Nielsen A. L, Debosz K. K. 1994. Botanical composition, yield and herbage quality of swards of different age on organic meadowlands. V: Grassland and society. Proceedings of the 15th General Meeting of the European Grassland Federation, Wageningen, June 6–9. 1994. Mannetje L.'t, Frame J. (ed.). Wageningen, Wageningen Pers: 324–327.

Nösberger J., Lehmann J., Jeangros B., Dietl W., Kessler W., Bassetti P., Mitchley J. 1994. Grassland production systems and nature conservation. V: Grassland and society. Proceedings of the 15th General Meeting of the European Grassland Federation, Wageningen, June 6–9. 1994. Mannetje L.'t, Frame J. (ed.). Wageningen, Wageningen Pers: 255–265.

Nösberger J., Rodriguez M. 1996. Increasing biodiversity through management. V: Grassland and land use systems. Proceedings of the 16th General Meeting of the European Grassland Federation, Grado (Gorizia), September 15–19. 1996. Parente G., Frame J., Orsi S. (ed.). Wageningen, Wageningen Pers: 949–957.

Pomembnejši podatki popisa kmetijstva: Raba zemljišč in število kmetijskih gospodarstev, Slovenija, 2010 – začasni podatki. 2011. Ljubljana, Statistični urad RS (5.10.2011).

http://www.stat.si/novica_prikazi.aspx?id=3818 (10.jan. 2012).

Poročilo Slovenije na podlagi 10.člena Direktive sveta 91/676/EEC, ki se nanaša na varstvo voda pred onesnaževanjem z nitrati iz kmetijskih virov. Ministrstvo za okolje in prostor, Ljubljana, 1991, 1–39

Pregled stanja biotske raznovrstnosti in krajinske pestrosti v Sloveniji. 2001. Ljubljana, Ministrstvo za okolje in prostor, Agencija RS za okolje: 224 str.

Program razvoja podeželja Republike Slovenija za obdobje 2007–2013. 2007. Ljubljana, MKGP: 321. http://www.pora-gr.si/PRP%202007-2013.pdf (15. mar. 2010).

Pust Vučajnk M., Udovč A. 2008. Stališča kmetov ter kmetijskih svetovalcev do Slovenskega kmetijsko-okoljskega programa. V: Novi izzivi v poljedelstvu. Zbornik simpozija. Rogaška Slatina, 4. in 5. december 2008. Rogaška Slatina: 202–207.Suttie J. M., Reynolds S. G., Batello C. 2005. Grasslands of the world. FAO. http://www.fao.org/docrep/008/y8344e/y8344e00.htm (26.09. 2010).

Strokovne podlage za zavarovanje Radenskega polja. 2008. Juran V. Ljubljana, Zavod RS za varstvo narave: 58 str.

Udovč A., Čemažar M. 2002. Ekonomska analiza privlačnosti izbranih ukrepov slovenskega-kmetijsko okoljskega programa. V: Novi izzivi v poljedelstvu. Zbornik simpozija. Zreče, 5. in 6. december 2002. Ljubljana: 230–234.

Uredba Sveta (ES) št. 1782/2003 z dne 29. septembra 2003 o skupnih pravilih za sheme neposrednih podpor v okviru skupne kmetijske politike in o uvedbi nekaterih shem podpor za kmete ter o spremembi uredb (EGS) št. 2019/93, (ES) št. 1452/2001, (ES) št. 1453/2001, (ES) št. 1454/2001, (ES) 1868/94, (ES) št. 1251/1999, (ES) št. 1254/1999, (ES) št. 1673/2000, (EGS) št. 2358/71 in (ES) št. 2529/2001.

Uredba o izvedbi neposrednih plačil v kmetijstvu. Ur. l. RS št. 99/06, 5/07, 49/07, 124/07, 31/08 in 107/08.

Uredba o Krajinskem parku Radensko polje. Ur. l. RS št. 104/11.

Uredba o predpisanih zahtevah ravnanja ter dobrih kmetijskih in okoljskih pogojih. Ur. l. RS št. 34/07.

Wraber, T., Skoberne P. 1989. Rdeči seznam ogroženih praprotnic in semenk SR Slovenije. Varstvo narave 14–15:9–428.

Žvikart M. 2010. Uresničevanje varstvenih ciljev iz programa upravljanja območij Natura 2000 v kmetijski kulturni krajini. Varstvo narave, 10: 21–34; http://www.zrsvn.si/dokumneti/63/2/2010/Zvikart_2231 (10.11.2010).

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Enhanced growth of cabbage and red beet by *Trichoderma viride*

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ABSTRACT

The main agricultural importance of *Trichoderma* species was not so far away mainly suppression of plant diseases. Novel investigations emphasize their potential to stimulate plant growth independently of suppression of any plant disease. However, *Trichoderma* spp. and even biotypes of same species vary between each other in its effectiveness both in the control of plant pathogenic fungi and in the promotion of plant growth and increasing yield. The subject of this study was to evaluate growth promoting effect of two indigenous *T. viride* stains STP16 and STP8 on cabbage and red beet. Increment of fresh and dry weight accomplished by STP16 was statistically significant so, it could be concluded that growth enhancement of 27% at red beet and 29% at cabbage is significant.

Key words: cabbage, plant growth, red beet, *Trichoderma*

IZVLEČEK

POSPEŠENA RAST ZELJA IN RDEČE PESE Z DODATKOM GLIVE *Trichoderma viride*

Agronomski pomen gliv iz rodu *Trichoderma* je bil do nedavnega v zatiranju rastlinskih bolezni. Novejše raziskave poudarjajo njihov potencial vzpodbujanja rasti rastlin neoodvisno od zaviranja katerekoli bolezni. Pospeševanje rasti in povečevanje pridelka kot nadzor patogenih gliv pa se pri vrstah iz rodu *Trichoderma* razlikuje med biotipi iste vrste v učinkovitosti. Predmet te raziskave je bil ovrednotiti učinek na povečanje rasti zelja in rdeče pese dveh samoniklih sojev glive *T. viride*, STP16 in STP8. Povečanje sveže in suhe teže obeh vrtnin je bilo ob prisotnosti seva STP16 statistično značilno, 27 % pri rdeči pesi in 29 % pri zelju.

Ključne besede: zelje, rdeča pesa, rast, *Trichoderma*

1 INTRODUCTION

The members of the genus *Trichoderma* are rhizosphere competent fungi which taking part in the decomposition of plant debris in the soil. But, since the early 1930s when Weidling (1934) reported that *T. lignorum* produce and excretes a "lethal principle" in the surrounding, the scientists become involved in investigation of antifungal ability of various *Trichoderma* species although *T. harzianum* arisen as the most prominent species of the genus. Along with revelation of diverse antifungal mechanisms of *Trichoderma* the ability to promote plant growth, to increase plant height, leaf area and dry weight were perceived. Firstly was this ability treated as side effect of suppression of plant pathogenic fungi (Baker, 1988; Chang at al., 1986; Inbar at al., 1994; Ousley at al., 1994). Other possible explanations of this phenomenon include: control of minor pathogens leading to stronger root growth and nutrient uptake (Ousley at al., 1993), secretion of plant growth regulatory factors such as phytohormones (Windham at al., 1986; Chang at al., 1986; Baker, 1988) and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil (Ousley at al., 1994a). Furthermore, positive influence of *Trichoderma* spp. to a faster germination and increases in percentage of emergency were perceived also (Celar and Valič, 2005; Koch, 2001; Gupta and Sharma, 1995). Recently, it is speculated that *Trichoderma*

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positive effect on plant growth is independent ability and equally remarkable and significant as its antifungal ability because growth enhancement has been observed in the absence of any detectable disease and in sterile soil (Celar and Valič, 2005; Altomare at al., 1999). Therefore, today is considered that the direct effects of these fungi on plant growth and development are crucially important for agricultural uses and for understanding the roles of *Trichoderma* in natural and managed ecosystems.

Metabolic products of various *Trichoderma* strains are no identical and they have selective character to different plant species and even a variety (Celar and Valič, 2005; Gupta and Sharma, 1995). There are numerous papers about positive influence on plant growth of *T. harzianum* and *T. viride* in lettuce, cucumbers and bell peppers (Bal and Altinatas, 2006; Bal and Altinatas, 2008; Poldma at al., 2000; Yedidia at al., 2001) and *T. virens*, *T. tomentosum* and *T. longipile* in cabbage (Rabeendran at al., 2000). In progress are investigations of effectiveness differences between species and biotypes of same species in the plant growth promotion. Seems that may be due to better interaction of some *Trichoderma* species or some biotypes with certain plant species because root exudates may induce or inhibit their mycelial growth (Bal and Altinatas, 2008).

Nowadays are *Trichoderma* species considered as opportunistic plant symbionts because they colonise root surface and even penetrate into the epidermis (outer layer of root tissue) and a few cell layers below this level establishing pseudo-mycorrhizal relationship with plant host (Harman, 2006). This intimate relationship between *Trichoderma* sp. and the host root cells is what induces localized and systemic resistance plant responses to pathogen attack. For the fungus, abundant healthy roots are environment where it grows and proliferates best because plant derived sucrose which is an important resource provided to the *Trichoderma* sp. cells. Furthermore, roots are resort of plant pathogenic fungi and nematodes, the target for *Trichoderma* as micoparasite and nematofag. The plants also benefits from this relationship through increased root and shoot growth and increased macro- and micronutrient uptake. Therefore, *Trichoderma* sp. may be benefit as growth promotant (biofertiliser) as well as pathogen control agent (mycofungicide) and their application may lower the production costs and environmental impact.

The *Trichoderma* potential as biocontrol agent is utilized through the commercial production of. *Trichoderma* – based biofungicides, which account for about 60% of the biofungicides market. The spectra of *Trichoderma* – based products signed as biofertilizer in Croatia has tendency to expand due to the easier registrations. The availability and diffusion of *Trichoderma sp*. based biofertilizers is more widespread then commonly known. Mostly permitted for use in organic farming in Europe are: RootShield, Plant Box and Bio Trek (northern Europe), Binap (Switzerland, Sweden, UK), Bio fungus (Belgium), Supersivit (Czech Republic), Trichodex (Italy), Trifender (Hungary) and Trianum (Avantagro, Spain) (Topolovec-Pintarić at al., 2011; Robson at al., 2007). Today in Croatia only *Trichoderma* based biofertilizer is Trifender (Bioved, Hungary) and is legal to sell from 2009 (distributed by ZKI Sljeme) while, only biofungicide is Trichodex (Makhteshim, Israel) registered as mycofungicide for uses against grey mould in vineyards.

Since 2007 we have conducted investigation of antagonistic ability of indigenous *T. viride* isolates against some of important plant pathogenic fungi like *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Botrytis cinerea*. As two indigenous *T. viride* strains, STP16 and STP8 achieved good antagonistic activity it seemed appropriate to also investigate whether, would have a direct effect on plant growth when no disease pressure was present. The mode of effective dispersal of fungal inocula became an issue.

The development of formulation and delivery systems for antagonistic microorganisms is of great importance in the field of biological control. After rearing beneficial organism in laboratory their reintroduction in rhizosphere can be difficult even if it is soil borne organism such as *T. viride.* Rhizosphere is environment fulfil with spectrum of microbes with whom reintroduced *T. viride* must compete, trying to colonize available sites along the plant roots. Therefore, it need to be applied in low-cost formulation with highly densities inocula and engineer to maintain fungal propagules viable

during the transport, storage and application. To accomplish mentioned goals wet or dry fungal biomass needs to be immobilized within a matrix forming material, such as gelatinized polysaccharide or an oil emulsion. Matrix is serving as carrier of fungal inoculums. Most of the examples on different types of fungal inocula for biological control include peat, granular vermiculite or clay mixtures, grains and alginate pellets. Encapsulation in formulation of alginate pellets has been found to be successful for the delivery of *Trichoderma sp*. (Walker and Connick, 1983; Fravel at al., 1985; Lewis and Papavizas, 1985; Knudsen and Bin, 1990, Nipoti at al., 1990; Leštan and Lamar, 1996; Mafia at al., 2003). In this work we chose to encapsulate fungal inocula into form of alginate pellets.

2 MATERIALS AND METHODS

Two indigenous *Trichoderma viride* Pers. strains were used: STP16 originated from parasited sclerotia of *Athelia rolfsii* while STP8 originated from sclerotia of *Sclerotinia sclerotiorum*. Cultures were maintained at 25 °C on potato dextrose agar (PDA, Biolife, Italy) slants.

The alginate pelletes were prepared as described previously (Topolovec-Pintarić at al., 2011). The culture of isolates were grown on Petri dishes 10 cm in diameter containing 20 ml of PDA and incubated in humid chamber at 25 °C for 7 days until conidiation occurred. After incubation the substrate altogether with hyphal biomass and conidia from two Petri dishes were upraised with spatula and transferred into glass with 50 ml sterile DI water. These were mixed by common blender at low speed for 3-5 min in order to make a suspension. The final concentration to be used contained 4 x 10^6 spore ml^{-1}. The suspension was mixed with 100 g l^{-1} talcum (Kemig, Croatia) and 10 g l^{-1} sodium alginate (Fluka, Switzerland). The formed matrix was then placed in a separator funnel modified in order to allow suspension to dripping into a 0.1 M suspension of calcium gluconate (Kemig, Croatia) under stirring on magnetic agitator. Drops of alginate matrix dripped into calcium gluconate suspension transformed to gelatinized spherules or pellets. Pellets were removed from suspension within 10 min, rinsed with distilled water and allow to dry on waxed paper under a sterile vertical flow for 12 -24 h.

Too sets of experiments (white cabbage (Ditmar) and red beet (Crimson Globe)) were carried out in the plastic tunnel according to randomised complete block design in 5 replications (4 plants per replication). The seedlings were planted in raised beds 250 x 100 cm wide and 250 mm deep edged with scaffold planks and fulfil with sterilized potting compost Potgrond P (Klasmann-Deilmann GmbH, Germany). Pellets of *T. viride* strains STP16 and STP8 were added manually during planting into the root zone of seedlings. The equal number of cabbage and red beet plants was grown without addition of pellets as control. The planting was conducted at the beginning of May. After that first application the pellets were added manually four times more at two weeks intervals in the root zone. Control beds were omitted. The 4 g of pellets were added per plant in each application. Plant protection practices were not applied during the growing period. Mature plants were pullout from the beds 48 days after planting. The roots of plants treated with pellets were rinsed with tap sterile distillate water. In such manner obtained suspension from each test plant were poured onto PDA in the Petri dish. After incubation in humid chamber at 25 °C for five days examination was conducted for *T. viride* presence. All plants were weighed, each cabbage head and red beet root, and after the fresh weight (*FW*) was recorded they were dried to constant weight, dry weight (*DW*). An analysis of variance ($p=0.05$) and t-test were performed on *FW* and *DW* data.

Evaluation of growth promotion by *T. viride* was interpreted as Index of growth and calculated as follows: $I = 100\ (T-C)\ C^{-1}$ where *I* or index growth is percent of growth promotion with respect to control, *C* is plant growth represented with *FW* and *DW* at control and *T* is plant growth represented with *FW* and *DW* in the treatment.

3 RESULTS AND DISCUSSION

Two indigenous *T. viride* strains STP16 and STP8 were evaluated for growth promotion effects in order to confirm the hypothesis that biotypes of same species differ in their abilities to inducing plant growth. The most effective strains will colonize roots and provide benefits for at least the life of annual crops (Harman, 2006). Furthermore, even if the *T. viride* is present only on roots the enhancement of growth; both on the root and on the foliage can be asses (Harman, 2000; Harman, 2004a; Harman, 2004b; Harman, 2004c). Therefore, the trial was set to investigate whether strains would have a direct effect on plant growth when no disease pressure was present, although they showed good antagonisms against soil-borne pathogens in previous investigation. In order to estimate the hypothesis that presence of *T. viride* on root can enhance the foliage we choose cabbage as represent of leafy green vegetables and red beet as root vegetable.

In conducted trial the indigenous *T. viride* isolates STP16 and STP8 enhanced plant growth in only one trial vegetation season. Influence of strains STP16 and STP8 on red beet growth was estimated by weighing the root (table 1). Fresh weight was increased by both isolates, STP16 (*FW* =725 g) and STP8 (*FW* = 607.5 g). There was no statistically significant difference between isolate's influences although STP16 significantly increased fresh weight in comparison to control (*FW* = 569 g) while STP8 did not. Dry weight was greater at STP16 treatment (*DW* = 13.1 g) and statistically significant in comparison to STP8 treatment (*DW* = 12.2 g) and control (*DW* = 11.6 g). Growth-promotion index (*I)* showed that STP16 treatment increased root fresh weight for 27.42% while STP8 treatment for only 6.44%. Index calculated for dry weight showed that STP16 increased dry weight for 12.93% and STP8 for 5.17%.

Table 1: Effect of *Trichoderma viride* strains on red beet yield.

Treatment	Fresh weight (*g*)	SEM	Dry weight (g)	SEM	I_{FW} (%)	I_{DW} (%)
STP16	725[a]	150	13.1[a]	0.218	27.42	12.93
STP8	607.5 [ab]	133	12.2[b]	0.213	6.44	5.17
Control	569 [b]	202	11.6[b]	0.277	–	–

[a, b] Mean in each column, with same letters are not significantly different at $p < 0.05$.
SD = standard deviation: I_{FW} = index of growth promotion for fresh weight; I_{DW} = index of growth promotion for dry weight.

Influence of strains STP16 and STP8 on cabbage growth was estimated by weighing the heads (table 2). Fresh weight was greater at STP16 treatment (*FW* = 1666.5 g) than at STP8 treatment (*FW* = 1372.5 g) but not statistically significant. In comparison to control (*FW* = 1291 g) STP16 and STP8 significantly increased fresh weight. Dry weight was slightly but statistically significant increased at STP16 treatment (*DW* = 8.2 g) against STP8 treatment (*DW* = 7.2 g) which was statistically equal to control (*DW* = 6.3 g). Growth-promotion index showed that STP16 treatment promote fresh weight for 29% while STP8 treatment only for 6.3% and dry weight for 30.16% while STP8 for 14.29%.

Calculation of growth-promoting index allowed comparison of strain's abilities in relation to control. Taking into account that increment of fresh and dry weight accomplished by STP16 was statistically significant it could be concluded that growth enhancement of 27.42% at red beet and 29% at cabbage is significant. Here represented result of *T. viride* influence on cabbage growth differs from previous reports of Rabeendran at al. (2000). They reported that *T. longipile* and *T. tomentosum* promote cabbage growth of seedlings old 28 days but the effect was not maintained through to the 42 days assessment. We consider the discrepancy between here presented results and Rabeendran observations (Rabeendran at al., 2000). as confirmation of speculation that different *Trichoderma* species acting differently on various host plants. Unfortunately, we were unable to compare growth promotion effect of *T. viride* on red beet owing to lack of similar investigation.

Observations here described, different ability of two new *T. viride* autochthonous isolates in enhancing growth of different plant species,

supporting hypothesis that growth promotion of *Trichoderma* is not species dependent as well as that biotypes of same species differ in their abilities for inducing plant growth.

To accomplish introduction of *T. viride* into the rhizosfere, the fungal culture was encapsulated into the alginate pellets. Pellets allowed effective dispersal of fungal inocula into the soil and enable fungal transfer onto the roots of plants. Colonisation of the roots was confirmed with isolation of strains from root of every plant originated from beds amended with pellets. The alginate matrix used for the formation of pellets remained in the soil after the harvesting. With the time the alginate matrix slowly decayed, possibly because the substrate was sterile, without microbiological activity and only the regular watering enables the decomposition of the pellets. Some of the remaining pellets were taken to the laboratory where the isolates STP16 and STP8 were regenerated from the pellets to the PDA medium. Therefore, the alginate pellets can be considered as good formulation for encapsulation and preservation of *T. viride* inocula. Furthermore, alginate pellets shown to be formulation easy for application. Obtained results warrant further investigation toward field application and perhaps commercialization of the product for the market.

Table 2: Effect of *Trichoderma viride* strains on cabbage yield.

Treatment	Fresh weight (*g*)	SEM	Dry weight (g)	SEM	I_{FW} (%)	I_{DW} (%)
STP16	1666.5 [a]	409	8.2[a]	0.184	29	30.16
STP8	1372.5 [a]	484	7.2[b]	0.242	6.3	14.29
CONTROL	1291 [b]	595	6.3[b]	0.293	–	–

[a, b] Mean in each column, with same letters are not significantly different at $p < 0.05$.
SD = standard deviation: I_{FW} = index of growth promotion for fresh weight; I_{DW} = index of growth promotion for dry weight.

4 REFERENCES

Altomare C., Norvell W.A., Björkman T., Harman G.E. 1999. Solubilisation of phosphates and micronutrients by the plant – growth – promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295 – 22. Applied and Environmental Microbiology, 65: 2926-2933

Baker R. 1988. *Trichoderma* spp. as plant stimulants. Critical Reviews in Biotechnology 7: 97-106

Bal U., Altintas S. 2006. Application of the antagonistic fungus *Trichoderma harzianum* (TrichoFlow WPTM) to root zone increases yield of bell peppers grown in soil. Biological Agriculture & Horticulture, 24: 149-163

Bal U., Altintas S, 2008. Effects of *T.harzianum* on lettuce in protected cultivation. Central European Agriculture Journal, 9; 63-70

Celar F., Valic N. 2005. Effects of *Trichoderma* spp. and *Gliocladium roseum* culture filtrates on seed germination of vegetables and maize. Journal of Plant Diseases and Protection, 112, 343–350

Chang Ya-Chun, Chang Yih-Chang, Baker R, Kleifeld O., Chet I. 1986. Increased growth of plants in presence of the biological control agent *Trichoderma harzianum*. Plant Disease, 70: 145-148

Fravel D.R., Marois J.J., Lumsden R.D., Connick W.J.J. 1985. Encapsulation of potential biocontrol agent in an alginate-clay matrix. Phytopathology, 75: 774-777

Gupta O., Sharma N.D. 1995. Effect of fungal metabolites on seed germination and root length of black gram (*Phaseolus mungo* L.). Legume Research. 18**,** 64–66

Harman G.E. 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease, 84: 377-393

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M. 2004a. *Trichoderma* species—Opportunistic, avirulent plant symbionts. Nature Reviews Microbiology, 2: 43-56

Harman G.E., Lorito M., Lynch J.M. 2004b. Uses of *Trichoderma* spp. to remediate soil and water pollution. Advances in Applied Microbiology, 56: 313-330

Harman G.E., Petzoldt R., Comis A., Chen J. 2004c. Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17 and effects of this interaction on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology, 94: 147-153

Harman G.E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology, 96: 190-194

Inbar J., Abramsky M., Cohen D., Chet I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings growth under commercial conditions. European Journal of Plant Pathology, 100: 337-346

Knudsen G.R., Bin L. 1990. Effects of temperature, soil moisture, and wheat bran on growth of *Trichoderma harzianum* from alginate pellets. Phytopathology, 80: 724-727

Koch E. 2001. Effect of biocontrol agents on plant growth in the absence of pathogens. Bull. OILB/SROP, 24**,** 81–89

Leštan D., Lamar R.T. 1996. Development of fungal inocula for bioaugmentation of contaminated soils. Applied and Environmental Microbiology, 62: 2045-2052

Lewis J.A., Papavizas G.C. 1985. Characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the proliferation of the fungi in soil. Plant Pathology, 34: 571-577

Mafia R.G., Alfenas A.C., Maffia L.A., Ventura G.M., Sanfuentes E.A. 2003. Encapsulamento de *Trichoderma inhamatum* para ocontrole biologico de *Rhizoctonia solani* na propagacao clonal de Eucalyptus. Fitopatologia Brasileira, 28**:** 101-105

Nipoti P., Manzali D., Gennari S., D´Ercole N., Rivas F. 1990. Activity of *Trichoderma harzianum* Rifai on the germination of asparagus seeds: I – Seed treatments. Acta Horticulturae, 271: 403-407

Ousley, M.A., Lynch, J.M. and Whipps, J.M., 1993. Effect of *Trichoderma* on plant growth: a balance between inhibition and growth promotion. *Microb. Ecol. 26*:277-285.

Ousley M.A., Lynch J.M., Whipps J.M. 1994. Potential of *Trichoderma* spp. as consistent plant growth stimulators. Biological Fertilization of Soils, 17: 85-90

Ousley, M.A., Lynch, J.M.,Whipps, J.M., 1994a. The effects of addition of *Trichoderma* inocula on flowering and shoot growth of bedding plants. Sciencia Horticulturae, 59: 147-155.

Poldma P., Jaakson K., Merivee A., Albrecht A. 2000. *Trichoderma viride* promotes growth of cucumber plants. In 'Proc. Int. Conf. on Development of Environmentally Friendly Protection in the Baltic Region' Transactions of Estonian Agricultural University, 209: 162-164, Tartu, Estonia, Sept 28-29, 2000 (BIOSIS, UK).

Robson G.D., van West P., Gadd G.M., 2007. Exploitation of fungi. Symposium of the British Mycological Society held at the University of Manchester, September 2005. Cambridge University Press, Cambrideg, UK.

Rabeendran N., Moot D.J., Jones E.E., Stewart A. 2000. Inconsistent growth promotion of cabbage and lettuce from *Trichoderma* isolates. New Zeland Plant Protection, 53: 143-146

Topolovec-Pintarić S., Žutić I., Lončarić I. 2011. Enhancing plant growth with *Trichoderma viride* based pellets. Proceedings papers of the X. Alps-Adria Scientific Workshop, Opatija, Croatia, Novenyterm.60. Suppl.1, pp 177-180. I DOI: 10.1556 ISSN: 0546-8191 (Print) 2060-8543 (Online)

Walker H.L., Connick W.J. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Science, 31: 333-338

Weidling R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lingorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology, 24: 1153-1179

Windham, M.T., Elad, Y.,Baker, R., 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology, 6: 518-521.

Yedidia I., Srivastva A.K., Kapulnik Y., Chet I. 2001. Effects of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant and Soil, 235: 235-242

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The suitability of malolactic fermentation for the Cviček wine

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ABSTRACT

Cviček is a traditional, light red Slovenian wine made by blending the grapes, must or wine of red and white grapevine varieties. The malic acid concentration in Cviček wine is relatively high, so the use of malolactic fermentation (MLF) during its production was studied in two consecutive vintages, particularly with respect to the varieties with which it might be appropriate. The concentrations of organic acids were analysed using HPLC and UV-Vis detection. The simple ranking test and directional difference test were used for sensory assessments. They showed that partial MLF, concerning 15-50% of the wines in the final blend, contributed to a balanced acidity of Cviček. A suitable technique concerned either the MLF of Blaufränkisch or that of Blaufränkisch and white wine together. The MLF of Blauer Kölner or white wine alone was not appropriate. Despite our analysis of only a limited number of bottled wines, it appeared that MLF is already used in Cviček production and the resulting quality is promising.

Key words: wine, acidity, biological deacidification, *Oenococcus oeni*, sensory evaluation

IZVLEČEK

PRIMERNOST UPORABE JABOLČNO MLEČNOKISLINSKE FERMENTACIJE V PRIDELAVI CVIČKA PTP

Cviček PTP je tradicionalno rdečkasto vino, ki ga pridelujejo z mešanjem grozdja, mošta ali vina rdečih in belih sort žlahtne vinske trte. Vsebnost jabolčne kisline v njem je precej velika, zato smo v dveh zaporednih letih proučevali uporabo jabolčno mlečnokislinske fermentacije (JMKF) s poudarkom na izboru sort, ki bi bile primerne za izvedbo JMKF. Vsebnost organskih kislin smo merili s HPLC in UV-Vis detektorjem. Za senzorično ocenjevanje smo uporabili test razvrščanja in test parov. Pokazali smo, da je delna uporaba JMKF (na 15-50 % vina v končni zvrsti) pozitivno vplivala na uravnoteženost kislinske zaznave vina cviček PTP. Primerna sta bila bodisi JMKF vina modra frankinja, bodisi modra frankinja in belo vino skupaj. Uporaba JMKF samo pri vinu žametovka ali belem vinu ni bila primerna. Čeprav smo analizirali le omejeno število stekleničenih vin, se zdi, da pridelovalci že uporabljajo JMKF v pridelavi cvička PTP in kakovost procesa je obetavna.

Ključne besede: vino, kislina, biološki razkis, *Oenococcus oeni*, senzorično ocenjevanje

1 INTRODUCTION

Cviček is a traditional, light red Slovenian wine made by blending the grapes, must or wine of red and white grapevine varieties. Some researches have been already performed on the wine, namely an investigation of the yeast community on grapes and during alcoholic fermentation relative to the three most important grapevine varieties used in its production (Raspor et al., 2002; Raspor et al., 2009). The total acidity (expressed as tartaric acid) in Cviček wine should be between 6.0 to 9.5 g l^{-1}. The addition of acids to must or wine is not allowed (Pravilnik o vinu …, 2006). The level of malic acid in Cviček is relatively high as it tends to be dependent on grapevine variety and climate. However, malolactic fermentation (MLF, also referred to as biological deacidification) has rarely

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been applied during the production of Cviček wine. Davis et al. (1985) considered that it was difficult to describe MLF as being distinctly desirable or undesirable in terms of final wine quality. A grey area exists where the benefits and drawbacks depend on the region of production, grape variety, wine composition, winemaking techniques and style objectives of the winemaker. Chemical deacidification is more common in Cviček production, during which the use of potassium and calcium carbonates and tartrates is allowed.

The most important change that results from the activity of lactic acid bacteria (LAB) in wine is a reduction in wine acidity due to the decarboxylation of L-malic acid to form L-lactic acid. LAB may also influence aroma and flavour through the production of volatile secondary metabolites and modifications to grape and yeast-derived metabolites (Davis et al., 1985). Laaboudi et al. (1995) demonstrated differences between young MLF and non-MLF wines resulting from the lower acidity level of MLF wines, but their flavour was not significantly modified. However, certain LAB also degrade citric acid into various products of metabolism, amongst which acetoin compounds, diacetyl, acetoin and 2,3-butanediol play important roles. A moderate diacetyl content is desirable in wine; however, excessive acetic acid, the synthesis of glucane, biogenic amines and ethyl carbamate precursors are not (Beneduce et al., 2010; Liu, 2002; Lonvaud-Funel, 1999). In terms of the colour of red wines, the concentrations in monomeric anthocyanins were shown to fall during MLF (Garcia-Falcon et al., 2007; Mazza et al., 1999). However, García-Falcón et al. (2007) confirmed the absence of a correlation between changes in colour density and monomeric anthocyanins content in two young Spanish red wines during a year of storage; suggesting that co-pigmentation and polymerization with other phenolic compounds prevailed over pigment degradation. Among other factors, the colour of anthocyanins is dependent on the pH of the wine (Brouillard et al., 1978; Vivar-Quintana et al., 2002), which rises during MLF. LAB also consume pyruvic acid and thereby limit the production of vitisin A, which forms from pyruvic acid and malvidin-3-glucoside and contributes to colour stability (Asenstorfer et al., 2003). Together with the fact that monomeric anthocyanins may account for more than 85% of total anthocyanins in Cviček wine, considerable caution should therefore be adopted when applying MLF.

Of the chromatographic methods, liquid chromatography (HPLC) is the most widely employed to determine the organic acids present in wine (Lopez and Gomez, 1996; Mato et al., 2005) and it was also used in our study. Attribute difference tests are used for the sensory evaluation of a single attribute, e.g. acidity, by comparing one sample with one or more others. Various tests should be used in this context, depending on the number of samples under analysis (Meilgaard et al., 1999).

During a two-year experiment, the use of MLF was studied in the production of Cviček wine, particularly with respect to the main grapevine varieties used and, consequently, the proportions in the final blend. Furthermore, the use of MLF at an industrial scale was investigated through the analysis of organic acids and sensorial evaluations of bottled Cviček wines.

2 MATERIALS AND METHODS

2.1 Biological deacidification

Eighteen, 25-litre samples of wine, vintage 2008 were collected after alcoholic fermentation and transferred to our microvinification cellar. Three of the main wines used to produce Cviček: a white wine (a blend of different white grapevine varieties, containing a majority of ´Königstraube´ and ´Welschriesling´), ´Blaufränkisch´ (red wine) and ´Blauer Kölner´ (red wine) were sampled in duplicate at their sites of production (three producers). Inoculated MLF was applied to one of these duplicate samples: *Oenococcus oeni* commercial strain VP 41 (Lallemand Inc., Rexdale, Ontario, Canada) was used (10 mg l^{-1}) and the temperature of the wine was maintained at between 20 °C and 22 °C for 10-14 days. For the other sample of each wine, free SO_2 was maintained at 20-30 mg l^{-1}. The experimental procedure described for 2008 was only completed

in six wines of 2009 vintage (one producer). MLF was performed as described above, except for Blauer Kölner wine.

2.2 Determining the concentrations of organic acids

MLF was followed by the identification and quantification of citric, lactic, malic and tartaric acids in the wines, using the reference method with an Agilent 1100 HPLC with a DAD detector (Agilent Technologies, Palo Alto, USA). The wine was filtered prior to injection using a Minisart® RC25 syringe filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany) with a pore size of 0.45 µm. The organic acids were separated using an Aminex HPX-87H ion-exchange resin column, with dimensions of 300 x 7.8 mm i.d. (Bio-Rad Laboratories, Hercules, CA) and detected with UV-Vis absorption spectroscopy at 210 nm. An isocratic technique was used with sulphuric acid (0.0125 mmol l^{-1}) as the mobile phase at a constant flow rate of 0.5 ml min^{-1}. The injection volume was 10 µl, the column temperature 65 °C and the time of analysis 30 min. Quantification was performed by comparing the peak areas of the sample and those of the external standards in a linear relationship (Lopez and Gomez, 1996; OIV, 2013).

2.3 Sampling of bottled wines

Twenty bottled Cviček wines of the 2009 vintage were sampled at their production sites. After the determination of organic acids, only twelve samples with different concentrations of malic and lactic acids were subjected to sensory evaluation with respect to their acidic taste. The purpose was to examine the use of MLF at an industrial scale and to verify the results obtained during microvinification experiments relative to the preference for the acidic taste of wines with or without partial MLF.

2.4 Blending the wine and chemical analysis

During our experiments, the proportions of each grapevine variety in Cviček wines were as follows: 50% ´Blauer Kölner´, 35% white wine and 15% ´Blaufränkisch´. After five months of wine maturation of 2008 vintage, four different blends containing different proportions of biologically deacidified wines were prepared for each producer (3x4 wines): C - control, none of the base wines underwent MLF; W - white wine which underwent MLF (35% of the final blend); BW - ´Blaufränkisch´ and white wine which underwent MLF (50% of the final blend); K - ´Blauer Kölner´ which underwent MLF (50% of the final blend).

Concerning the total acidity of the wines and the results of sensory evaluations in 2008, it was once again decided to include 15-50% of biologically deacidified wines in the blends of the vintage 2009, but not to use MLF for the ´Blauer Kölner´ wine. The proportions of each grapevine variety (´Blauer Kölner´, white wine and ´Blaufränkisch´) in the final blend were the same as in the previous vintage. After five months of maturation, four different blends containing different proportions of biologically deacidified wines were prepared (1x4 wines): C – control, none of the wine underwent MLF; B - ´Blaufränkisch´ which underwent MLF (15% of the final blend; this blend was not prepared in the previous vintage); W – white wine which underwent MLF (35% of the final blend); BW - ´Blaufränkisch´ and white wine which underwent MLF (50% of the final blend).

The levels of organic acids were measured in the wines before and after blending using the reference method described above. The total acidity and pH of the wines were determined using the reference methods published by the OIV (2013). The colour of all wines was evaluated by sensory evaluation alone.

2.5 Sensory evaluation

Sensory evaluations of 2008 vintage were performed in our sensory unit by two groups of assessors: one representing wine experts experienced in the sensory evaluation of Cviček (12 assessors) and the second representing consumers (12 assessors also). All wines were served at 12 °C. Ranking tests were used for the following attributes: colour, flavour and acidity. Both panels evaluated three series of four wines. The three series represented the different producers (1, 2, and 3) and the four wines represented the different blends of Cviček (C, W, BW, and K) described above. The assessors ranked the wines from the best – liked extremely (grade 1) to the most inferior – disliked extremely (grade 4) for each of the attributes (Koak et al., 2010). The procedure was applied separately for each attribute, using new samples that were coded

differently. The panelists assessed the samples in random order (Meilgaard et al., 1999).
Wines from 2009 vintage were evaluated using a paired comparison test (two-sided) on three pairs of samples: one wine in each pair was a control (C) and the other was one of the blends described above (B, W, and BW). The samples were offered simultaneously to all subjects who were asked to decide which sample they preferred relative to the attribute being tested (colour, flavour, and acidity). The procedure was carried out separately for each attribute, using new samples that were coded differently. The number of respondents was 13 (p_{max} = 75%, α = 0.10 and β = 0.50). The minimum numbers of correct responses required for significance at the stated α-level for the corresponding number of respondents were taken from the table (Meilgaard et al., 1999).

The acidity of the 12 samples of bottled Cviček wines from the 2009 vintage was also evaluated. Eleven assessors (experts) in the sensory panel scored the wines according to their acidic taste on a scale of 1 to 3, where 1 = poor, 2 = good, 3 = excellent (Meilgaard et al., 1999).

2.6 Statistical analysis

The results of all sensory evaluations were analysed statistically using the Friedman analysis and the tables for the critical number of correct responses for the directional difference test. The acidity scores for bottled Cviček wines were evaluated by ANOVA using a randomized block design and an LSD multiple comparison procedure at the 95% confidence level (Meilgaard et al., 1999).

3 RESULTS AND DISCUSSION

During this two-year experiment, the use of MLF in Cviček production was studied, particularly with respect to the varieties used and consequently their proportions in the final blend enabling its application.

3.1 2008 vintage

The total acidity, pH, lactic and malic acid levels of the white wine, Blaufränkisch and Blauer Kölner before and after MLF and after blending (C, W, BW and K blends) are shown in Table 1, 2, and 3 (Producers 1, 2, and 3). The total acidity of the control blends ranged from 6.93 g l^{-1} (Producer 1) to 7.91 g l^{-1} (Producer 3). The choice of wine samples from the 2008 vintage was indeed excellent because the total acidity of control blends had a range of almost 1.0 g l^{-1}. The total acidity of Cviček wine with MLF of Blauer Kölner (K - Producer 1) was lower than the lower limit fixed by the regulations (6 g l^{-1}). Together with the increased proportion of biologically deacidified wine in the final blends, the total acidity of the wines decreased; in particular the difference between the controls (C) and wines with MLF of Blauer Kölner (K) ranged from 1.34 (Producer 2) to 1.59 g l^{-1} (Producer 3). Consequently, the pH values of the blends with MLF of Blauer Kölner rose from 0.07 (Producer 2) to 0.12 (Producers 1 and 3). At the same time, the malic acid levels fell and those of lactic acid rose.

Table 1: Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine, Blaufränkisch, Blauer Kölner, and in the different blends of 2008 Cviček wines – Producer 1. The results of sensory evaluations of the different blends are also shown

Producer 1	*Wines for blending*					
	White wine (without MLF)	White wine (with MLF)	Blaufränkisch (without MLF)	Blaufränkisch (with MLF)	Blauer Kölner (without MLF)	Blauer Kölner (with MLF)
Before MLF						
Total acidity (g l^{-1} of tartaric acid)	7.21	7.18	7.17	7.08	8.93	9.01
pH	3.37	3.37	3.42	3.42	3.23	3.22
Malic acid (g l^{-1})	3.75	3.71	2.91	2.73	3.53	3.04
Lactic acid (g l^{-1})	0.26	0.26	0.30	0.42	0.23	0.15
After MLF						
Malic acid (g l^{-1})	2.96	0.05	2.28	0.19	3.69	0.24
Lactic acid (g l^{-1})	0.20	2.37	0.40	2.05	0.33	2.00

	Cviček – blends with and without partial MLF[1)]			
	C	W	BW	K
Chemical parameter				
Total acidity (g l^{-1} of tartaric acid)	6.93	6.34	6.32	5.51
pH	3.30	3.33	3.32	3.42
Malic acid (g l^{-1})	2.81	1.86	1.70	1.04
Lactic acid (g l^{-1})	0.63	1.16	1.27	1.80
Sensory attributes	*Panel 1 (experts; n = 12) - Rank sum*			
Colour[2]	20 (the best)	22	32	46 (the most inferior)
Flavour[2]	24	21 (the best)	28	47 (the most inferior)
Acidity[2]	25	24 (the best)	27	44 (the most inferior)
	Panel 2 (consumers; n = 12) - Rank sum			
Colour[2]	19 (the best)	30	30	41 (the most inferior)
Flavour[2]	19 (the best)	36 (the most inferior)	29	36 (the most inferior)
Acidity[2]	22 (the best)	32	27	39 (the most inferior)

[1)] C – control blend (all wines without MLF); W – white wine with MLF (35% of final blend); BW – white wine and Blaufränkisch with MLF (50% of final blend); K – Blauer Kölner with MLF (50% of final blend)
[2)] Samples differing significantly regarding that parameter. The critical value (LSD_{rank}) of the multiple comparisons was 12.4. Any two samples whose sums differed by more than 12 were rated significantly different at $\alpha = 0.05$

Table 2: Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine, Blaufränkisch, Blauer Kölner, and in the different blends of 2008 Cviček wines – Producer 2. The results of sensory evaluations of the different blends are also shown

Producer 2	*Wines for blending*					
	White wine (without MLF)	White wine (with MLF)	Blaufränkisch (without MLF)	Blaufränkisch (with MLF)	Blauer Kölner (without MLF)	Blauer Kölner (with MLF)
Before MLF						
Total acidity (g l^{-1} of tartaric acid)	9.38	9.32	7.79	7.90	8.54	8.76
pH	3.17	3.18	3.40	3.43	3.23	3.24
Malic acid (g l^{-1})	4.72	4.81	2.93	3.17	2.92	3.25
Lactic acid (g l^{-1})	0.15	0.16	0.19	0.27	0.14	0.14
After MLF						
Malic acid (g l^{-1})	4.44	0.10	2.40	0.21	3.31	0.25
Lactic acid (g l^{-1})	0.13	3.45	0.24	1.85	0.36	2.48

	Cviček – blends with and without partial MLF[1)]			
	C	W	BW	K
Chemical parameter				
Total acidity (g l^{-1} of tartaric acid)	7.61	6.46	6.37	6.27
pH	3.24	3.33	3.33	3.31
Malic acid (g l^{-1})	3.29	1.83	1.70	1.54
Lactic acid (g l^{-1})	0.53	1.57	1.71	1.80
Sensory attributes	*Panel 1 (experts; n = 12) - Rank sum*			
Colour[2)]	14 (the best)	30	30	46 (the most inferior)
Flavour	26 (the best)	30	26 (the best)	38 (the most inferior)
Acidity[2)]	35	23	22 (the best)	40 (the most inferior)
	Panel 2 (consumers; n = 12) - Rank sum			
Colour[2)]	22 (the best)	30	26	42 (the most inferior)
Flavour	24 (the best)	31	29	36 (the most inferior)
Acidity	27 (the best)	31	29	33 (the most inferior)

[1, 2)] *See notes to Table 1*

Table 3: Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine, Blaufränkisch, Blauer Kölner, and in the different blends of 2008 Cviček wines – Producer 3. The results of sensory evaluations of the different blends are also shown

Producer 3	*Wines for blending*					
	White wine (without MLF)	White wine (with MLF)	Blaufränkisch (without MLF)	Blaufränkisch (with MLF)	Blauer Kölner (without MLF)	Blauer Kölner (with MLF)
Before MLF						
Total acidity (g l^{-1} of tartaric acid)	9.43	9.40	8.34	8.31	9.00	9.02
pH	3.14	3.16	3.29	3.30	3.25	3.24
Malic acid (g l^{-1})	4.91	5.12	2.97	3.12	3.64	3.90
Lactic acid (g l^{-1})	0.19	0.28	0.20	0.26	0.27	0.17
After MLF						
Malic acid (g l^{-1})	4.70	0.06	2.14	0.30	3.80	0.12
Lactic acid (g l^{-1})	0.10	3.48	0.30	1.31	0.37	2.60

	Cviček – blends with and without partial MLF[1)]			
	C	W	BW	K
Chemical parameter				
Total acidity (g l^{-1} of tartaric acid)	7.91	6.63	6.57	6.32
pH	3.20	3.30	3.29	3.32
Malic acid (g l^{-1})	3.44	2.17	1.97	1.58
Lactic acid (g l^{-1})	0.47	1.49	1.64	1.92
Sensory attributes	*Panel 1 (experts; n = 12) - Rank sum*			
Colour[2)]	25	21 (the best)	31	43 (the most inferior)
Flavour	30	27 (the best)	27 (the best)	36 (the most inferior)
Acidity[2)]	35	22 (the best)	23	40 (the most inferior)
	Panel 2 (consumers; n = 12) - Rank sum			
Colour[2)]	30	25	20 (the best)	45 (the most inferior)
Flavour	27 (the best)	27 (the best)	30	36 (the most inferior)
Acidity[2)]	33	27	21 (the best)	39 (the most inferior)

[1, 2)] *See notes to Table 1*

The results of sensory evaluations with respect to wine colour, flavour and acidity are shown in Tables 1, 2, and 3. A lower score means a higher quality for the attribute evaluated.

Colour. Panel 1 (experts) determined the colour of blends containing biologically deacidified Blauer Kölner (K – all three producers) as being inferior to all the other blends (Tables 1, 2, and 3). At the same time, the control blends (C) had a better colour than blends with MLF of Blaufränkisch and white wine (BW) (Producer 1) or blends containing either white wine with MLF (W) or Blaufränkisch and white wine together (BW) (Producer 2). Panel 2 (consumers) also found the colour of K blends to be inferior to the other blends (Producers 2 and 3), while for Producer 1, only the colour of the control blend was significantly better.

Flavour. Panel 1 found the flavour of the K blend to be inferior to that of the other blends (Producer 1), while the blend from Producer 2 scored significantly lower than the control and BW blend. The same trend towards lower scores (by both panels) for the K blend could be also seen for Producer 3. Panel 2 found the flavour of K blend to

be significantly inferior for Producers 1 and 2 when compared to the control. For Producer 1, the W blend also received a significantly lower score than the control.

Acidity. Panel 1 ranked the acidity of the W and BW blends as being better than the control and K blends (Producers 2 and 3). For producer 1, the K blend was evaluated as being inferior to the other three blends in terms of acidity. Panel 2 scored the acidity of the control and BW blend higher than the K blend (Producer 1). This panel did not recognize any differences in acidity between the wines from Producer 2. For Producer 3, the W and BW blends displayed a more appropriate acidity than the K blend. At the same time, the consumers also ranked the acidity of the BW blend as being better than the control.

3.2 2009 vintage

The total acidity, pH, lactic and malic acid levels of the two replicates of white wine and Blaufränkisch (before and after MLF) and one replicate of Blauer Kölner, are shown in Table 4. These parameters were also determined for Cviček wines after blending (C, B, W, and BW). The total acidity in the blends fell from 6.40 g l^{-1} (C) to 5.60 g l^{-1} (BW). The total acidity of the W and BW blends were lower than the limit fixed by the regulations (6 g l^{-1}), but a sensory comparison with the control blend was nonetheless performed. By increasing the proportion of biologically deacidified wine in the final blends, the pH of the wines rose from 3.15 (C) to 3.22 (BW); malic acid levels fell, while lactic acid levels rose.

Table 4: Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine and Blaufränkisch and one of Blauer Kölner, respectively, and in the different blends of 2009 Cviček wines. The results of sensory evaluations comparing the control and other blends are also shown

	Wines for blending				
	White wine (without MLF)	White wine (with MLF)	Blaufränkisch (without MLF)	Blaufränkisch (with MLF)	Blauer Kölner (without MLF)
Before MLF					
Total acidity (g l^{-1} of tartaric acid)	8.01	8.04	8.21	8.28	7.52
pH	3.18	3.18	3.25	3.24	3.28
Malic acid (g l^{-1})	3.59	3.57	2.23	2.36	1.83
Lactic acid (g l^{-1})	0.23	0.29	0.25	0.22	0.22
After MLF					
Malic acid (g l^{-1})	3.50	0.60	2.05	0.10	1.73
Lactic acid (g l^{-1})	0.20	1.95	0.30	1.76	0.27

	Cviček – blends with and without partial MLF[1)]			
	C	B	W	BW
Chemical parameter				
Total acidity (g l^{-1} of tartaric acid)	6.40	6.10	5.70	5.60
pH	3.15	3.16	3.21	3.22
Malic acid (g l^{-1})	2.30	2.06	1.01	0.80
Lactic acid (g l^{-1})	0.24	0.47	0.85	1.16

Sensory attributes	*Panel 1 (experts; n = 13) – Number of preferred choices in directional difference test*					
	C	B	C	W	C	BW
Colour	6	7	3	10b[2)] (better)	8	5
Flavour	6	7	5	8	6	7
Acidity	3	10b (better)	3	10b (better)	3	10b (better)
	Panel 2 (consumers; n = 13) - Number of preferred choices in directional difference test					
	C	B	C	W	C	BW
Colour	7	6	10 (better)	3b	5	8
Flavour	6	7	10 (better)	3b	6	7
Acidity	2	11a (better)	11 (better)	2a	5	8

[1)] C – control (without MLF); B – Blaufränkisch with MLF (15% of final blend); W – white wine with MLF (35% of final blend); BW – white wine and Blaufränkisch with MLF (50% of final blend)

[2)] The samples differed significantly from the control for the parameter at $\alpha = 0.05$ (a) or $\alpha = 0.10$ (b), respectively. Higher number of preferred choices denotes better wine for that sensory attribute

The results of sensory evaluations by both panels are shown in Table 4. A higher number of preferred choices in the directional difference test indicated a higher quality of the parameter being assess in the chosen wine. Parameter quality differed significantly when 10 (10% level) or 11 (5% level) assessors preferred the same blend.

Colour. Panel 1 assessed the colour of the W blend as being significantly better than the colour of the control, in complete contrast with the findings of panel 2.

Flavour. Panel 1 did not distinguish between the flavour of blends with partial MLF and the control. By contrast, panel 2 evaluated the flavour of the W blend as being significantly worse.

Acidity. Panel 1 assessed the acidity of blends with partial MLF (B, W and BW) as being significantly better than the control. Panel 2 assessed the acidity of the B blend as being significantly better, but the W blend as being significantly worse, than the control.

Based on the results obtained with the vintage 2008, it could be seen that if the total acidity of the

control blend was low (Producer 1), then the use of MLF was not necessary. As the acidity rose in control wines, the acidity of blends involving either the MLF of white wine, or of white wine and Blaufränkisch together, was preferred by the experts, a finding that was confirmed for the 2009 vintage. However, this preference was not clearly confirmed by the consumers in either 2008 or 2009. It was certainly possible to confirm that the MLF of Blauer Kölner was not appropriate to Cviček production as it caused significant changes to the colour, flavour and acidity of the wine. As for the MLF of white wine alone, a cautious attitude should also be adopted, because the consumer panel did not prefer that blend.

3.3 Analysis of bottled Cviček wines

The malic and lactic acid contents and the results of sensory evaluations of wine acidity by the panel of 11 experts are shown in Table 5. Some correlation between the concentrations of malic or lactic acid, respectively, and the sensory scores for wine acidity, could be seen. The preference for an acidic taste increased as the malic acid content in the wine fell to a concentration of 0.76 g l^{-1} in sample 10, which obtained significantly the highest sensory score. For the two samples with lower malic acid content, the sensory scores were lower again.

Table 5: Malic and lactic acid levels and the results of acidity scores for 12 bottled Cviček wines (2009 vintage)

Wine sample	1[1)]	2	3	4	5	6	7	8	9	10	11	12
Organic acids												
Malic (g l^{-1})	2.08	2.06	1.98	1.97	1.87	1.85	1.72	1.49	1.10	0.76	0.53	0.42
Lactic (g l^{-1})	0.17	0.62	0.98	0.33	0.49	0.64	0.29	0.83	0.77	0.90	1.73	1.67
Sensory attribute												
Acidity (1-3)	2.00a[2)]	2.00a	2.18a	2.00a	2.00a	2.09a	2.36ab	2.27ab	2.27ab	2.73b	2.09a	2.18a

[1)] Samples are presented in order from the highest to the lowest concentration of malic acid in the wine. Eleven experts in the sensory panel scored the wines according to the suitability of their acidic taste on a scale of 1 to 3, where 1 = poor, 2 = good, 3 = excellent

[2)] Mean scores followed by the different letters are significantly different at the 95% confidence level – $LSD_{95\%}$ = 0.48

The analysis of organic acids in bottled wines showed that some producers applied MLF in the production of Cviček in 2009. Its positive influence on wine acidity, as shown in microvinification experiments, was confirmed, while sample 10 of bottled Cviček received the best score; this contained malic and lactic acid levels that were very similar to those in our blend involving the MLF of Blaufränkisch and white wine together in 2009.

4 CONCLUSIONS

It was possible to conclude that a limited use of MLF in Cviček production, namely concerning 15-50% of the wine in the final blend, exerted a positive impact on its acidity. The higher the expected total acidity in the final blend, the greater the proportion of base wines that could be biologically deacidified. MLF should be used on either Blaufränkisch or on Blaufränkisch and white wine together. The upper limit of total acidity at which MLF is considered to be necessary is dependent on each vintage. In this case, measuring the concentration of malic acid could also be used, since we determined its concentration to be inappropriate if it exceeded 3.0 g l^{-1} (2008) or 2.0 g l^{-1} (2009) in the final blend. However, it is clear that MLF does not only modify the acidic taste; colour and flavour attributes are also affected (Liu, 2002; Lonvaud-Funel, 1999), as was also shown by our study. Consequently, producers should be cautious when considering the use of MLF, because an excessive rise in the pH negatively influences the colour of light red wines

(Brouillard et al., 1978; Vivar-Quintana et al., 2002). MLF is not always beneficial and may cause undesirable changes to the sensory properties of wine. Greater attention therefore needs to be paid to managing the MLF with respect to the production of diacetyl and other metabolites that might influence the aroma, flavour and healthiness of the wine (Bartowsky, 2009; Bauer and Dicks, 2004). However, despite our analysis of only a limited number of bottled wines, it appeared that MLF is already used in Cviček production and the resulting quality is promising.

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6 REFERENCES

Asenstorfer R.E., Markides A.J., Iland P.G., Jones G.P. 2003. Formation of vitisin A during red wine fermentation and maturation. Australian Journal of Grape and Wine Research, 9, 1: 40-46

Bartowsky E.J. 2009. Bacterial spoilage of wine and approaches to minimize it. Letters in Applied Microbiology, 48, 2: 149-156

Bauer R., Dicks L.M.T. 2004. Control of malolactic fementation in wine. A review. South African Journal for Enology and Viticulture, 25, 2: 74-88

Beneduce L., Romano A., Capozzi V., Lucas P., Barnavon L., Bach B., Vuchot P., Grieco F., Spano G. 2010. Biogenic amine in wines. Annals of Microbiology, 60, 4: 573-578

Brouillard R., Delaporte B., Dubois J.E. 1978. Chemistry of anthocyanin pigments. 3. Relaxation amplitudes in pH-jump experiments. Journal of the American Chemical Society, 100, 19: 6202-6205

Davis C.R., Wibowo D., Eschenbruch R., Lee T.H., Fleet G.H. 1985. Practical Implications of Malolactic Fermentation: A Review. American Journal of Enology and Viticulture, 36, 4: 290-301

Garcia-Falcon M.S., Perez-Lamela C., Martinez-Carballo E., Simal-Gandara J. 2007. Determination of phenolic compounds in wines: Influence of bottle storage of young red wines on their evolution. Food Chemistry, 105, 1: 248-259

Koak J.H., Kang B.S., Hahm Y.T., Park C.S., Baik M.Y., Kim B.Y. 2010. Blending of Different Domestic Grape Wines Using Mixture Design and Optimization Technique. Food Science and Biotechnology, 19, 4: 1011-1018

Laaboudi I., Sauvageot F., Gerbaux V. 1995. Effect of Malolactic Fermentation on Flavour in Young Wines. Sciences des Aliments, 15, 3: 251-260

Liu S.Q. 2002. Malolactic fermentation in wine – beyond deacidification. Journal of Applied Microbiology, 92, 4: 589-601

Lonvaud-Funel A. 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie Van Leeuwenhoek, 76, 1-4: 317-331

Lopez E.F., Gomez E.F. 1996. Simultaneous determination of the major organic acids, sugars, glycerol, and ethanol by HPLC in grape musts and white wines. Journal of Chromatographic Science, 34, 5: 254-257

Pravilnik o vinu z oznako priznanega tradicionalnega poimenovanja – cviček. Ur. l. RS, št. 3/2000: 286-288

Mato I., Suarez-Luque S., Huidobro J.F. 2005. A review of the analytical methods to determine organic acids in grape juices and wines. Food Research International, 38, 10: 1175-1188

Mazza G., Fukumoto L., Delaquis P., Girard B., Ewert B. 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. Journal of Agricultural and Food Chemistry, 47, 10: 4009-4017

Meilgaard M., Civille G.V., Carr B.T. 1999. Sensory evaluation techniques. Boca Raton etc., CRC Press: 99-121

OIV. 2013. Compendium of International Methods of Analysis of Wine and Musts. Vol 1. 2013 Ed. Paris, Organisation International de la Vigne et du Vin: 498 str.

Raspor P., Čuš F., Jemec K.P., Zagorc T., Čadež N., Nemanič J. 2002. Yeast population dynamics in spontaneous and inoculated alcoholic fermentations of Zametovka must. Food Technology and Biotechnology, 40, 2: 95-102

Raspor P., Milek D.M., Polanc J., Možina S.S., Čadež N. 2006. Yeasts isolated from three varieties of grapes cultivated in different locations of the Dolenjska vine-growing region, Slovenia. International Journal of Food Microbiology, 109, 1-2: 97-102

Vivar-Quintana A.M., Santos-Buelga C., Rivas-Gonzalo J.C. 2002. Anthocyanin-derived pigments and colour of red wines. Analytica Chimica Acta, 458, 1: 147-155

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The impact of environmental factors on the infection of cereals with *Fusarium* species and mycotoxin production – a review

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ABSTRACT

Several phytopathogenic *Fusarium* species occurring worldwide on cereals as causal agents of 'head blight' (scab) of small grain cereals and 'ear rot' of maize, are capable of accumulating, in infected kernels, several mycotoxins some of which of notable impact to human and animal health. *Fusarium graminearum, F. culmorum*, *F. poae*, *F. avenaceum* and *Microdochium nivale* predominantly cause *Fusarium* diseases of small-grain cereals. Maize is predominantly attacked by *F. graminearum*, *F. moniliforme*, *F. proliferatum* and *F. subglutinans*. The review is focused on the influence of climatic variables, particularly temperature, humidity and rainfall on growth, reproduction, survival, competitive ability, mycotoxicity and pathogenicity of *Fusarium* fungi commonly isolated from wheat, barley and maize.

Key words: *Fusarium* spp., mycotoxins, smal grain cereals, maize, climatic factors

IZVLEČEK

VPLIV OKOLJSKIH DEJAVNIKOV NA OKUŽBO ŽIT Z GLIVAMI *FUSARIUM* SPP. IN TVORBO MIKOTOKSINOV – PREGLEDNI ČLANEK

Številne fitopatogene glive rodu *Fusarium*, ki povzročajo plesnivost klasov žit in koruznih storžev, je sposobnih v okuženih zrnih akumulirati številne mikotoksine, med katerimi so nekateri škodljivi za zdravje ljudi in živali. Žita prvenstveno okužujejo vrste *Fusarium graminearum, F. culmorum*, *F. poae*, *F. avenaceum* in *Microdochium nivale,* medtem ko koruzo *F. graminearum*, *F. moniliforme*, *F. proliferatum* in *F. subglutinans*. V pregledu je poudarek na vplivu vremenskih dejavnikov (temperatura, vlaga in padavine) na rast, razmnoževanje, preživetje, tekmovalno sposobnost, mikotoksičnost in patogenost *Fusarium* vrst, običajno izoliranih iz pšenice, ječmena in koruze.

Ključne besede: *Fusarium* spp., mikotoksini, strna žita, koruza, klimatski dejavniki

1 INTRODUCTION

Fusarium is a common mould in cereal fields. The infestation (superficial contamination) and infection of *Fusarium* in cereals are of great concern worldwide – as plant pathogens and producers of mycotoxins.

The genus *Fusarium* comprises a diverse array of fungi, members of which are phytopathogenic to a wide range of plants under diverse environmental conditions. Phytopathogenic *Fusarium* fungi cause several diseases of small-grain cereals, including seedling blight and foot rot, fusarium head blight (FHB) (also known as 'scab' or ear blight) and ear rot of maize (Parry et al., 1995). The *Fusarium* species *Fusarium graminearum* (teleomorph *Gibberella zeae*), *F. culmorum, F. poae*, *F. avenaceum* (teleomorph *G. avenacea*) and *Microdochium nivale* (formerly known as *Fusarium nivale*, teleomorph *Monographella nivalis*) are common pathogens of wheat and

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barley (Sutton, 1982; Parry et al., 1995; Miedaner, 1997; Tekauz et al., 2000; Brennan et al., 2003).
Three *Fusarium* species are frequently isolated from infected maize: *F. graminearum, F. moniliforme* (syn. *F. verticillioides*, teleomorph *G. fujikuroi* mating population A) and *F. subglutinans* (teleomorph *G. fujikuroi* mating population E). Other species responsible for ear rot of maize include *F. culmorum*, *F. proliferatum* (teleomorph *G. fujikuroi* mating population D) and *F. equiseti* (Sutton, 1982; Leslie et al., 1986; Pomeranz et al., 1990; Odiemah and Manninger, 1994; Vigier et al., 1997; Velluti et al., 2000; Torres et al., 2001).

Fusarium diseases of wheat, barley and maize cause significant yield losses world-wide and are therefore of great economic importance (Sutton, 1982; Parry et al., 1995; Miedaner, 1997; Mesterhazy et al., 1999). In addition, many of these *Fusarium* species have the potential to produce a range of toxic secondary metabolites known as mycotoxins that cause a potential health risk when contaminated grain is consumed in human and animal food products (D'Mello and Macdonald, 1997; D'Mello et al., 1999; Placinta et al., 1999).

Host and climatic factors influence the growth, survival, dissemination and hence the incidence of *Fusarium* fungi and the disease severity. The influence of host cultivars on the pathogenicity and toxicity of *Fusarium* fungi has been extensively reviewed (Miedaner, 1997; Miedaner et al., 2001; Mesterhazy et al., 1999; Magg et al., 2002).

The influence of climatic factors on *Fusarium* diseases is complicated by the fact that *Fusarium* fungi can cause disease individually or in complex infections (Doohan et al., 1998), and there are numerous reports on how species differentially respond to different environmental variations, particularly temperature and humidity.

Also, host susceptibility to fungal disease is directly influenced by temperature and osmotic stress. This review is focused on the influence of climatic variables, particularly temperature, humidity and rainfall, on grain infection, growth, reproduction, survival, competitive ability, mycotoxicity and pathogenicity of *Fusarium* fungi commonly isolated from wheat, barley and maize.

2 CLIMATIC DISTRIBUTION OF *FUSARIUM* SPP.

Several factors influence the occurrence of *Fusarium* in the soil and the infestation and infection it generates in cereal plants. Geographical factors including climate are of superior importance for the occurrence of *Fusarium* and for the pattern of infestation by various *Fusarium* species.

The incidence of the causal organisms of FHB of wheat, barley and ear rot of maize is often correlated to different climatic conditions (temperature and rainfall) in different geographic locations. *F. culmorum*, *F. poae, F. avenaceum* and *M. nivale* are common pathogens of wheat and barley in the cooler temperate regions of the world, while *F. graminearum* tends to be the predominant *Fusarium* species pathogenic to these cereals in hotter regions of the world (Parry et al., 1995; Brennan et al., 2003).

F. graminearum, *F. moniliforme* and *F. subglutinans* are the *Fusarium* species most frequently isolated from infected maize, but depending on geographical location, other causal species of ear rot include *F. culmorum*, *F. proliferatum* and *F. equiseti* (Leslie et al., 1986; Vigier et al., 1997; Pomeranz et al., 1990; Odiemah and Manninger., 1994; Velluti et al., 2000; Torres et al., 2001).

Varying the temperature in a simple model ecosystem produces changes in the community structure of *Fusarium* species that mimic those found along climatic temperature and rainfall gradients (Saremi et al., 1999). The influence of climatic conditions on the incidence of *Fusarium* species is probably both direct (e.g. an effect on mode of reproduction) and indirect (e.g. an effect of soil and vegetation type).

More research is required to determine the indirect effect of climate on the incidence of *Fusarium* fungi and how this affects species-specific factors.

3 GERMINATION, GROWTH AND COMPETITION BETWEEN *Fusarium* spp.

Germination, growth and competition between *Fusarium* spp. are dependent upon the availability of nutrients and environmental factors such as temperature, pH, humidity, aeration and light. The influence of nutritional availability is outside the scope of this review. It is generally not a limiting factor during infection and colonisation of host tissue, but may be limiting or growth-inhibiting during saprophytic survival (e.g. humic acids in soil) (Moliszewska and Pisarek, 1996).

3.1 Germination

Germination is influenced by water availability (*a*w) and temperature: warm humid conditions favour this developmental stage. Marín et al. (1996) found that the *a*w minima for the microconidial germination of Spanish isolates of *F. moniliforme* and *F. proliferatum* were 0.88 on maize meal extract medium.

Microconidia of *F. moniliforme* germinated optimally at 25–37 °C and 0.96–0.98 *a*w, but at 30 °C when the *a*w was 0.90–0.94, with intraisolate variation. The germination of microconidia of *F. proliferatum* was optimal at 30 °C, regardless of *a*w, and with significant intra-isolate variation. However, Etcheverry et al. (2002) found that Argentinean isolates of *F. moniliforme* and *F. proliferatum* grew very slowly, if at all, at *a*w 0.93 and 25 °C. At marginal temperatures and *a*w levels, the germination lag time increases (Marín et al., 1996; Etcheverry et al., 2002). Earlier, Francis and Burgess (1977) found that the percentage germination of conidia, ascospores and chlamydospores of *F. graminearum* Group II isolates was reduced as water potential was lowered from −1 to −20 bars.

3.2 Growth

Temperature and *a*w differentially affect the growth of *Fusarium* species (Table 1, Figure 1). *Fusarium* species differed in their temperature requirements for optimal growth on potato dextrose agar (Cook and Christen, 1976; Pettitt et al., 1996; Brennan et al., 2003). Irrespective of the European origin of isolates, *in vitro* culture experiments showed that optimal growth occurred at 25 °C for *F. graminearum*, at 20–25 °C for *F. culmorum and F. poae* and at 20 °C for *F. avenaceum* and *M. nivale*.

In general, *F. culmorum* had the fastest growth rate of all five species over the range 10–30 °C. Species accounted for 51–63% and country of origin accounted for 23–52% of growth rate variation. At the low temperature of 5 °C, Pettitt et al. (1996) found that of *F. culmorum*, *F. avenaceum* and *M. nivale*, the latter species was significantly the fastest growing. At the higher temperature of 35 °C, Cook and Christen (1976) found that *F. graminearum* did not grow, even after 30 days. Marín et al. (1998a) found that the maize pathogens *F. moniliforme* and *F. proliferatum* had a faster growth rate than *Eurotium* and *Penicillium* species and on sterile layers of maize grew best at 30 °C (Table 1).

The temperature optima for growth of *Fusarium* spp. appears to be dependent on *a*w. Cook and Christen (1976) found that the optimal growth temperature for European isolates of *F. graminearum* (24–28 °C) increased slightly when lower water potentials prevailed. *Fusarium graminearum* grew optimally at −10 to −20 bars and *F. culmorum* at −8 to −14 bars. Increasing *a*w (>0.925) favoured growth of *F. moniliforme* and *F. proliferatum* on sterile layers of maize at 30 °C (Marín et al., 1995). More research is required to better understand the influence of *a*w on the growth of *F. culmorum*, *F. poae* and *M. nivale.* It must be noted that drawing comparisons between growth rate studies is difficult, as the rates are very dependent on the growth substrates used. For example, on maize culture media *F. subglutinans* grew optimally at 20–25 °C, but faster on rice culture media at 15 °C (Castellá et al., 1999).

Table 1: Optimum temperature and water potential/availability for the *in vitro* growth of *Fusarium* species.
Preglednica 1: Optimalne temperature in vodni potencial/dostopnost za *in vitro* rast *Fusarium* vrst.

Species	Substrate[a]	Optimum growth conditions		References
		Temperature (°C)	Water potential/availability[b]	
F. graminearum	BM, PDA	24–28	−10 to −20 bars	Cook and Christen (1976), Brennan et al. (2003)
F. culmorum	BM, CMA, PDA	20–25	−8 to −14 bars	Cook and Christen (1976), Parry et al. (1994), Brennan et al. (2003)
F. avenaceum	PDA	20–25	ND	Parry et al. (1994), Brennan et al. (2003)
F. poae	PDA	20–25	ND	Brennan et al. (2003)
M. nivale	PDA	15–20	ND	Parry et al. (1994), Brennan et al. (2003)
F. moniliforme	Sterile maize layers	30	a_w > 0.925	Marín et al. (1995)
F. proliferatum	Sterile maize layers	30	a_w > 0.925	Marín et al. (1995)
F. subglutinans	MCM, RCM	15–25	ND	Castellá et al. (1999)

[a] BM = basal medium, PDA = potato dextrose agar, CMA = corn meal agar, MCM = maize culture media, RCM = rice culture media.
[b] ND = no data.

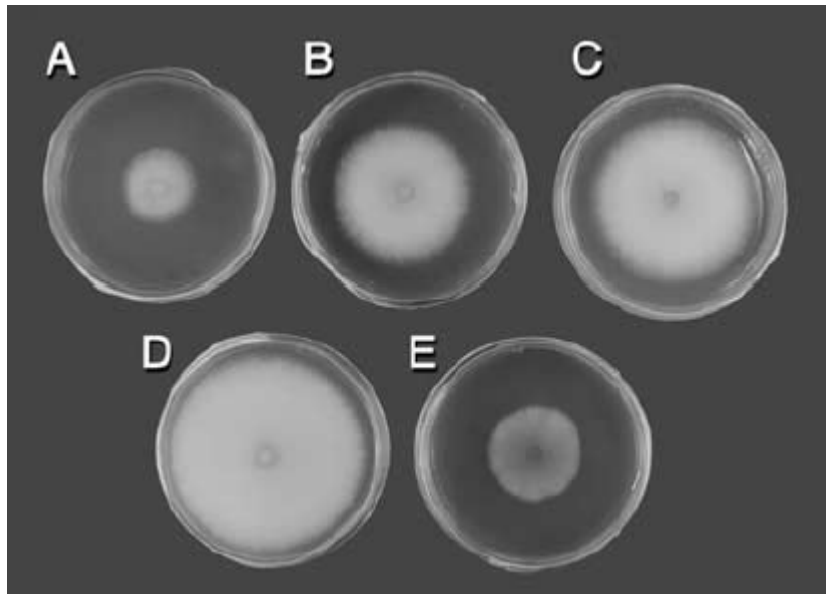

Figure 1: *In vitro* growth rate of *F. poae* (strain CC359B) at 10 (A), 15 (B), 20 (C), 25 (D) and 30 °C (E) (Brennan et al., 2003).
Slika 1: *In vitro* priraščanje glive *F. poae* (izolat CC359) pri 10 (A), 15 (B), 20 (C), 25 (D) in 30 °C (E) (Brennan et al., 2003).

3.3 Competition: Temperature and water availability (*a*w)

Fusarium fungi do not exist in isolation, either in the soil, on debris, or on the host, but are continually competing with other organisms, particularly microorganisms. Microbial interactions and the balance between microbial communities are influenced by the prevailing environmental conditions. It has previously been shown that temperature and *a*w significantly influence the growth and interaction between *F. moniliforme* and *F. proliferatum*, and between *F. graminearum*, *F. subglutinans*, *F. proliferatum, Aspergillus, Penicillium*, *Eurotium* and *Trichoderma* species (Marín et al., 1998a,b).

In a study of the competing abilities of *Fusarium*, *Aspergillus*, *Penicillium*, *Eurotium* and *Trichoderma* species, Marín et al.(1998a) found that *Fusarium* species were only dominant at high *a*w (0.995). Magan and Lacey (1984) found that of a range of field fungi, *F. culmorum* was the only one able to compete with and dominate other fungi, particularly at *a*w > 0.95. Within the *Fusarium* genus, *F. graminearum* appears to have a competitive advantage over other species under cooler conditions (Marín et al., 1998b; Velluti et al., 2000). Marín et al. (1998b) suggested that *F. graminearum* has a competitive advantage over *F. moniliforme* and *F. proliferatum* at 15 ^{o}C, while at 25–30 ^{o}C, these species coexisted in the same niche. Similar results were found by Velluti et al. (2000), regardless of *a*w (0.93, 0.95 and 0.98). Later in this seminar, the occurrence of *Fusarium* complexes and their impact on mycotoxin production will be discussed.

4 *FUSARIUM* SPECIES INVOLVED AND MYCOTOXIN PRODUCED

4.1 *Fusarium* species involved

The species of *Fusarium* (teleomorph *Gibberella*) causing fusariosis of cereals are worldwide in distribution and can cause several diseases generally recognized, according to the host, as *Gibberella* seedling blight, foot rot, and head blight (scab) of small grain cereals (wheat, oats, barley, rye, triticale); and *Gibberella* stalk and ear rot, and seedling blight of maize. From the mycotoxicological point of view, the phases of disease of greatest concern are certainly scab of small cereals and ear rot of maize, for the potential accumulation of mycotoxins in grains. The etiological characteristic of both these phases, is the co-occurrence or the quick succession of several species of *Fusarium* usually referred to as a ‘complex’. In fact, it is quite common to isolate up to nine different *Fusarium* species, from a single fragment of infected tissues or up to seventeen different species from freshly harvested wheat samples collected in a limited area. However only a restricted number of species have been regarded as pathogenic and generally only very few of them predominate in a particular host-agroclimatic system (Burgess et al., 1997).

But, like the strains of the pathogenic and predominant *Fusarium* species, also several strains of the other less pathogenic or opportunistic *Fusarium* species are capable of producing considerable amounts of toxins. Therefore, the toxigenic profile of a contaminated crop is determined not only by the predominant pathogenic species, but also by the opportunistic species included in the “complex” (Burgess et al., 1997).

The species predominantly found associated with head blight of wheat and other small cereals are *F. graminearum* Schwabe and its widespread teleomorph *G. zeae* (Schw.) Petch, *F. culmorum* (Wm.G. Sm.) Sacc. and *F. avenaceum* (Fr.) Sacc. (*G. avenacea* R.J. Cooke). Among the other less frequently isolated species there are *F. poae* (Peck) Wollenw., *F. crookwellense* L.W. Burgess, P.E. Nelson & T.A. Toussoun (syn. *F. cerealis* Cooke), *F. equiseti* (Corda) Sacc. (syn. *F. scirpi*) (*G. intricans* Wollenw.), *F. sporotrichioides* Sherb., and *F. tricinctum* (Corda) Sacc. Several other species may be sporadically encountered, including *F. acuminatum* Ellis & Everh. (*G. acuminata* Wollenw.), *F. subglutinans* (Wollenw. & Reink.) P.E. Nelson, T.A. Toussoun & Marasas (syn. *F. sacchari*), *F. solani* (Mart.) Sacc. (*Nectria haematococca* Berk. & Broome), *F. oxysporum* Schlecht., and *F. semitectum* Berk. & Rav. (syn. *F. pallidoroseum*, *F. incarnatum*) (Burgess et al., 1997).

Fusarium species may be responsible for at least two kinds of maize ear rot, commonly called as 'red ear rot' mainly caused by species of the *Discolor* section, and 'pink ear rot', mainly caused by representatives of the *Liseola* section. The predominant species causing maize 'red ear rot' are *F. graminearum*, *F. culmorum* and *F. crookwellense*. Among the other less frequently isolated species there are *F. subglutinans*, *F. avenaceum*, *F. moniliforme* J. Sheld. [syn. *F. verticillioides* (Sacc.) Nirenberg]. The species more frequently isolated from maize 'pink ear rot' and related 'random kernel rot', are essentially the widespread anamorphs of the rather rare *G. fujikuroi* (Sawada) Ito in Ito & K. Kimura, namely, *F. moniliforme*, *F. proliferatum* (T. Matsushima) Nirenberg and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson. Among the other toxigenic *Fusarium* species less frequently isolated from molded maizeears, there are: *F. equiseti*, *F. poae*, *F. sporotrichioides*, *F. acuminatum*, *F. semitectum*, *F. solani* and *F. oxysporum* (Burgess et al., 1997).

Finally, there are many other species only sporadically isolated from cereals, but in some occasion reported as emerging problem, such as *F. anthophilum* (A. Braun) Wollenw., *F. chlamydosporum* Wollenw. & Reink. (syn. *F. fusarioides*), *F. compactum* (Wollenw.) Gordon, *F. flocciferum* Corda, *F. heterosporum* Nees (syn. *F. reticulatum*, *F. graminum*), *F. lateritium* Nees, *F. sambucinum* Fuckel, *F. torulosum* (Berk. & Curt.) Nirenberg, and *F. venenatum* Nirenberg (Burgess et al., 1997).

Within *F. graminearum* (*G. zeae*) were characterized two populations designated as Group 1 and Group 2, with almost the same toxigenic potentiality. The Group 1 very rarely forms perithecia in nature and mainly causes crown rot of cereals and grasses; Group 2 readily forms abundant perithecia in nature and mainly causes head blight of grain cereals and stalk and ear rot of maize. Studies on genetic diversity indicated that *F. graminearum* Group 2 have greater affinity to *F. culmorum* and *F. crookwellense* than to *F. graminearum* Group 1 (Burgess et al., 1997).

In addition, the toxigenic strains of *F. graminearum* were classified in two chemotypes: DON and NIV producers, according to the main type B trichothecenes synthesized. Furthermore, DON-chemotype strains of *F. graminearum* were subclassified into two types: 3-AcDON and 15-AcDON producers (Miller *et al*., 1991; Logrieco et al., 1992; Szécsi and Bartok, 1995; Yoshizawa, 1997).

Ecological differences in chemotype distribution may contribute to characterizing a regional grain contamination. Toxigenic strains of *F. culmorum* can be divided into two types: DON and NIV chemotypes, according to the main type B trichothecenes produced. DON-type strains produced also AcDON (3-AcDON) (Gang et al., 1998; D'Mello et al., 1997).

The species G. *fujikuroi* has been subdivided into seven distinct mating populations (biological species), indicated as A to G, and covering several *Fusarium* anamorphs (Leslie, 1995). From these, the most frequently found on maize were *F. moniliforme* (A), *F. proliferatum* (D), and *F. subglutinans* (E), which were also differentiated by their toxigenic capability (Moretti et al., 1997). *F. nivale* Ces. ex Berl. & Voglino is a well known pathogen of cereals, very frequently found among the major fungi included in the species complex causing 'foot rot' and 'head blight (scab)' of small cereals. *F. nivale* is no longer considered a *Fusarium*, first it was placed in the genus *Gerlachia* [*G. nivalis* (Ces. ex Berl. & Voglino) W. Gams & E. Müller], and then transferred to *Microdochium* as *M. nivalis* (Fr.) Samuels & I.C. Hallett [teleomorph *Monographella nivalis* (Schaff.) E. Müller]. Therefore *M. nivalis* is not included in this paper dedicated to cereal fusarioses, also because it has a very low toxicity, and proved to be incapable of producing the typical *Fusarium* mycotoxins (Logrieco *et al*., 1991).

4.2 Mycotoxin production

One of the most serious consequences of FHB and ear rot of cereals is the contamination of grain with mycotoxins (D'Mello and Macdonald, 1997; D'Mello et al., 1999; Placinta et al., 1999). The most important classes of *Fusarium* mycotoxins, based on their harmful effects on human and animal health, are the trichothecenes, fumonisins, moniliformin and zearalenone (ZEA) (D'Mello et al., 1999).

Trichothecene mycotoxins are tricyclic sesquiterpenes and two classes; types A and B, are commonly found in cereals along with the oestrogenic mycotoxin ZEA (D'Mello and MacDonald, 1997; D'Mello et al., 1999). The fumonisin class of mycotoxins comprises a group of structurally related metabolites of which fumonisin B1 (FB1*)* and B2 (FB2*)* are commonly found in maize grain with moniliformin (D'Mello and Macdonald, 1997; D'Mello et al., 1999). Mycotoxin production in grain can begin in the field and continue throughout storage. Mycotoxin production is dependent mainly on both well-defined ranges of temperature and *a*w. But in turn, the optimum climatic conditions for mycotoxin production in infected grains depends on the substrate, *Fusarium* species and isolate. The influence of temperature and *a*w on mycotoxin production by *Fusarium* fungi is probably not entirely direct but rather a function of the influence of these parameters on fungal growth.

- Trichothecenes and zearalenone (ZEA)

Many *Fusarium* species, including *F. graminearum*, *F. culmorum*, *F. poae*, *F. oxysporum* and *F. sporotrichioides* are producers of trichothecenes and ZEA (D'Mello and Macdonald, 1997; D'Mello et al., 1999) (Table 2). *F. sporotrichioides* and perhaps *F. poae* predominately produce type A trichothecenes, which includes T-2 toxin, HT-2 toxin, neosolaniol and diacetooxyscirpenol (DAS). *F. culmorum* and *F. graminearum* predominately produce type B trichothecenes, including deoxynivalenol (DON, also known as vomitoxin), its 3-acetyl and 15-acetyl derivatives (3-ACDON and 15-ACDON, respectively) and nivalenol (NIV). Most studies indicate that high moisture favours the production of both classes of mycotoxins, but the optimum temperatures for trichothecene and ZEA production in *Fusarium*-infected grain appears to be specific to the substrate, species and individual metabolites (Table 2). Moderate rather than warm temperatures favour the production of type A trichothecenes by *F. sporotrichioides* (Miller, 1994; Mateo et al., 2002) (Table 2). While the optimum production conditions varied depending on the substrate and toxic metabolite, in general *F. sporotrichioides*-infected maize, wheat and rice grains contained more type A trichothecenes when moistened with 35% water (*a*w = 0.990) and incubated at 20 ^{o}C for 3 weeks than when incubated at higher temperatures or *a*w. However, Rabie et al. (1986) detected relatively large amounts of T-2 toxin in *F. acuminatum*-infected oats stored at 25 ^{o}C, although a comparison was not drawn between different incubation conditions. In the case of type B trichothecenes, warm humid conditions favour their production during storage of *F. culmorum* and *F. graminearum*-infected grain (Greenhlagh et al., 1983; Lori et al., 1990; Beattie et al., 1998; Homdork et al., 2000; Martins and Martins, 2002) (Table 2).

Table 2: The major classes of *Fusarium* mycotoxin, their principal producers and optimal production conditions on cereal grains

Preglednica 2: Glavni razredi fuzarijskih mikotoksinov, vrste gliv, ki jih tvorijo in optimalni pogoji za njihovo tvorbo na žitnem zrnju

Toxin	Species	Substrates	Optimum production conditions[a]	References
Type A trichothecenes [T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol (DAS)]	*F. sporotrichioides* *F. poae*	Barley, oats, rice, wheat, maize	Moderately warm and humid (20–25 °C, a_w = 0.990)	Mateo et al. (2002), Miller (1994), Rabie et al. (1986)
Type B trichothecenes [deoxynivalenol (DON), 3-acetyl DON, 15-acetyl DON, nivalenol (NIV)]	*F. graminearum* *F.culmorum*	Barley, wheat, rice, maize	Warm and humid (25–28 °C, a_w = 0.97)	Greenhalgh et al. (1983), Lori et al. (1990), Beattie et al. (1998), Homdork et al. (2000)
ZEA	*F. graminearum* *F. culmorum*	Wheat, rice, maize	Warm (17–28 °C), or temperature cycles (e.g. 25–28 °C for 14–15 days; 12–15 °C for 20–28 days) and humid (a_w = 0.97 or 90% RH)	Jiménez et al. (1996), Lori et al. (1990), Ryu and Bullerman (1999), Homdork et al. (2000), Martins and Martins (2002)
Fumonisins	*F. moniliforme* *F. proliferatum* *F. subglutinans*	Maize	Cool to warm conditions and humid (15–30 °C, a_w = 0.98)	Cahagnier et al. (1995), Marín et al. (1999a,b)
Moniliformin	*F. subglutinans* *F. moniliforme* *F. avenaceum*	Wheat, rye, barley, oats, maize	Warm temperatures (25–30 °C)	Kostecki et al. (1999), Schütt (2001)

[a] Optimum temperature and humidity vary depending on substrate, species and isolate; typical conditions are given in parentheses. Time of production varies from 3 to 8 weeks.

Martins and Martins (2002) found that on *F. graminearum*-infected cracked corn (*aw* = 0.97), more of the type B trichothecene DON was produced following incubation at 28 °C for 35 days, rather than at 22 or 28 °C for 15 days followed by 12 °C for 20 days; their results agreed with those of Greenhlagh et al. (1983). Also, maximal DON was produced by *F. graminearum* on infected wheat and polished rice following incubation in the dark at 27 °C, but in hulled rice, DON production was maximised when incubated at 27 °C in the light (Lori et al., 1990).

The effect of initial infection level may outweigh the effect of environmental conditions on mycotoxin contamination of grain, depending on the toxic metabolite in question. Following 7 months storage of barley grain with high initial *Fusarium* infection levels (85%), DON contents did not change significantly, irrespective of conditions (−4, 20 or 24 °C, quiescent or forced aeration), although it was lowest in malt produced from the grain stored at 24 °C (Beattie et al., 1998). Initial infection levels would not normally be so high. In wheat stored for 6–8 weeks under warm humid conditions (25 °C, 90% RH), Homdork et al. (2000) found that, while the DON content significantly increased in grain with a low to moderate (4–15%) initial *F. culmorum* infection level, it did not increase in samples with high (>50%) initial infection levels.

However, the influence of initial infection levels on mycotoxin production may be toxin specific, as

while these conditions were optimal for the production of NIV, unlike DON, it was not present at harvest and levels increased irrespective of initial infection level. As for trichothecenes, the conditions for optimal ZEA production appear to be species, isolate and substrate specific, and may vary from those for DON production. Several studies have found that maximum ZEA was produced in *F. graminearum* and *F. oxysporum*-infected maize at *a*w 0.97 and by cycling the incubation temperatures from 25 to 28 °C for 14–15 days, followed by 12–15 °C for 20–28 days (Jiménez et al., 1996; Ryu and Bullerman, 1999; Martins and Martins, 2002) (Table 2).

However, the optimum temperature for ZEA production may vary with isolate and substrate. Jiménez et al. (1996) found that, while the aforementioned conditions were optimal for ZEA production in maize grain infected by two isolates each of *F. graminearum* and *F. oxysporum*, another *F. graminearum* and two *F. culmorum* isolates produced maximal ZEA after 30 days incubation at room temperature (16–25 °C) rather than at 28 or 37 °C (*a*w = 0.97). In wheat grain with moderate to high levels (4–15%) of *F. culmorum* infection, ZEA production was favoured by warm and humid (25 °C, 90% RH) rather than cool and dry storage conditions.

Most ZEA was produced towards the end of the storage period (6–8 weeks) (Homdork et al., 2000). Lori et al. (1990) reported a lower optimal substrate-dependent temperature for ZEA production by a *F. graminearum* isolate. ZEA production was maximised by incubation of *F. graminearum*-infected wheat and polished rice in the dark at 17 and 21 °C, respectively, while production was maximised in hulled rice incubated at 27 °C in the light (Lori et al., 1990).

- Fumonisins and moniliformin

Fumonisins and moniliformin are commonly produced in maize infected by *F. moniliforme* and *F. proliferatum*, species which tend to grow better at higher temperatures (Keller et al., 1997; Kostechi et al., 1999; Miller, 2001; Marín et al., 1999a,b). Moniliformin has also been detected in cereals infected with *F. avenaceum* and *F. subglutinans* (Kostechi et al., 1999; Torres et al., 2001; Kiecana et al., 2002). While the temperature optima for the production of fumonisins by these pathogens vary, they all prefer *a*w~ 0.98 and fumonisin production generally decreases with temperature and higher *a*w (Cahagnier et al., 1995; Marín et al., 1999a,b). Marín et al. (1999a,b) found that *a*w had a more significant effect than temperature on total fumonisin production in maize grain and ground maize by *F. moniliforme* and *F. proliferatum*. In general, fumonisin production and fungal biomass decreased with temperature and *a*w and was optimal at 15–30 °C and 0.98 *a*w, depending on the isolate. At marginal temperatures (especially 15 °C), there was an increase in fumonisin production at lower *a*w levels (0.92 and 0.95) when compared to the concentrations produced at higher temperatures and higher *a*w levels. But even at 37 °C, Marín et al. (1999b) found that an isolate of *F. moniliforme* could produce significant amounts of fumonisin.

Ono et al. (1999) attributed the higher fumonisin content of maize in the Northern region of the State of Párana, Brazil compared to the Central-South to higher rainfall in the former during the month preceding harvest (202 and 92.8 mm, respectively). Oxygen limitation retards the growth of *F. moniliforme* and *F. proliferatum* and under such conditions it was found that no FB1 was produced (Keller et al., 1997).

Higher temperatures favour moniliformin production in cereal grains infected by *F. avenaceum* or *F. subglutinans* (Kostecki et al., 1999; Schütt, 2001). Moniliformin production by a *F. subglutinans* isolate from maize was higher at 30 than 20 or 25 °C and on rice rather than on wheat, rye, barley, oat or maize grains (Kostecki et al., 1999). Temperature greatly influenced moniliformin production by *F. avenaceum* on wheat, with more being produced under mediterranean rather than temperate conditions (Schütt, 2001).

5 CONCLUSIONS

Temperature and humidity/wetness are the main climatic factors influencing the development of *Fusarium* fungi causing diseases of cereals, although the influence of these climatic factors is not independent of other environmental and host factors. Many gaps exist in our knowledge of the influence of environmental parameters on *Fusarium* diseases of cereals. A risk assessment model for the forecasting of FHB epidemics in Ireland, based on environmental conditions is currently being developed (van Maanen, Cook and Doohan, unpubl. data). These data will also form part of an EU risk assessment model (EU RAMFIC project QLRT-1999-31517).

Particularly interesting questions for future research are: the influence of humidity on *Fusarium* diseases of small-grain cereals and the influence of environmental parameters on both the mycotoxin profiles (rather than individual metabolites) and biological control of *Fusarium* spp. Knowledge of the influence of climatic conditions on *Fusarium* diseases may prove useful towards developing novel disease control methods.

6 REFERENCES

Beattie S., Schwarz P.B., Horsley R., Barr J., Casper H.H. 1998. The effect of grain storage conditions on the viability of *Fusarium* and deoxynivalenol production in infested malting barley. Journal of Food Protection, 61: 103–106

Brennan J.M., Fagan B., Van Maanen A., Cooke B.M., Doohan F.M. 2003. Studies on *in vitro* growth and pathogenicity of *Fusarium* fungi. European Journal of Plant Pathology, 109: 577–587

Burgess L.W., Summerell B.A., Backhouse D., Benyon F., Levic J. 1997. Biodiversity and population studies in *Fusarium*. *Sydowia* (Special issue March 30, 1997): 1-11

Cahagnier B., Melcion D., Richard-Molard D. 1995. Growth of *Fusarium moniliforme* and its biosynthesis of fumonisin B1 on maize grain as a function of different water activities. Letters in Applied Microbiology, 20: 247–251

Castellá G., Munkvold G.P., Imerman P., Hyde W.G. 1999. Effects of temperature, incubation period and substrate on the production of fusaproliferin by *Fusarium subglutinans* ITEM 2404. Natural Toxins, 4: 129–132

Cook R.J., Christen A.A. 1976. Growth of cereal root rot fungi as affected by temperature–water potential interactions. Phytopathology, 66: 193–197

D'Mello J.P.F., Macdonald A.M.C. 1997. Mycotoxins. Animal Feed Science and Technology, 69: 155–166

D'Mello J.P.F., Placinta C.M., Macdonald A.M.C. 1999. *Fusarium* mycotoxins: A review of global implications for animal health, welfare and productivity. Animal Feed Science and Technology, 80: 183–205

Doohan F.M., Parry D.W., Jenkinson P., Nicholson P. 1998. The use of species-specific PCR-based assays to analyse *Fusarium* ear blight of wheat. Plant Pathology, 47: 197–205

Etcheverry M., Torres A., Ramirez M.L., Chulze S., Magan N. 2002. *In vitro* control of growth and fumonisin production by *Fusarium verticillioides* and *F. proliferatum* using antioxidants under different water availability and temperature regimes. Journal of Applied Microbiology, 92: 624–632

Francis R.G., Burgess L.W. 1977. Characteristics of *Fusarium roseum 'Graminearum'* in Eastern Australia. Transactions of the British Mycological Society, 68: 421–427

Gang G., Miedaner T., Schuhmacher U., Schollenberger M., Geiger H.H. 1998. Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in aggressiveness toward winter rye. Phytopathology, 88: 879-884

Greenhlagh R., Neish G.A., Miller J.D. 1983. Deoxynivalenol, acetyl deoxynivalenol, and zearalenone formation in Canadian isolates of *Fusarium graminearum* on solid substrates. Applied and Environmental Microbiology, 46: 625–629

Homdork S., Fehrmann H., Beck R. 2000. Influence of different storage conditions on the mycotoxin production and quality of *Fusarium*-infected wheat grain. Journal of Phytopathology, 148: 7–15

Jiménez M., Máñez M., Hernández E. 1996. Influence of water activity and temperature on the production of zearalenone in corn by three *Fusarium* species.

International Journal of Food Microbiology, 29: 417–421

Keller S.E., Sullivan T.M., Chirtel S. 1997. Factors affecting the growth of *Fusarium proliferatum* and the production of fumonisin B1: oxygen and pH. Journal of Industrial Microbiology and Biotechnology, 19: 305–309

Kiecana I., Mielniczuk E., Kaczmarek Z., Kostecki M., Golinski P. 2002. Scab response and moniliformin accumulation in kernels of oat genotypes inoculated with *Fusarium avenaceum* in Poland. European Journal of Plant Pathology, 108: 245–251

Kostecki M., Wisniewska H., Perrone G., Ritieni A., Golinski P., Chelkowski J., Logrieco A. 1999. The effects of cereal substrate and temperature on production of beauvericin, moniliformin and fusaproliferin by *Fusarium subglutinans* ITEM-1434. Food Additives and Contaminants, 16: 361–365

Leslie J.F. 1995. *Gibberella fujikuroi*: available populations and variable traits. *Canadian Journal of Botany* 73 (Suppl. 1): 282-291

Leslie J.F., Marasas W.F., Shephard G.S., Sydenham E.W., Stockenström S., Thiel P.G. 1986. Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and E mating populations of *Gibberella fujikuroi* (*Fusarium moniliforme*). Applied and Environmental Microbiology, 62: 1182–1187

Logrieco A., Altomare C., Mulè G., Bottalico A. 1992. Alcuni dati sulla presenza e patogenicità di chemiotipi di *Fusarium graminearum* in Europa. Atti Giornate Fitopatologiche 2: 287-294

Logrieco A., Vesonder R.F., Peterson S.W., Bottalico A. 1991. Reexamination of the taxonomic disposition of and deoxynivalenol production by *Fusarium nivale* NRRL 3289. Mycologia, 83: 367-370

Lori G.A., Henning C.P., Violante A., Alippi H.E., Varsavsky E. 1990. Relation between the production of deoxynivalenol and zearalenone and the mycelial growth of *Fusarium graminearum* on solid natural substrates. Microbiologia, 6: 76–82

Magan N., Lacey J. 1984. Effect of water activity, temperature and substrate on interactions between field and storage fungi. Transactions of the British Mycological Society, 82: 83–93

Marín S., Companys E., Sanchis V., Ramos A.J. 1999a. Twodimensional profiles of fumonisin B1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain. International Journal of Food Microbiology, 51: 159–167

Marín S., Homedes V., Sanchis V., Ramos A.J., Magan N. 1999b. Impact of *Fusarium moniliforme* and *Fusarium proliferatum* colonisation of maize on calorific losses and fumonisin production under different environmental conditions. Journal of Stored Food Products, 35: 15–26

Marín S., Companys E., Sanchis V., Ramos A.J. 1998a. Effect of water activity and temperature on competing abilities of common maize fungi. Mycological Research, 120: 959–964

Marín S., Sanchis V., Ramos A.J., Vinas I., Magan N. 1998b. Environmental factors, *in vitro* interactions, and niche overlap between *Fusarium moniliforme, F. proliferatum* and *F. graminearum*, *Aspergillus* and *Penicillium* species from maize grain. Mycological Research, 102: 831–837

Marín S., Sanchis V., Teixidó A., Sáenz R., Ramos A.J., Vinas I., Magan N. 1996. Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *Fusarium proliferatum* from maize. Canadian Journal of Microbiology, 42: 1045–1050

Marín S., Sanchis V., Magan N. 1995. Water activity, temperature and pH effects on growth of *Fusarium moniliforme* and *Fusarium proliferatum* isolates from maize. Canadian Journal of Microbiology, 41: 1063–1070

Martins M.L., Martins H.M. 2002. Influence of water activity, temperature and incubation time on the simultaneous production of deoxynivalenol and zearalenone in corn (*Zea mays*) by *Fusarium graminearum*. Food Chemistry, 79: 315–318

Mateo J.J., Mateo R., Jiménez M. 2002. Accumulation of type A trichothecenes in maize, wheat and rice by *Fusarium sporotrichioides* isolates under diverse culture conditions. International Journal of Food Microbiology, 72: 115–123

Mesterhazy A., Bartok T., Mirocha C.G., Komoroczy R. 1999. Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breeding, 118: 97–110

Miedaner T. 1997. Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breeding, 116: 201–220

Miller J.D. 2001. Factors that affect the occurrence of fumonisin. Environmental Health Perspectives, 109: 321–324

Miller J.D. 1994. Epidemiology of *Fusarium* diseases of cereals. In: Miller J.D., Trenholm H.L. (eds)

Mycotoxins in Grain: Compounds other than Aflatoxins. Eagon Press, St. Paul, MN, USA: 19-36

Miller J.D., Greenhalgh R., Wang Y.Z., Lu M. 1991. Trichothecene chemotype of three *Fusarium* species. Mycologia, 83: 121-130

Moliszewska E., Pisarek I. 1996. Influence of humic substances on the growth of two phytopathogenic soil fungi. Environment International, 22: 579–584

Moretti A., Logrieco A., Bottalico A., Ritieni A., Fogliano V., Randazzo G. 1997. Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Sydowia* (Special issue March 30, 1997): 44-56

Odiemah M., Manninger I. 1994. Inheritance of resistance to Fusarium ear rot in maize. Acta Phytopathological Academiae Scientarum Hungaricae, 17: 91–99

Ono E.Y., Sugiura Y., Homechin M., Kamogac M., Vizzoni E., Ueno Y., Hirooka E.Y. 1999. Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the state of Párana Brazil. Mycopathologia, 147: 139–148

ParryD.W., Jenkinson P., McLeod L. 1995. *Fusarium* ear blight (scab) in small-grain cereals – a review. Plant Pathology, 44: 207–238

ParryD.W., Pettitt T.R., Jenkinson P., Lees A.K. 1994. The cereal *Fusarium* complex. In: Blakeman P., Willamson B. (eds) Ecology of Plant Pathogens. CAB International, Wallingford, UK: 301-320

Pettitt T.R., Parry D.W., Polley R.W. 1996. Effect of temperature on the incidence of nodal foot rot symptoms in winter wheat crops in England and Wales caused by *Fusarium culmorum* and *Microdochium nivale.* Agricultural and Forest Meteorology, 79: 233–242

Placinta C.M., D'Mello J.P.F., Macdonald A.M.C. 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Animal Feed Science and Technology, 78: 21–37

Pomeranz Y., Bechter D.B., Sauer D.B., Seitz L.M. 1990. *Fusarium* headblight (scab) in cereal grains. Advances in Cereal Science and Technology, 10: 373–433

Rabie C.J., Sydenham E.W., Thiel P.G., Lübben A., Marasas W.F. 1986. T-2 toxin production by *Fusarium acuminatum* isolated from oats and barley. Applied and Environmental Microbiology, 52: 594–596

Ryu D., Bullerman L.B. 1999. Effect of cycling temperatures on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883. Journal of Food Protection, 62: 1451–1455

Saremi H., Burgess L.W., Backhouse D. 1999. Temperature effects on the relative abundance of *Fusarium* species in a model plant-soil ecosystem. Soil Biology and Biochemistry, 31: 941–947

Schütt F. 2001. Moniliformin production of *Fusarium* species under defined conditions. Ph.D. Thesis, Technical University of Berlin, Germany: 135-137.

Sutton J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum.* Canadian Journal of Plant Pathology, 4: 195–209

Szécsi A., Bartok T. 1995. Trichothecene chemotypes of *Fusarium graminearum* from corn in Hungary. *Mycotoxin Research*, 11: 85–92

Tekauz A., McCallum B., Gilbert J. 2000. Review: Fusarium head blight of barley in western Canada. Canadian Journal of Plant Pathology, 22: 9–16

Torres A.M., Reynoso M.M., Rojo F.G., Ramirez M.L., Chilze S.N. 2001. *Fusarium* species (section Liseola) and its mycotoxins in maize harvested in northern Argentina. Food additives and Contaminants, 18: 836–843

Velluti A., Marín S., Bettucci L., Ramos A.J., Sanchis V. 2000. The effect of fungal competition on colonisation of maize grain by *Fusarium moniliforme*, *F. proliferatum* and *F. graminearum* and on fumonisin B1 and zearalenone formation. International Journal of Food Microbiology, 59: 59–66

Vigier B., Reid L.M., Seifert K.A., Stewart D.W., Hamilton R.I. 1997. Distribution and prediction of *Fusarium* species associated with maize ear rot in Ontario. Canadian Journal of Plant Pathology, 19: 60–65

Yoshizawa T. 1997. Geographic differences in trichothecene occurrence in Japanese wheat and barley. Bulletin of the Institute for Comprehensive Agricultural Sciences Kinki University, Nara, Japan, 5: 23-29

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Antioksidativni potencial otrobov pire, navadne in tatarske ajde

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IZVLEČEK

Pira, navadna in tatarska ajda vsebujejo antioksidante in še nekatere snovi, ugodne za ohranjanje zdravja ljudi. V raziskovalni nalogi smo preučevali predvsem koliko in katere antioksidante vsebujejo otrobi posameznih vrst rastlin in v kakšnem zaporedju po količini antioksidantov, si vrste sledijo. Antioksidante smo tako v vzorcih rastlin določali spektrofotometrično. Ugotovili smo, da otrobi tatarske ajde vsebuje največ antioksidantov. Njena skupna antioksidativna aktivnost znaša 87,23 % DPPH razbarvanja, sledi ji navadna ajda z 11,71 % DPPH razbarvanja, najmanjšo skupno antioksidativno aktivnost pa ima pira z 1,01 % DPPH razbarvanja.

Ključne besede: antioksidanti, fenoli, polifenoli, tanini, navadna ajda, tatarska ajda, pira

ABSTRACT

ANTIOXIDATIVE POTENTIAL OF SPELT, COMMON BUCKWHEAT AND TARTARY BUCKWHEAT BRAN

Species as spelt, common buckwheat and tartary buckwheat contain antioxidants and some other substances, which are important for maintaining human health. In our research we tried to determine which and how many antioxidants contained bran and which species of plants contained the the highest quantity of antioxidants. We determined the amount of antioxidants in samples spectrophotometrically. We found out that bran of tartary buckwheat contained the the highest quantity of antioxidants. Total antioxidant activity of tartary buckwheat was 87.23 % DPPH discoloration, followed by common buckwheat with 11.71 % DPPH discoloration, and the lowest total antioxidant activity was found in spelt (1.01 % DPPH discoloration).

Key words: antioxidants, phenols, polyphenols, tannins, common buckwheat, tartary buckwheat, spelt

Prispevek je del magistrskega dela „Antioksidativni potencial pire (*Triticum aestivum* L. var. *spelta*), navadne ajde (*Fagopyrum esculentum* Moench) in tatarske ajde (*Fagopyrum tataricum* Gaertn.)", ki ga je pod mentorstvom akad. prof. dr. Ivana Krefta napisala Lea Lukšič.

The manuscript is a part of the master thesis „Antioxidative potential of spelt (*Triticum aestivum* L. var. *spelta*), common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.)" written by Lea Lukšič (supervisor: Prof. Ph. D. Ivan Kreft).

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1 UVOD

Tržno zanimivejše postajajo zadnje čase nekatere stare poljščine, pa tudi neobičajne mlevske frakcije, kot so otrobi, ki niso v množični uporabi in katerih antioksidativni potencial smo preučevali v našem poskusu. Te rastline so navadna ajda (*Fagopyrum esculentum* Moench), tatarska ajda (*Fagopyrum tataricum* Gaertn.) in pira (*Triticum aestivum* L. var. *spelta*). Z vidika vnosa eksogenih antioksidantov v naš organizem je zlasti pomembna hrana rastlinskega izvora, ki vsebuje veliko teh snovi, zato smo od take hrane tudi močno odvisni. Količina antioksidantov, ki jih zaužijemo s hrano, je odvisna od vrste rastlin, od vsebnosti antioksidantov v rastlinah, kar je pri rastlinah genetsko pogojeno, vplivajo pa tudi ekološke razmere in agrotehnični dejavniki. Vsebnost antioksidantov je različna tudi v posameznih rastlinskih delih, zato je pomembno, katere rastlinske dele uporabimo v naši prehrani. Antioksidanti imajo pomembno vlogo tudi pri rastlinah, saj jih ščitijo pred poškodbami, ki bi lahko nastale pod vplivom ultravijoličnega sevanja (Kreft in sod., 2000).

Prosti radikali so ioni, atomi ali molekule z vsaj enim prostim elektronom brez para. To so visoko reaktivne molekule, ki poškodujejo celične strukture, vključno z nukleinskimi kislinami in geni. Prosti radikali nastajajo pri cepitvi kovalentne vezi. So rezultat normalne celične presnove (dihanje) in posledica dejavnikov okolja, kot so sevanje, toplota, kajenje, uživanja alkohola, zdravil, onesnaženega okolja in nekaterih drugih dejavnikov. V organizmu zdravega človeka so antioksidanti in prosti radikali v stalnem ravnotežju. Če se to ravnotežje poruši nastane za organizem neugodno stanje, ki ga imenujemo oksidativni stres. Antioksidanti ga preprečujejo z lovljenjem prostih radikalov v našem telesu (Korošec, 2000).

Antioksidanti se med presnovo pri ljudeh lahko kemijsko spremenijo pod vplivom prebavnih encimov ter drugih snovi, ki jih izloča organizem v prebavni trakt in zaradi različnih interakcij med snovmi. V debelem črevesu pri rastlinojedih in vsejedih organizmih pa pri presnovi antioksidantov sodeluje tudi črevesna mikroflora. Biotska dostopnost različnih oblik antioksidantov je odvisna od njihove vključenosti v strukture hrane, interakcije med snovmi tekom presnove, topnosti antioksidantov v prebavnem traktu in od prenosnih mehanizmov preko črevesne stene v krvni obtok. Za antioksidante še ni dovolj znano v kolikšni meri, na kakšen način in s kolikšno zakasnitvijo se pojavljajo v celicah, oziroma na posameznih mestih delovanja (Kreft in sod., 2000).

Rutina do sedaj niso našli v nobenem drugem nepravem ali pravem žitu, razen v ajdi (Kreft in sod., 1999). Ugotovili so, da je v zeli tatarske ajde do 3 % rutina (suhe teže), kvercitrina pa je bilo v zeli od 0,01 do 0,05 % suhe teže. Kvercetin so v sledovih odkrili le v nekaterih vzorcih tatarske ajde. Ugotovljeno je bilo tudi, da zrnje tatarske ajde vsebuje več rutina (od 0,8 do 1,7 % suhe teže) kot zrnje navadne ajde (0,01 % suhe teže) Zrnje tatarske ajde je prav tako vsebovalo antioksidanta kvercetin in kvercitrin, ki pa ju v navadni ajdi niso zaznali (Fabjan in sod., 2003).

Dokazali so da rutin zavira oksidacijo LDL in HDL holesterola, zavira pa tudi toksičnost snovi v pljučih podgan, ki so jih podgane vdihale. Rutin in taninske kisline prav tako znižujejo raven holesterola v krvi in jetrih podgan. Poskus na hrčkih je pokazal, da rutin uspešno zavira nastanek poškodb na molekuli DNK in znižanje vrednosti LDL holesterola pri teh živalih. Rutin lahko zmanjša pojav arteroskleroze. Ugotovljeno je bilo, da prav tako pri miših blaži vnetja (Wieslander in sod., 2011).

Tanine uvrščamo v skupino polifenolov in so sekundarni produkti metabolizma rastlin. Imajo močan antioksidativen učinek in delujejo zavirajoče proti nekaterim vrstam raka. Tanine najdemo v sadju, čaju, vinu, sokovih in tudi v ajdi (Gadžo in sod., 2010). Tanini so pomemben dejavnik kakovosti, saj preprečujejo procese staranja in kvarjenja semen. Pozitivna lastnost taninov za ljudi je njihovo antibakterijsko, antitumorsko, antivirusno in antimutageno delovanje. Negativna lastnost pa je njihova inhibicija encimov α-amilaz za prebavo škroba, večje količine tudi negativno vplivajo na prebavljivost aminokislin in močno poudarjajo trpek okus (Luthar, 1992).

Zaradi množice pozitivnih učinkov antioksidantov na naš organizem in pomembnosti rastlinske hrane kot vira le teh smo se odločili raziskati, koliko antioksidantov vsebujejo otrobi pire, navadne in tatarske ajde.

2 MATERIALI IN METODE

Pira (*Triticum aestivum* L. var. *spelta* 'Ostro'), navadna ajda (*Fagopyrum esculentum* 'Pyra') in tatarska ajda (*Fagopyrum tataricum* Gaertn.; domača populacija) so bile pridelane in zmlete s tradicionalnim mlinom na kamne na kmetiji Rangus v Vrhpolju pri Šentjerneju. Pri ločitvi mlevskih frakcij so bili dobljeni ustrezni otrobi. Ugotavljali smo skupno vsebnost antioksidantov v otrobih pire, navadne ajde in tatarske ajde spektrofotometrično. Ugotavljali smo tudi vsebnost surovih beljakovin po Kjeldahlovi metodi z razklopom in destilacijo in vsebnost pepela v suhi snovi na princip sežiga vzorca in tehtanja dobljenega ostanka v otrobih pire, navadne ajde in tatarske ajde. V otrobih smo analizirali tudi vsebnost antioksidantov rutina in kvercetina, taninov (z oceno ekvivalentov katehina) in antioksidativno sposobnost otrobov pire, navadne ajde in tatarske ajde.

2.1 Vsebnost surovih beljakovin, pepela v suhi snovi, antioksidantov in antioksidativne aktivnosti otrobov pire, navadne in tatarske ajde

2.1.1 Ugotavljanje surovih beljakovin

Dušik smo v vzorcih določali po Kjeldahlovi metodi z razklopom in destilacijo. Metoda temelji na segrevanju in razklopu organske substance (1 g vzorca otrobov) s koncentrirano žveplovo kislino (H_2SO_4) ob prisotnosti katalizatorja. Razklop organske substance smo izvedli tako, da smo vzorce (otrobe) segrevali 60 do 75 minut na 400 °C. Pri razklopu izločeni dušik preide v amonijak in se veže s kislino, kot amonijev sulfat. Ob dodatku natrijevega hidroksida se dušik ponovno sprosti in destilira v posodo, v kateri je določena količina kisline znane koncentracije. Količino preostale kisline smo določili s končno titracijo. Metoda, ki smo jo izbrali, je bila predpisana po Pravilniku o metodah fizikalnih in kemičnih analiz za kontrolo kakovosti žit, mlevskih in pekarskih izdelkov, testenin in hitro zamrznjenega testa (1988). Vsebnost beljakovin smo določili s korektivnim faktorjem za preračunavanje dušika v beljakovine, in sicer s faktorjem N x 6,25 (Vombergar, 2010).

2.1.2 Ugotavljanje vsebnosti pepela

Princip ugotavljanja vsebnosti pepela temelji na sežigu vzorca in tehtanju dobljenega ostanka. Da bi določili vsebnost pepela smo 2 do 3 grame vzorca posameznih otrobov (pira, navadna ajda in tatarska ajda) žarili v žarilni peči pri temperaturi 900±20 °C eno uro. Kar je po žarjenju ostalo je vsebnost pepela, ki je preračunana na delež začetne mase vzorca pred žarjenjem.

2.1.3 Spektrofotometrične analize flavonoidov, skupnih fenolov in taninov

Natehtali smo otrobe posameznih vzorcev, in sicer po 600 mg za analizo taninov in 50 mg za analizo flavonoidov in fenolov. Vsaki natehti vzorca smo dodali po 10 mL 60 % etanola. Vzorce smo stresali eno uro, potem pa smo jih pustili ekstrahirati preko noči. Naslednji dan smo jih centrifugirali 10 minut pri 4000 obr./min., bistri supernatant smo uporabili za analizo. Vzporedno z raztopino vzorcev smo analizirali tudi raztopine standardnih in slepih vzorcev.

2.1.3.1 Ugotavljanje vsebnosti polifenolnih spojin s Folin-Ciocalteu-jevo metodo

S Folin-Ciocalteu-jevim reagentom (FC) smo določali vsebnost celokupnih fenolnih spojin v vzorcu. Reakcija med fenolnimi spojinami in FC reagentom je oksidacija fenolatov in redukcija reagenta FC (heteropolnih kislin), pri čemer nastane moder kompleks.

Reakcijo smo izvajali v šibko alkalnem mediju ob visoki koncentraciji reagenta, saj so fenolati prisotni le v alkalnem mediju, v katerem pa so tako reagent kot nastali produkti nestabilni. Da smo zagotovili večjo stabilnost reakcije, smo raztopini vzorca, ki vsebuje polifenolne spojine, dodali FC reagent, nekaj minut za tem pa še raztopino

natrijevega karbonata. Ko je reakcija potekla smo pomerili absorbanco raztopine pri valovni dolžini 750 nm.

S Folin-Ciocalteu reagentom lahko določimo vse fenolne skupine, tudi tiste, ki so vezane na beljakovine (Vombergar, 2010).

2.1.3.2 Ugotavljanje koncentracije rutina in kvercetina z metodo HPLC

Uporabili smo HPLC (HPLC - visoko tlačna tekočinska kromatografija) aparaturo Spectra-Physics (Mountain View, Kalifornija, ZDA) inštrument Spectra System P4000 in Spectra Focus optical scanning detector, kolono Hibar – LiChrospher 100, RP-18 (5 µm) (E. Merck, Darmstadt, Nemčija, 250 mm x 4 mm).

Topila za HPLC so bila: A: acetonitril in metanol v razmerju 1:2 in B: 0,75 % raztopina H_3PO_4.

Vzorce smo spustili po koloni (razmerje topil je bilo 60 % A in 40 % B) v času 20 minut. Potem za nadaljnjih 20 minut pri razmerju topil 100 % A in 0 % B in na koncu še za 10 minut z 100 % topila B.

Spojinam, ki smo jih dobili po filtriranju skozi kolono, smo pomerili absorbanco pri valovni dolžini 380 nm. Pomerjene vrednosti smo primerjali s pomerjenimi vrednostmi standardne raztopine.

2.1.3.3 Ugotavljanje vsebnosti taninov z vanilin-HCl metodo

Metoda določanja taninov z vanilinom temelji na principu, da v kislih razmerah poteče reakcija med vanilinom in kondenziranimi tanini, pri tem pa nastane rdeče obarvana raztopina, ki ima absorbcijski maksimum med 480 in 550 nm (Vombergar, 2010).

Reagent smo pripravili tako, da smo 400 mg 4 % vanilina raztopili v 10 mL 96 % etanola.

Standardno raztopino smo pripravili tako, da smo 1 mg standarda (epikatehin) raztopili v 10 mL 60 % etanola.

Pripravili smo 50 µL vzorčne raztopine, ki smo ji dodali reagent (100 µL vanilin, 50 µL 32 % HCl). Vzporedno smo pripravili tudi slepe vzorce (100 µL 96 % etanola, 50 µL 32 % HCl). Po pretečenem času 60 minut smo vzorcem pomerili absorbanco pri valovni dolžini 500 nm. Pomerjene vrednosti smo primerjali s pomerjenimi vrednostmi standardne raztopine (0,1 mg/mL epikatehina).

2.1.3.4 Ugotavljanje antioksidativne aktivnosti z metodo DPPH

Antioksidativno aktivnost skupnih antioksidantov v otrobih pire, navadne ajde in tatarske ajde, smo ugotavljali z metodo DPPH (2,2-diphenil-1-picrilhidrazil). Metoda temelji na principu, da s strani antioksidantov poteče redukcija raeagenta DPPH, ki je vijolične barve, po reakciji z antioksidanti pa pride do spremembe barve v rumeno (Molyneux, 2004).

Pripravili smo po 1 g vsakega vzorca otrobov in mu dodali 25 mL 80 % metanola. Tako pripravljene vzorce smo nato pri sobni temperaturi stresali 8 ur na 250 rpm. Po pretečenem času smo vzorce prefiltrirali skozi filter papir in jih shranili na temperaturo 8 °C. Iz vsakega vzorca otrobov smo pripravili po tri metanolne izvlečke, da smo imeli ponovitve.

Reagent smo pripravili tako, da smo 0,025 g DPPH raztopili v 100 mL metanola. Tako pripravljeno raztopino smo shranili v hladen in temen prostor.

Neposredno pred analizo smo pripravili slepe vzorce raztopine DPPH in metanola v razmerju 1:10. Takoj za tem smo pripravili še vzorce, ki so vsebovali metanolne izvlečke otrobov (0,1 mL izvlečka smo dodali v kiveto z reagentom DPPH) Po 10 minutah smo pomerili absorbanco (A10) pri valovni dolžini 515 nm.

Končno skupno antioksidativno aktivnost posameznega vzorca smo določili tako, da smo izmerili stopnjo razbarvanja (inhibicije) DPPH, ki je premo sorazmerna s količino antioksidantov v vzorcu (Brand-Williams in sod., 1995). Stopnjo razbarvanja DPPH smo določali po 10 minutah. Vzorce z antioksidanti (A10) smo primerjali s slepimi vzorci brez antioksidantov (A0).

2.2 Statistične analize

Rezultate smo analizirali z uporabo programov Microsoft Excel 2007, ter programom STAT G (Statgraphics 5.0, Statistical Graphics Corporation, ZDA). Statistične analize so bile opravljene po statistični metodi ANOVA, statistične značilnosti pa smo ugotavljali pri $p<0,05$.

3 REZULTATI IN RAZPRAVA

Iz preglednice št. 1 je razvidno, da otrobi navadne ajde vsebujejo v povprečju treh ponovitev največ surovih beljakovin v suhi snovi, kar 24,2 (% N x 6,25), sledi ji tatarska ajda z 21,6 (% N x 6,25), najmanj surovih beljakovin pa vsebuje pira, samo 16,3 (% N x 6,25).

Vsebnost pepela, torej anorganskega dela, je največja pri otrobih tatarske ajde in znaša 3,9 %, neznačilno manjša je pri navadni ajdi 3,8 %, vendar sta rezultata primerljiva za obe vrsti ajde, odstopa pira z le 2,4 % pepela v suhi snovi.

Pri vsebnosti polifenolov v preglednici št. 1 vidimo, da otrobi tatarske ajde vsebujejo izrazito več polifenolov (13,08 mg/g suhe snovi), kot otrobi navadne ajde (8,02 mg/g suhe snovi) in pire, ki ima majhno vsebnost polifenolov v primerjavi z obema vrstama ajde (1,79 mg/g suhe snovi).

Po vsebnosti rutina prav tako odstopajo otrobi tatarske ajde, saj je njegova vsebnost kar 8,67 mg/g suhe mase, bistveno manj rutina vsebuje navadna ajda, le 0,11 mg/g suhe mase, za vzorec otrobov pire pa vsebnost rutina ni bila ugotovljena, ker je ta pod mejo detekcije, oziroma ker pira rutina ne vsebuje.

Izkazalo se je tudi, da največ antioksidanta kvercetina vsebujejo otrobi tatarske ajde (0,49 mg/g suhe mase), vsebnost kvercetina pri navadni ajdi pa je bila pod mejo detekcije. Pri otrobih pire prav tako nismo določili vsebnosti kvercetina, saj ga pira ne vsebuje.

Vsebnost taninov oziroma ekvivalentov katehina je največja pri otrobih tatarske ajde (15,9 mg/g suhe mase) in je skoraj štirikrat večja kot vsebnost taninov oziroma ekvivalentov katehina pri navadni ajdi (4,0 mg/g suhe mase). Zelo malo taninov oziroma ekvivalentov katehina pa je v otrobih pire (0,07 mg/g suhe mase).

Po metodi določanja antioksidativne sposobnosti z 10 minutnim razbarvanjem reagenta DPPH imajo, kot je razvidno iz preglednice št. 2, daleč največjo antioksidativno aktivnost otrobi tatarske ajde (87,23 % DPPH se je razbarvalo zaradi vsebnosti antioksidantov), bistveno manjšo antioksidativno sposobnost izraža navadna ajda (11,71 % DPPH razbarvanja), zelo majhno antioksidativno sposobnost pa ima pira (1,01 % DPPH razbarvanja).

Preglednica 1: Vsebnost surovih beljakovin v suhi snovi, pepela v suhi snovi, polifenolov, rutina, kvercetina, taninov, oziroma ekvivalentov katehina ter antioksidativna sposobnost v otrobih pire, navadne ajde in tatarske ajde

VZORCI	Vsebnost surovih beljakovin v s.s.* (% N x 6, 25)	Vsebnost pepela v s.s.* (%)	Polifenoli (mg/g suhe mase)	Rutin (mg/g suhe mase)	Kvercetin (mg/g suhe mase)	Tanin, ekvivalenti katehina (mg/g suhe mase)	Antioksidativna sposobnost (% DPPH razbarvanja), po 10 min.
Pira, otrobi	16,3 [a]	2,4 [a]	**1,79**±0,12 [a]	N.u.*	N.u.*	**0,07** ±0,01 [a]	**1,01**± 0,39 [a]
Navadna ajda, otrobi	24,2 [b]	3,8 [b]	**8,02**±0,17 [b]	**0,11**± 0,04 [a]	N.u.*	**4,0**±0,90 [b]	**11,71**± 0,63 [b]
Tatarska ajda, otrobi	21,6 [c]	3,9 [b]	**13,08**±0,26 [c]	**8,67**± 0.09 [b]	**0,49** ± 0,05	**15,9**±1,19 [c]	**87,23** ± 1,94 [c]

Povprečni rezultati treh ponovitev ± standardni odklon *s.s. – suha snov *N. u. – ni ugotovljeno.
[a] Enaka črka v stolpcu pomeni, da se označene vrednosti ne razlikujejo značilno.

Z analiziranjem otrobov pire, navadne ajde in tatarske ajde smo ugotovili, da po vsebnosti antioksidantov otrobi tatarske ajde zelo odstopajo od otrobov navadne ajde in pire. Skupna vsebnost polifenolov v tatarski ajdi je kar 1,6-krat večja kot v navadni ajdi in kar 7,3-krat večja, kot v piri. Količina antioksidanta rutina je v tatarski ajdi kar za 78,8-krat večja kot v navadni ajdi. Tatarska ajda odstopa tudi po vsebnosti taninov oziroma ekvivalentov katehina, saj presega vsebnost taninov oziroma ekvivalentov katehina v navadni ajdi kar za 4-krat. Vsebnost taninov v tatarski ajdi pa je kar za 227-krat večja od vsebnosti taninov v piri. Skupna antioksidativna aktivnost je zaradi na sploh večje vsebnosti antioksidantov v otrobih tatarske ajde največja.

Največ surovih beljakovin v suhi snovi vsebujejo otrobi navadne ajde, sledi ji tatarska ajda, najmanj surovih beljakovin pa je v otrobih pire.

Vsebnost pepela v suhi snovi je v otrobih navadne ajde in tatarske ajde podobna, nekoliko manj pepela pa ostane po žarjenju otrobov pire.

4 SKLEPI

Iz rezultatov poskusa sklepamo, da otrobi tatarske ajde po vsebnosti antioksidantov odstopajo od otrobov navadne ajde in še posebej od otrobov pire.

Otrobi ajde so dober vir antioksidanta rutina. Do sedaj je bila izmerjena vsebnost rutina v moki tatarske ajde (11,67 mg/g,) kvercetina pa 0,63 mg/g. Vsebnost rutina v kruhu narejenem iz 100 % moke tatarske ajde je bila 0,44 mg/g, vsebnost kvercetina pa 5,00 mg/g. Vsebnost skupnih polifenolnih snovi je v kruhu znašala 7,84 mg GAE/g (Vogrinčič in sod., 2010).

Količina rutina izmerjenega v izvlečku iz kaše tatarske ajde znaša 187,60 mg/g izvlečka, količina kvercetina pa 28,50 mg/g izvlečka. V izvlečku kaše navadne ajde so bile vsebnosti manjše. Rutina je bilo 2,93 mg/g izvlečka, kvercetina pa 6,47 mg/g izvlečka (Cao in sod., 2007). V prekuhani kaši navadne ajde je bilo rutina 87,9 mg/kg suhe teže (Kreft in sod, 2006).

Količina rutina izmerjena v piškotih narejenih iz moke tatarske ajde je znašala 2,530 mg/kg suhe teže, kvercetina pa 1,620 mg/kg suhe teže. Vsebnost rutina v piškotih narejenih iz moke navadne ajde pa je bila 270 mg/kg suhe teže, med tem ko je bila vsebnost kvercetina pod mejo detekcije (Wieslander in sod., 2012).

Iz rezultatov naših raziskav v primerjavi s predhodnimi raziskavami sklepamo, da je vsebnost antioksidantov rutina in kvercetina v otrobih tatarske ajde nižja od izmerjene vrednosti teh dveh antioksidantov v moki. Vsebnost kvercetina je večja v kruhu narejenem iz moke tatarske ajde. Prav tako je znatno večja vsebnost antioksidantov rutina in kvercetina v izvlečku kaše navadne ajde in tatarske ajde, kot v otrobih obeh vrst ajde.Večja vsebnost rutina je tudi v prekuhani kaši navadne ajde v primerjavi z otrobi te ajde. V otrobih tatarske ajde pa je bila izmerjena znatno večja vsebnost rutina kot v kruhu narejenem iz moke tatarske ajde. Prav tako, je bila količina rutina in kvercetina večja v otrobih tatarske ajde v primerjavi s piškoti narejenimi iz moke tatarske ajde. Enako velja za vsebnost rutina v navadni ajdi. Kvercetin pa je bil pri navadni ajdi tako v otrobih, kot tudi v piškotih pod mejo detekcije in tako njegove vrednosti ni bilo moč izmeriti. Otrobi tatarske ajde imajo večjo vsebnost skupnih polifenolnih snovi, kot kruh narejen iz moke tatarske ajde.

Žita so osnovno živilo mnogim ljudem. Izboljšava vsebnosti mineralnih snovi v žitih pa predstavlja potencialno strategijo za izboljšanje prehrane ljudi (Regvar in sod., 2011). Moka tatarske ajde je lahko prav tako dober vir mineralov kot so železo, cink, baker, mangan, magnezij, kalij in kalcij, podobno velja za moko navadne ajde (Ikeda in sod., 2004).

Izmerjena vsebnost pepela v suhi snovi je v otrobih navadne ajde (cv. Siva) znašala 4,08 % suhe teže, v otrobih tatarske ajde (domača populacija, Luksemburg) pa 4,97 % suhe teže (Bonafaccia in sod., 2003). Vsebnost pepela pa je bila podobna tudi v otrobih, ki smo jih analizirali v našem poskusu. Na podlagi prejšnjih in v našem poskusu pridobljenih rezultatov, sklepamo, da ni bistvenih razlik v vsebnosti mineralov v navadni in tatarski ajdi. Znatna razlika pa se pojavlja med vsebnostjo mineralnih snovi v obeh vrstah ajde in vsebnostjo mineralnih snovi v piri.

Beljakovine ajde imajo izredno biološko vrednost, kar pomeni, da aminokisline ajde zelo ustrezajo človeškim potrebam po aminokislinah (Kreft, 1995).

Pojavljajo se značilne razlike med vsebnostjo surovih beljakovin med otrobi obeh vrst ajde in otrobi pire. Vsebnost surovih beljakovin pa je v obeh vrstah ajde podobna, malo večja je vsebnost surovih beljakovin v otrobih navadne ajde, kar je ravno nasprotno ugotovitvi v raziskavi, ki so jo leta 2003 opravili Bonafaccia in sodelavci, kjer so ugotovili, da je vsebnost surovih beljakovin sicer podobna v obeh vrstah ajde a nekoliko večja v otrobih tatarske ajde. Vsebnost surovih beljakovin v suhi snovi v otrobih je v navadni ajdi znašala 21,6 % suhe teže, v otrobih tatarske ajde pa 25,3 % suhe teže (Bonafaccia in sod., 2003).

Iz v poskusu pridobljenih rezultatov lahko sklepamo, da so otrobi lahko ugodna komponenta dietne hrane za ljudi. Predvsem so otrobi tatarske ajde dober vir antioksidantov. Ugodna mineralna in beljakovinska sestava otrobov navadne in tatarske ajde pa lahko zagotavlja dober vir teh sestavin v prehrani ljudi.

5 ZAHVALA

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6 VIRI

Bonafaccia G., Marocchini M., Kreft I. 2003. Composition and technological properties of the flour and bran from common and Tartary buckwheat. Food Chemistry, 80: 9-15

Brand-Williams W., Cuvelier M. E., Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie, 28: 25-30

Cao W., Chen W. J., Suo Z. R., Yao Y. P. 2008. Protective effects of ethanolic exstracts of buckwheat groats on DNA damage caused by hydroxyl radicals. Food Research International, 41: 924-929

Fabjan N., Rode J., Košir I. J., Wang Z., Zhang Z., Kreft I. 2003. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. Journal of Agricultural and Food Chemistry, 51: 6452-6455

Gadžo D., Djikić M., Gavrić T., Štrekelj P. 2010. Comparison of tannin concentration in young plants of common and Tartary buckwheat. Acta agriculturae Slovenica, 95: 75-78

Ikeda S., Tomura K., Lin L., Kreft I. 2004. Nutritional characteristics of minerals in Tartary buckwheat. Fagopyrum, 21:79-84

Korošec L. 2000. Prosti radikali in vloga antioksidantov v bioloških sistemih. V: Antioksidanti v živilstvu, 20. Bitenčevi živilski dnevi 2000. Ljubljana, Biotehniška fakulteta, oddelek za živilstvo: 11-21

Kreft I. 1995. Ajda. Ljubljana, ČZD, Kmečki glas: 112 str.

Kreft I., Fabjan N., Yasumoto K. 2006. Rutin content in buckwheat (Fagopyrum esculentum Moench) food materials and products. Food Chemistry, 98: 508-512

Kreft I., Škrabanja V., Bonafaccia G. 2000. Temelji prehranskih in biotskih vplivov antioksidantov. V: Antioksidanti v živilstvu, 20. Bitenčevi živilski dnevi 2000. Ljubljana, Biotehniška fakulteta, oddelek za živilstvo: 33-37

Kreft S., Knapp M., Kreft I. 1999. Extraction of rutin from buckwheat (Fagopyrum esculentum Moench) seeds and determination by capillary electrophoresis. Journal of Agricultural and Food Chemistry, 47: 4649-4652

Luthar Z. 1992. Tanin v semenih navadne in tatarske ajde (*Fagopyrum esculentum* Moench. in *Fagopyrum tataricum* Gaertn.). Zbornik Biotehniške fakultete Univerze v Ljubljani, 59: 55

Molyneux P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity**.** Songklanakarin Journal of Science and Technology, 26: 211-219

Pravilnik o metodah jemanja vzorcev in metodah fizikalnih in kemičnih analiz za kontrolo kakovosti žit, mlevskih in pekarskih izdelkov, testenin in hitro zmrznjenega testa. 1988. Ur. l. SFRJ 74/1988

Regvar M., Eichert D:, Kaulich B., Gianoncelli A., Pongrac P., Vogel-Mikuš K., Kreft I. 2011. New insights into globoids of protein storage vacuoles in wheat aleurone using synchrotron-soft-X-ray microscopy. Journal of Experimental Botany, 11: 3929–3939

Vogrinčič M., Timoracka M., Melichacova S., Vollmannova A., Kreft I. 2010. Degradation of utin and polyphenols during the preparation of Tartary buckwheat bread. Journal of Agricultural and Food Chemistry, 58: 4883-4887

Vombergar B. 2010. Rutin v frakcijah zrn navadne ajde (*Fagopyrum esculentum* Moench) in tatarske ajde (*Fagopyrum tataricum* Gaertn.). Doktorska disertacija. Ljubljana, Biotehniška fakulteta, Odd. za agronomijo: 147 str.

Wieslander G., Fabjan N., Vogrinčič M., Kreft I., Janson C., Spetz-Nystrom U., Vombergar B., Tagesson C., Leanderson P., Norback D. 2011. Eating buckwheat cookies is associated with the reduction in serum levels of myeloperoxidase and cholesterol: a double blind crossover study in day-care centre staffs. Tohoku Journal of Experimental Medicine, 225: 123-130

Wieslander G., Fabjan N., Vogrinčič M., Kreft I., Vombergar B., Norbäck D. 2012. Effects of common and Tartary buckwheat consumption on mucosal symptoms, headache and tiredness: A double-blind crossover intervention study. Journal of Food, Agriculture and Environment, 10: 107-110

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Vpliv različnih tehnoloških postopkov na kakovost vina malvazija

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IZVLEČEK

Namen raziskave je bil ugotoviti vpliv različnih tehnoloških postopkov predelave grozdja in pridelave vina na fizikalne in kemijske parametre vina ter njegovo senzorično kakovost. Za izvedbo poskusa smo izbrali vina sorte malvazija (letnik 2007), pridelana na območju Slovenske in Hrvaške Istre. Vina so pridelana po treh tehnoloških postopkih: klasičnem postopku, z maceracijo drozge in z zorenjem vina na drožeh. Pri nekaterih vzorcih je bila uporabljena kombinacija več postopkov (hladna maceracija in zorenje vina na drožeh). Pri vinih smo določili pH, vsebnost titrabilnih kislin, vsebnost hlapnih kislin, pufrno kapaciteto, sladkorja prostega ekstrakta, vsebnost alkohola, relativno gostoto, vsebnost reducirajočih sladkorjev, vsebnost prostega in skupnega SO_2, vsebnost fenolov, taninskih fenolov, intenziteto barve ter vsebnost nekaterih hlapnih aromatičnih snovi. Poleg tega smo vina tudi senzorično ocenili in rezultate statistično izvrednotili. Ugotovili smo, da uporaba različnih tehnoloških postopkov predelave grozdja in pridelave vina značilno vpliva na vse fizikalne in kemijske parametre vina. Analiza korelacijskih koeficientov je pokazala številne pozitivne in negativne korelacije med izmerjenimi parametri. Analiza glavnih komponent je pokazala, da je večina variabilnosti med vzorci posledica različne intenzitete barve ter različnih koncentracij fenolov metanola, etil acetata in izoamil acetata. Pri senzorični analizi so najboljše ocenjena vina, pridelana po tehnologiji zorenja vina na drožeh.

Ključne besede: vino, Malvazija, tehnološki postopki, klasična predelava, maceracija drozge, zorenje vina na drožeh, sur lie, kemijska sestava, kemijske lastnosti, senzorična kakovost

ABSTRACT

INFLUENCE OF DIFFERENT TEHNOLOGICAL PROCEDURES ON QUALITY OF MALVASIA WINE

White variety Malvasia (vintage 2007) from the winegrowing districts in Slovenian and Croatian Istria was chosen for the experiment. Three different technological procedures were included in the study: classical method, maceration and ageing wine on less. In some cases combinations of more than one technological were used (maceration and ageing on lees). The following parameters were determined: pH value, total acidity, volatile acid content, buffer capacity, extract , alcohol content, relative density, sugars level, free and total, SO_2 content, total phenols content, tannin content, colour intensity and content of some volatile aromatic compounds. Wines were also evaluated and the results were statistically analysed. The results showed that different technological procedures have significant influences on all physical and chemical parameters included in this question. Principal component analyses showed that the most of the variability among wine samples is due to colour intensity and contents of total phenols, methanol, ethyl acetate and isoamyl acetate. According to sensory evaluation the top grades were given to wines produced using wine ageing on lees.

Key words: wines, cv. Malvasia, winemaking, classical method, maceration, ageing on lees, sur lie, chemical composition, physico-chemical properties, sensory quality

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1 UVOD

Istra je vinorodno območje, na katerem uspevajo številne rdeče in bele sorte vinske trte. Ena izmed glavnih sort je Malvazija. Sorta, stara več tisoč let, se je iz Grčije razširila na vse kontinente. Udomačila se je na severnem Jadranu, dobila ime Istrska malvazija in postala sinonim za to območje. V zahodni Istri raste Malvazija na rdeči zemlji, na srednjem delu na beli zemlji in na območju slovenske Istre na laporju. Na istrskem polotoku zavzema Malvazija več kot polovica pridelka vseh sort grozdja (Hrček in Korošec-Koruza, 1996).

Novejše tehnologije pridelave omogočajo vinarjem, da pridelajo vina, bogata na ekstraktu in alkoholu. S sodobnim kletarjenjem se je Malvazija oddaljila od tradicionalnega načina pridelave, ki je dajala težka, oksidirana vina. Iz grozdja sorte Malvazija se lahko pridelajo mirna in suha, sladka ter celo peneča vina. V kategoriji mirnih suhih vin prevladuje klasičen postopek takojšnje predelave grozdja, ki daje lahka, sveža vina. Danes se vse več vinarjev odloča za novejše tehnologije pridelave, ki dajejo vina različnih senzoričnih lastnosti. Uporablja se kombinacija nerjavnega jekla in lesa (barrique, hrastov ali akacijev sod). Vse bolj pomembne postajajo tehnologije, ki vključujejo maceracijo grozdja ter zorenja vina na drožeh. Nekateri vinarji se odločajo za uporabo dolijev (ki jim ponekod pravijo tudi amfore). Malvazijo lahko po tradicionalni šampanjski metodi pridelajo tudi v kakovostno peneče vino. Takšna raznolikost pridelanih vin iz ene same sorte grozdja lahko ustreza številnih kulinaričnim in gastronomskim zahtevam ter vsekakor prispeva k bogastvu Istrskega polotoka.

V okviru naših raziskav smo se osredotočili na klasično pridelavo vina, obdelavo drozge z maceracijo in tehnologijo zorenja vina na drožeh. Nekatera vina so bila pridelana s kombinacijo več tehnologij. Klasičen postopek daje sveže, prijetno lahko vino, medtem ko drozga, tretirana s hladno maceracijo, poveča aromatičnost vina, intenzivnost vonja in okusa ter ohrani prijetno svežino vina. Pri predelavi drozge z daljšo maceracijo je ekstrakcija barvnih, aromatskih in taninskih snovi iz grozdne jagode intenzivnejša, saj poleg kožic v maceraciji aktivno sodelujejo tudi pečke. Pri zorenju vina na drožeh pride do interakcije med kvasovkami, organskimi in anorganskimi snovmi ter vinom. Ta tehnologija prispeva k različni senzorični strukturi vina, beljakovinski stabilnosti, stabilizaciji vina na vinski kamen in vezavi taninov, barvil in hlapnih aromatičnih spojin v stabilne komplekse. (Radeka in sod., 2007).

2 CILJ RAZISKAVE

Vina pridelana iz ene sorte grozdja se lahko senzorično močno razlikujejo. Cilj raziskave je senzorično vrednotenje vin sorte malvazija, ki so bila pridelana po različnih tehnoloških postopkih in tudi s pomočjo kemijskih analiz sestavin vina pokazati vpliv tehnologije na heterogenost končnega pridelka. Rezultati kemijskih analiz so lahko pokazatelj primernosti tehnološkega postopka, senzorična analiza pa kaže na primerno kakovost in senzorično sprejemljivost vina.

3 KLASIČEN POSTOPEK PRIDELAVE BELEGA VINA

Pot do dobrega vina se začne pri razmišljanju o zasaditvi vinograda, nadaljuje z zasaditvijo, predelavo grozdja, nego in zorenjem, ponudbo in prodajo vina ter konča šele v kozarcu porabnika. Celotna tehnologija predelave grozdja mora biti usmerjana k ustrezni kakovosti vina. Ob pravi obremenitvi trte in v ugodnih vremenskih razmerah je kakovost grozdja primerna. Obremenitev trte je odvisna od namena pridelave grozdja in ciljane kakovosti vina. Pridelava je lahko usmerjana v namizno, kakovostno ali vrhunsko kakovost vina. Za primerno kakovost vina je odločilno tudi zdravstveno stanje grozdja. Zato je potrebno skrbno in strokovno varstvo pred boleznimi in škodljivci.

Bela vina pridelujemo v svetu in pri nas po različnih tehnologijah in stilih pridelave. Nekateri

svetovni trendi se obračajo k izenačevanju in stalni kakovosti vina, ne glede na letnik. To pa je mogoče le ob dobrem poznavanju tehnologij in postopkov pridelave.

Klasičen postopek pridelave belega vina temelji na takojšnji predelavi grozdja: pecljanje, drozganje; nato stiskanje in bistrenje grozdnega soka tem čimprejšnji alkoholni fermentaciji. Po zaključenem alkoholnem vrenju sledi žveplanje in prvi pretok mladega vina, nato nekaj mesečno zorenje ter drugi pretok. Po stabilizaciji vina sledi še stekleničenje in predhodna filtracija (Ribéreau-Gayon in sod., 2006).

4 TEHNOLOGIJA PRIDELAVE VINA Z MACERACIJO DROZGE

Maceracija pomeni podaljšan stik grozdnega soka s trdimi deli grozdne jagode, predvsem z jagodno kožico, pa tudi s pečkami v drozgi. Postopek je splošno znan pri vinifikaciji rdečih vinskih sort, že dlje časa pa se uporablja tudi pri belih sortah. Glede na različno sestavo posameznih delov grozdne jagode je v maceriranih vinih pričakovati večjo ekstrakcijo barve, arom, taninov in drugih snovi iz grozdne jagode v mošt, v primerjavi z nemaceriranimi vini. Učinkovitost ekstrakcije posameznih snovi je odvisna od sorte, dozorelosti in zdravstvenega stanja grozdja ter pogojev med maceracijo (temperature, trajanja maceracije, načina ekstrakcije) (Ribéreau-Gayon in sod., 2006).

Vsebnost taninov v grozdju je odvisna od sorte in stopnje dozorelosti. Pečke vsebujejo največ taninov, zato je pomembno, da pri predelavi grozdja te ostanejo nepoškodovane. Zunanji deli pečk vsebujejo fenole, dušikove in mineralne snovi, ki so različno topne in zato med predelavo v manjši ali večji meri prehajajo v mošt. Sestavine, ki jih vsebuje notranjost pečk, vplivajo negativno na kakovost vina. Endosperm pečk vsebuje 10 – 22 % olja. Pri nepravilni predelavi (mehanske poškodbe) lahko v hl mošta preide tudi do 0,2 l olja. Z večjo količino olja je povezana tudi večja količina hlapnih kislin in estrov, ki izvirajo iz reakcij saponifikacije in oksidacije olja, kar povzroča neprijeten, milnat priokus (Falgue in sod., 2008).

Jagodna kožica vsebuje barvne snovi, katerih vsebnost je odvisna od letnika, stopnje zrelosti, zdravstvenega stanja grozdja, podnebja, obremenitve trte, predvsem pa od sorte. Rumena barvila so pri belih vinskih sortah v kožici in mesu grozdne jagode; največ jih je ob polni zrelosti grozdja. V jagodni kožici so nakopičene primarne aromatične snovi, ki dajejo grozdju izrazit in značilen vonj. Pri mnogih sortah se značilni vonj razvije šele pri pretvorbi mošta v vino. Primarne aromatične snovi, ki jih prinese grozdje s seboj, predstavljajo osnovo za tvorbo sortne cvetice. Sekundarne aromatične snovi se razvijejo med alkoholno fermentacijo, terciarne pa nastanejo med zorenjem vina. Primarne aromatične snovi se nahajajo zlasti v zunanjih celičnih plasteh jagodne kožice, tako da se v večji meri izločijo med maceracijo drozge. Količina aromatičnih snovi je odvisna od zdravstvenega stanja, stopnje zrelosti grozdja, klime ter vremenskih razmer. Iz nagnitega grozdja je nemogoče pridelati kakovostno sortno vino, ker bakterije in plesni z encimi uničijo primarne aromatične snovi (Košmerl, 2005).

Jagodna kožica je zelo bogata z encimi, predvsem z invertazami (saharaza), pektolitičnimi encimi pa tudi škodljivimi oksidazami. Zlasti neželen je encim polifenol oksidaza iz plastidov celic jagodne kožice. Stopnja poškodbe jagodne kožice pri drozganju, pecljanju, maceraciji in stiskanju neposredno vpliva na stopnjo oksidacijskih procesov. Pri predelavi grozdja imajo negativno vlogo zlasti oksidaze, ki posredujejo prenos in vezavo kisika na druge sestavine. Količina oksidaz je bistveno povečana pri močno nagnitem grozdju, zato taki mošti in vina hitreje oksidirajo. Polifenol oksidaze se nahajajo v jagodni kožici neposredno pod njo, zato vsebujejo macerirana vina in prešanci več teh encimov v primerjavi z nemaceriranimi vini. Delovanje polifenol oksidaz preprečimo z žveplanjem. Pektinaze imajo pomembno vlogo pri spontanem bistrenju moštov, saj razgradijo makromolekulo pektina do galakturonskih kislin. V vinarstvu uporabljamo pektolitične encime za povečanje izkoristka grozdnega soka, sproščanje barvnih in aromatičnih snovi iz jagodne kožice ter za izboljšanje bistrenja mošta in filtrabilnosti vina. Pektinaze dodajamo takoj po pecljanju in drozganju grozdja. (Darias-Martin J.J., 2000).

Podaljšan stik grozdnega soka z jagodno kožico omogoča obsežnejšo ekstrakcijo primarnih aromatičnih snovi, njihovih prekurzorjev, fenolnih in dušikovih spojin, polisaharidov, kislin in mineralov. Njihova vsebnost in razmerje med sestavinami določata značilno aromo posameznih vinskih sort. Grozdje, namenjeno maceraciji, mora biti povsem zdravo in nepoškodovano. Želeno je tudi, da je razmerje med jagodo in sokom čim večje oziroma, da so jagode majhne z debelo kožico. Postopek maceracije vpliva na lažji potek in dokončanje alkoholne in jabolčno-mlečnokislinske fermentacije zaradi večje koncentracije aminokislin, ki se pri maceraciji ekstrahirajo v vino. Mošt iz maceriranega grozdja v primerjavi s klasično pridobljenim moštom je bogatejši z nefermentabilnimi sladkorji in proteini (Ribéreau-Gayon in sod., 2006). Vina, pridelana z maceracijo drozge, so tako po vonju in okusu kompleksna in harmonična. Najpomembnejša parametra maceracije sta čas trajanja in temperatura. Pri krajših maceracijah (manj kot 10 ur) je temperatura odločujoč parameter, pri daljših maceracijah (več kot 20 ur) pa je bistveni parameter čas. Glede na čas alkoholne fermentacije ločimo predfermentativno, fermentativno in postfermentativno maceracijo.

4.1 Hladna maceracija bele drozge

Hladna maceracija je postopek, pri katerem se pri nižji temperaturi (5 – 12 °C) vzpostavi stik med grozdnim sokom in jagodno kožico za določen čas (do 48 ur). Osnovni namen hladne maceracije je povečanje aromatičnosti snovi vina, intenzivnosti vonja in okusa ter ohranjanje prijetne svežine. Pri belih vinih vpliva na izboljšanje sadnega karakterja in barve.

Eden od osnovnih pogojev za varno izvedbo hladne maceracije je odsotnost nečistoč (listja, zemlje). Grozdje, pripeljano iz vinograda, se mora čimprej ohladiti, ker je hitrost ohladitve izredno velikega pomena. Pri nižjih temperaturah je ekstrakcija fenolnih snovi manjša, zato so vina manj trpka in manj grenka. Vina, pridelana z hladno maceracijo drozge, vsebujejo več skupnih dušikovih snovi (aminokislin, vitaminov, encimov), maščobnih kislin, polifenolov v primerjavi z nemaceriranimi vini. Poveča se tudi vsebnost skupnega ekstrakta in pepela, intenziteta barve in vrednost pH. Zmanjša se vsebnost nekaterih polisaharidov (pektinov) in skupnih kislin, kar je posledica povečanja mineralnih snovi, ki povzročajo nastanek in izločanje soli vinske kisline. Taki mošti vsebujejo več višjih alkoholov (izoamilalkohola, 2-feniletanola, izobutanola,...) in estrov (etil acetata, izoamil acetata, etil laktata,...). Večja je tudi koncentracija aromatskih snovi, ki pri nemaceriranih vinih dosežejo vrednosti od 2,5 – 5 mg/l, medtem ko so pri maceriranih vinih te vrednosti znatno večje, od 100 – 400 mg/l (Gomez-Miquez in sod., 2005). Večja vsebnost nekaterih aldehidov in alkoholov je posledica encimske oksidacije linolne in linolenske kisline v jagodni kožici. V svetu je hladna maceracija trenutno vodilna pri pridelavi mladih, svežih in sadnih belih vin. Zavedati pa se moramo, da se stroški pridelave vina povečajo za 10 – 15 % (Vrščaj-Vodošek, 2004).

4.2 Maceracija bele drozge z alkoholno fermentacijo

Tehnologija daljše maceracije belih sort je bila v preteklosti označena kot neprimerna za kakovost belih vin. Danes jo uporabljajo kot alternativen način vinifikacije belih sort. Najpogosteje ga uporabljajo na področju severozahodne Istre, kjer macerirajo predvsem avtohtone sorte.(Radeka in sod., 2007)

V procesu daljše maceracije je ekstrakcija snovi iz grozdne jagode intenzivnejša, saj poleg kožic aktivno sodelujejo tudi pečke, svoje pa prispeva tudi naraščujoča vsebnost alkohola in sproščanje ogljikovega dioksida. Maceracija drozge z alkoholno fermentacijo spodbuja jabolčno-mlečnokislinsko fermentacijo in vpliva na bistrenje vina. Mošti po daljši maceraciji vsebujejo več aminokislin, maščobnih kislin in bistveno več polifenolov. Od polifenolov so najbolj problematični tanini, ker vinu dajejo trpek ali celo grenek priokus, kar je seveda nezaželeno. Količina in sestava taninov v grozdu je odvisna predvsem od sorte in razmer med dozorevanjem grozdja. V procesu zorenja grozdja se vsebnost taninov v pečkah in kožicah zmanjšuje. Tanini iz pečk delujejo v primerjavi s tanini iz kožic bolj grobo oziroma celo trpko. (Gomez-Miquez in sod.,2005).

Na količino in spekter spojin, ki se iz trdih delov grozdne jagode ekstrahirajo v mošt, močno vplivata temperatura in čas. Višja temperatura maceracije pospešuje ekstrakcijo taninov, poveča

občutljivost vina za oksidacijo, vpliva na intenziteto barve in poudari grob karakter vina. Tudi dolžina maceracije vpliva na sestavo vina, saj daljši čas maceracije povečuje vsebnost taninov. Najprej se v vino ekstrahirajo tanini iz jagodnih kožic, ki po okusu delujejo bolj mehko, vendar lahko ob nepolni zrelosti grozdja delujejo grenko. Pozneje se pri večji koncentraciji etanola ekstrahirajo še tanini iz pečk, ki so manj grenki, vendar bolj trpki. (Vrščaj-Vodošek T. 2004).

Zaradi večje vsebnosti taninov v belih vinih po daljši maceraciji in seveda zaradi trpkosti, je treba pri nadaljnji vinifikaciji sestavine čimbolj harmonizirati. Vinarju so tako v pomoč nekatere tehnologije, ki lahko bistveno pripomorejo k zmanjšanju grobega okusa belih vin in ohranijo ali celo poudarijo polnost in aromatski profil vina. To so tehnologija zorenja vina na drožeh ali pa barrique tehnologija. Pri tehnologiji zorenja na drožeh se tanini vežejo na manoproteine kvasovk, kar zmanjša občutek trpkosti in grenkobe vina. Pri barrique tehnologiji pa prihaja do povezave med tanini lesa in tanini vina, kar enako omili občutek trpkosti in grenkobe (Darias-Martin in sod., 2000).

4.2.1. Postmaceracija

Podaljšana klasična maceracija s potapljanjem »klobuka« traja tudi do enega meseca pri temperaturi 25 – 30 °C. Po končani alkoholni fermentaciji, ko se klobuk tropin potopi, poteče še postmaceracija. Proces traja nekako 7 – 14 dni, da do konca poteče biološki razkis. Osnovni namen postmaceracije je povečanje ekstrakcije taninov iz pečk. Vina, pridelana po takem postopku, ohranijo nekoliko več sortnosti, so ekstraktno bogata in pripravljena za nadaljnje zorenje. Ta metoda ekstrakcije pa se le redko uporablja

5 TEHNOGIJA PRIDELAVE VINA Z ZORENJEM NA DROŽEH

Zorenje vina na drožeh (sur lie) je star postopek v vinarstvu, ki je v današnjem času spet postal pomemben. Tradicionalno se uporablja pri belih vinih v Burgundiji, predvsem pri muškatnih vinih. V osnovi gre za podaljšan stik vina z drožmi. Vino po končani fermentaciji pustimo v manjšem lesenem sodu na usedlini od 3 – 6 mesecev ali celo dlje. V tem času pride do interakcij med kvasovkami, lesom in vinom. Ta tehnologija vpliva na stabilnost in senzorične lastnosti vina. Primerna je za bela in rdeča vina. V procesu ležanja na drožeh poteka avtoliza kvasovk in se v vino sprostijo sestavine citoplazme (peptidi, aminokisline, nukleotidi, kratkoverižne maščobne kisline) in celičnih sten (manoproteini, glukani, hitin). Hitrost avtolize je odvisna od temperature, pH, vsebnosti etanola in drugih dejavnikov, vendar je na splošno zelo počasna.(Vrščaj-Vodošek T. 2004).

Droži v vinu lahko razdelimo na t.i. grobe (težke) droži in fine (lahke) droži. Izjemoma v vinih bogatih z glukanom (plesen vrste *Botrytis cinerea*) in/ali s pektinom, ta delitev ni možna, saj omenjena polisaharida preprečujeta bistrenje in usedanje (Gomez-Miquez in sod., 2005).

5.1 Grobe droži

Težke ali grobe droži so delci, ki se usedejo na dno posode v 24 urah po končani fermentaciji. Njihova velikost je od 100 nm do 2 mm. Grobe droži belih in rose vin so sestavljene iz rastlinskega materiala, če ni bilo zadostno opravljeno samobistrenje mošta pred alkoholno fermentacijo. Vsebujejo tudi skupke kristalov vinskega kamna, odmrlih kvasnih celic in izločenih koloidnih delcev ter delce čistilnih sredstev. Grobe droži rdečih vin pa poleg naštetih še barvne snovi, tanine, koloide in snovi, ki nastanejo pri reakcijah med beljakovinami, polisaharidi in tanini med maceracijo. Med zorenjem vina se količina grobih droži zmanjšuje, spreminja pa se tudi njihova sestava (Vrščaj-Vodošek. 2004).

5.1.1 Nevarnost grobih droži

Grobe droži vplivajo na rastlinski in vegetativni značaj vina (po zelenem, po travi), potencirajo zaznavo trpkosti ali grenkega okusa (po pecljevini). V vsaki posamezni fazi zorenja je potrebno oceniti njihovo vsebnost in jih ustrezno odstraniti. Njihova prisotnost omogoča takojšnjo vezavo z dodanim žveplovim dioksidom v vezani žveplov dioksid, kar izniči antioksidativne in protimikrobne lastnosti prostega žveplovega

dioksida. Vezava grobih droži in prostega žveplovega dioksida omogoča preživetje nekaterih mikroorganizmov, ki lahko povzročijo kvar vina. Nujna sta ustrezna higiena in učinkovita zaščita vina z žveplovim dioksidom, predvsem zaradi mikroorganizmov rodov *Brettanomyces*, *Lactobacillus* in *Pedioccocus* (Košmerl, 2005).

6 MATERIAL IN METODE

Analizirali smo devet vzorcev vin malvazija, letnik 2007: šest vzorcev iz hrvaške Istre, tri iz slovenske Istre. Analizirani vzorci so bili pridelani po treh različnih tehnoloških postopkih: klasična (takojšnja) pridelava vina, predelava drozge z maceracijo in tehnologija zorenja vina na drožeh. Nekatera vina so bila pridelana s kombinacijo več tehnologij.

Tabela: Predelava grozdja in pridelava vina po različnih tehnoloških postopkih:
Vzorec 1: klasična predelava grozdja in pridelava vina
Vzorec 2: klasična predelava grozdja in pridelava vina
Vzorec 3: klasična predelava grozdja; 3 mesece zorenja vina na drožeh
Vzorec 4: hladna maceracija drozge, 24 ur pri 10 °C; klasična pridelava vina
Vzorec 5: maceracija drozge 15 dni pri 25 °C; 10 mesecev zorenja vina na drožeh in spontana jabolčno-mlečna fermentacija
Vzorec 6: maceracija drozge 42 dni pri 17 °C; 6 mesecev zorenja vina na drožeh
Vzorec 7: Maceracija drozge 15 ur pri 20 °C; 4 mesece zorenja vina na drožeh in vodena jabolčno-mlečna fermentacija
Vzorec 8: maceracija drozge 8 ur pri 12 °C; 6 mesecev zorenja vina na drožeh in vodena jabolčno mlečna fermentacija.
Vzorec 9: klasična predelava grozdja; 6 mesecev zorenja vina na drožeh.

Vzorce smo analizirali s fizikalno-kemijskimi metodami ter senzorično ocenili. Rezultate smo tudi statistično izvrednotili. Fizikalno-kemijske analize so bile naslednje: pH, titrabilne kisline, hlapne kisline, pufrna kapaciteta, relativna gostota, skupni ekstrakt in alkohol, reducirajoči sladkorji, žveplov dioksid, fenolne spojine, barva, hlapne snovi in višji alkoholi v alkoholnem destilatu (Košmerl in Kač, 2007)).

Senzorična analiza je potekala po Buxbaumovi metodi, kjer smo ovrednotili pet kakovostnih parametrov: bistrost, barvo, vonj, okus in harmonijo ter podali tudi opisno oceno okusa (Nemanič, 2006). Rezultate smo statistično obdelali z analizo variance, s korelacijsko analizo in z analizo glavnih komponent: pH, skupne in titrabilne kisline, hlapne kisline in pufrna kapaciteta (PCA-Principal component analysis) (Matlab Software, 2004).

7 REZULTATI

Izmerjene parametre smo statistično obdelali in predstavili kot rezultate analize variance (preglednica 1) ter analize glavnih komponent (slike 1-4). Zaradi majhnega števila in velike heterogenosti vzorcev (različna vhodna surovina, različni pogoji pridelave) ne moremo trditi, da smo s statistično obdelavo parametrov dobili povsem zanesljive podatke. Kljub temu smo z obdelavo podatkov dobili predstavo o vplivu tehnološkega postopka na kakovost vina.

Preglednica 1: Vpliv različnih tehnoloških postopkov na fizikalno-kemijske parametre vina malvazija, letnik 2007 (Duncanov test, $\alpha = 0,05$)

Parameter/enota	Vzorec 1	Vzorec 2	Vzorec 3	Vzorec 4	Vzorec 5	Vzorec 6	Vzorec 7	Vzorec 8	Vzorec 9	*p* vrednost
pH (/)	3,36 ± 0,00^{f}	3,41 ± 0,00^{cd}	3,49 ± 0,00^{a}	3,21 ± 0,00^{h}	3,28 ± 0,00^{g}	3,44 ± 0,00^{c}	3,36 ± 0,00^{f}	3,38 ± 0,00^{e}	3,49 ± 0,00^{b}	<0,0001
Titrabilne kisline TK1 (g/L)	4,68 ± 0,00^{g}	4,81 ± 0,00^{c}	5,18 ± 0,00^{c}	5,91 ± 0,02^{a}	5,14 ± 0,02^{d}	4,53 ± 0,01^{h}	4,74 ± 0,01^{f}	4,75 ± 0,01^{f}	5,43 ± 0,03^{b}	<0,0001
Titrabilne kisline TK2 (g/L)	4,98 ± 0,01^{g}	5,14 ± 0,01^{e}	5,52 ± 0,02^{c}	6,31 ± 0,03^{a}	5,45 ± 0,02^{d}	4,88 ± 0,01^{h}	5,05 ± 0,01^{f}	5,06 ± 0,01^{f}	5,74 ± 0,02^{b}	<0,0001
Hlapne kisline (g/L)	0,391 ± 0,013^{g}	0,509 ± 0,004^{e}	0,659 ± 0,007^{b}	0,588 ± 0,016^{c}	0,421 ± 0,006^{g}	0,559 ± 0,022^{d}	0,450 ± 0,000^{f}	0,500 ± 0,013^{e}	0,718 ± 0,016^{a}	<0,0001
Pufrna kapaciteta (mmol/L/pH)	31,3 ± 0,0^{g}	33,1 ± 0,0^{e}	34,6 ± 0,0^{c}	36,8 ± 0,1^{b}	34,2 ± 0,0^{d}	31,6 ± 0,1^{f}	31,3 ± 0,0^{g}	31,2 ± 0,0^{g}	41,7 ± 0,2^{a}	<0,0001
Relativna gostota (/)	0,98382 ± 0,00004^{d}	0,98399 ± 0,00000^{c}	0,98298 ± 0,00001^{i}	0,98304 ± 0,00007^{h}	0,98425 ± 0,00001^{b}	0,98238 ± 0,00001^{k}	0,98280 ± 0,00002^{g}	0,98359 ± 0,00002^{e}	0,98481 ± 0,00001^{a}	<0,0001
Sladkorja prosti ekstrakt (g/L)	16,85 ± 0,21^{g}	16,80 ± 0,21^{g}	19,18 ± 0,10^{b}	20,80 ± 0,10^{a}	17,03 ± 0,04^{cd}	17,73 ± 0,11^{d}	18,45 ± 0,07^{c}	17,25 ± 0,07^{e}	21,08 ± 0,04^{a}	<0,0001
Alkohol (vol%)	12,26 ± 0,03^{g}	12,11 ± 0,00^{g}	13,00 ± 0,01^{b}	12,94 ± 0,06^{c}	11,89 ± 0,01^{h}	11,98 ± 0,01^{h}	13,16 ± 0,02^{a}	12,43 ± 0,01^{d}	11,40 ± 0,01^{i}	<0,0001
Reducirajoči sladkorji (g/L)	1,9 ± 0,1^{de}	1,6 ± 0,1^{f}	2,5 ± 0,0^{b}	3,3 ± 0^{a}	2,0 ± 0,1^{d}	2,3 ± 0,0^{c}	1,8 ± 0,0^{e}	1,6 ± 0,0^{f}	1,5 ± 0,1^{g}	<0,0001
Prosti SO_2 (mg/L)	14 ± 0^{h}	38 ± 0^{g}	35 ± 1^{g}	15 ± 0^{g}	32 ± 1^{c}	21 ± 1^{e}	16 ± 0^{f}	25 ± 0^{d}	7 ± 0^{h}	<0,0001
Skupni SO_2 (mg/L)	102 ± 1^{c}	82 ± 1^{e}	111 ± 1^{b}	140 ± 2^{a}	58 ± 1^{g}	46 ± 1^{h}	74 ± 1^{f}	95 ± 1^{d}	57 ± 1^{g}	<0,0001
Skupni fenoli (mg/L)	250 ± 4^{g}	272 ± 3^{cd}	288 ± 5^{cd}	397 ± 6^{c}	291 ± 2^{c}	482 ± 5^{b}	317 ± 3^{g}	267 ± 6^{f}	603 ± 4^{a}	<0,0001
Taninski fenoli (mg/L)	137 ± 2^{g}	158 ± 5^{f}	163 ± 5^{e}	226 ± 6^{c}	165 ± 1^{e}	269 ± 10^{b}	180 ± 1^{d}	153 ± 4^{f}	371 ± 8^{a}	<0,0001
Intenziteta barve (/)	0,077 ± 0,001^{k}	0,078 ± 0,001^{k}	0,063 ± 0,001^{g}	0,115 ± 0,000^{d}	0,103 ± 0,001^{e}	0,191 ± 0,000^{b}	0,135 ± 0,001^{c}	0,061 ± 0,000^{g}	0,304 ± 0,002^{a}	<0,0001

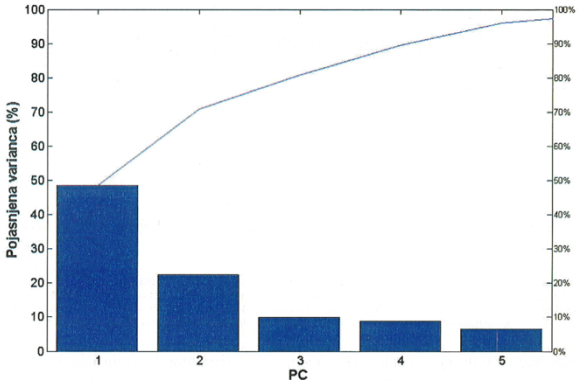
Preglednica 2: Koncentracije hlapnih snovi in višjih alkoholov (mg/L) v vinu malvazija, letnik 2007

Parameter (mg/L)	Vzorec 1	Vzorec 2	Vzorec 3	Vzorec 4	Vzorec 5	Vzorec 6	Vzorec 7	Vzorec 8	Vzorec 9
Acetaldehid	162.06	87.49	93.86	218.10	37.20	50.68	94.16	123.09	139.13
Metanol	60.06	49.15	50.38	78.82	80.44	107.22	57.17	52.51	114.50
1-propanol	21.94	22.36	24.38	20.41	20.14	21.29	27.09	21.39	29.84
Izoamilalkohol	119.84	107.40	100.15	148.52	151.18	162.30	119.95	137.03	164.90
2-feniletanol	4.53	/	/	6.70	7.17	5.03	2.28	5.97	16.45
Etil acetat	2.70	/	7.23	6.82	14.06	8.49	3.98	7.17	53.72
Izoamil acetat	30.57	31.12	32.53	34.19	34.91	36.07	30.46	32.25	45.06

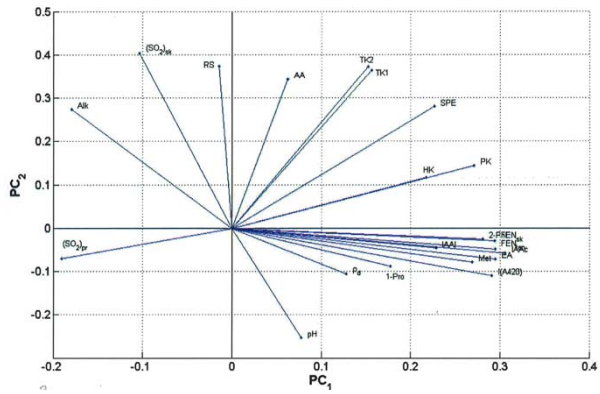
Iz preglednice 2 lahko zaključimo, da uporaba različnih tehnoloških postopkov predelave grozdja in pridelave vina vpliva na vsebnost hlapnih snovi in višjih alkoholov. Za skoraj polovico analiziranih vzorcev acetaldehid presega senzorični prag zaznave, ki je med 100 in 125 mg/L (Falgue in sod. 2008). Največjo koncentracijo metanola imajo vzorci, pridelani z maceracijo drozge (vzorci št. 4, 5 6) ter vzorec št. 9, ki je pridelan z zorenjem na drožeh. Noben vzorec ne presega dovoljene vrednosti metanola 150 mg/L (Falgue in sod. 2008). Največje koncentracije 1-propanola vsebujejo vina, pridelana z zorenjem na drožeh (vzorca št. 7, 9). Vina s predelavo drozge z maceracijo vsebujejo največ izoamilalkohola (cvetlična aroma), (vzorci št. 4. 5, 6) in 2-feniletanola (aroma po vrtnicah). Vzorec št. 9, ki je pridelan z zorenjem na drožeh, vsebuje značilno več 2-feniletanola v primerjavi z ostalimi vzorci. V vzorcih št. 2 in 3 je vsebnost 2-feniletanola pod mejo detekcije. Največje koncentracije etil acetata in izoamil acetata, ki dajeta vinu sadno aromo, smo določili v vinih, pridelanih z maceracijo drozge in z zorenjem vina na drožeh (vzorci št. 3, 5, 9).

Slika 1 prikazuje delež pojasnjene variabilnosti za pet najznačilnejših glavnih komponent (PC), pri čemer mora vsaka komponenta pojasniti najmanj

5 % variabilnosti pri vzorcih. Te glavne komponente so: pH, titrabilne kislilne, skupne in hlapne kisline, pufrna kapaciteta. Skupaj pojasnjujejo več kot 5 % variabilnosti (96,03 %). Zato smo prvih pet glavnih komponent uporabili za pojasnitev variabilnosti med vzorci. Preostalih 16 komponent lahko zanemarimo zaradi majhnega deleža variabilnosti, ki jo pojasnjujejo. Prva glavna komponenta (PC_1 pH) pojasnjuje skoraj 50 % skupne variabilnosti, druga glavna komponenta (PC_2 - titrabilne kisline) pa pojasnjuje več kot 20 % skupne variabilnosti. Vsaka naslednja PC pojasnjuje manj kot 10 % skupne variabilnosti in manj od predhodne.



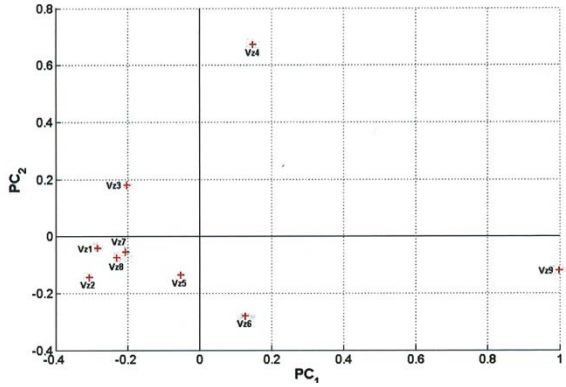
Slika 1: Delež pojasnjene variabilnosti za pet najznačilnejših glavnih komponent (PC).



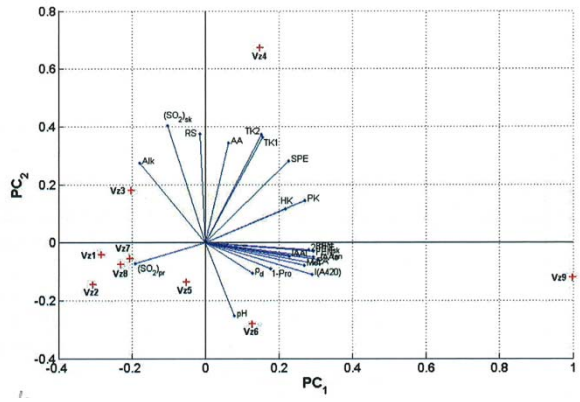
Slika 2: Dve različni glavni komponenti za izmerjene parametre v vinu malvazija, letnik 2007.

Dve najznačilnejši glavni komponenti, PC_1 in PC_2, ki pojasnjujeta 70,9 % variabilnosti v vzorcih vina malvazija sta predstavljeni na sliki 2. Prva glavna komponenta (PC_1) pojasnjuje največ variabilnosti. Na njo najbolj vplivajo intenziteta barve, vsebnosti fenolov, metanola, etil acetata in izoamil acetata. Ta podatek se ujema z dejstvom, da smo tudi v originalnih podatkih pri omenjenih parametrih ugotovili večjo variabilnost. Na drugo glavno komponento (PC_2) vplivajo predvsem vsebnosti skupnega žveplovega dioksida, reducirajočih sladkorjev, titrabilnih kislin in acetaldehida.

Slika 3 prikazuje variabilnost med analizirani vzorci vina malvazija, ki so opisani s prvima dvema glavnima komponentama, PC_1 in PC_2. Razvidno je, da se vzorec št. 9 (vino, pridelano po tehnologiji zorenja na drožeh) značilno razlikuje od ostalih vzorcev ter nosi največji delež variabilnosti med vzorci. Če ta podatek primerjamo z izmerjenimi parametri v vzorcih, lahko opazimo, da smo v tem vzorcu izmerili največ ekstremnih vrednosti med parametri (najpogostejše maksimalne vrednosti).



Slika 3: Dve najznačilnejši glavni komponenti (PC_1, PC_2) za analizirane vzorce vina malvazija, letnik 2007.



Slika 4: Dve najznačilnejši glavni komponenti (PC_1, PC_2) za parametre in vzorce vina malvazija, letnik 2007.

Na sliki 4 smo predstavili kombinacijo prvih dveh glavnih komponent za izmerjene parametre in za vzorce vina malvazija. Vzorec št. 9 (vino, pridelano po tehnologiji zorenja na drožeh) kaže v primerjavi z ostalimi vzorci intenzivnejšo barvo ter veliko vsebnost fenolov, metanola, etil acetata in izoamil acetata. To nazorno kaže tudi slika 4, saj se vzorec št. 9 in omenjeni parametri nahajajo v istem kvadrantu diagrama. Vzorec št. 4 (vino, pridelano z maceracijo drozge) kaže velike vsebnosti skupnega žveplovega dioksida, reducirajočih sladkorjev, titrabilnih kislin ter acetaldehida. Ta dva vzorca največ doprineseta k variabilnosti vzorcev.

Preglednica 3: Rezultati senzoričnega ocenjevanja vin malvazija po Buxbaumovi metodi.

Senzorični parameter	Vzorec 1	Vzorec 2	Vzorec 3	Vzorec 4	Vzorec 5	Vzorec 6	Vzorec 7	Vzorec 8	Vzorec 9
Bistrost (0 – 2)	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
Barva (0 – 2)	2,0	2,0	2,0	2,0	2,0	1,5	2,0	2,0	2,0
Vonj (0 – 4)	3,5	3,3	3,4	3,5	3,3	3,2	3,5	3,3	3,6
Okus (0 – 4)	5,0	4,9	5,4	5,2	5,2	5,5	5,4	5,3	5,4
Harmoničnost (0 – 6)	5,3	4,9	5,2	5,2	5,0	5,2	5,3	5,3	5,1
Senzorična ocena (0 – 20)	17,8	17,1	18,0	17,9	17,5	17,4	18,2	17,9	18,1

Preglednica 3 prikazuje rezultate senzoričnega vrednotenja vin malvazija, ki so bili pridelani po različnih tehnoloških postopkih. Zaradi standardizacije rezultatov smo vsem vzorcem pripisali maksimalno število točk za bistrost vina. Pri vrednotenju barve so vsa vina, z izjemo vzorca št. 6, prav tako dosegle maksimalno število točk. Vzorec št. 6 v času ocenjevanja še ni bil filtriran in je bil to moteč dejavnik pri ocenjevanju barve. V vrednotenju vonja je najbolje ocenjeno vino, pridelano s tehnologijo zorenja na drožeh (vzorec št. 9), vendar so tudi vina, pridelana po drugih tehnologijah, ocenjena z dokaj visokimi ocenami. Vina, zorena na drožeh, so dosegla najboljše ocene tudi pri vrednotenju okusa, (vzorci št. 3, 7, 8, 9) za njimi so vina, pridelana z maceracijo drozge in najslabše so ocenjena vina, pridelana po klasičnem postopku takojšnje predelave. Harmonija vina je bila najbolje ocenjena pri vzorcu št. 1, pridelanem po klasičnem postopku ter pri vzorcih št. 7 in 8, ki sta bila pridelana z zorenjem vina na drožeh. Tako so največjo končno oceno dosegla vina, pridelana po tehnologiji zorenja na drožeh, kar potrjuje našo hipotezo, da zorenje na drožeh pozitivno vpliva na senzorične lastnosti vina.

8 SKLEPI

- Različni tehnološki postopki značilno vplivajo na vse merjene fizikalno-kemijske parametre v analiziranih vzorcih.
- Največji vpliv na variabilnost vin imajo intenziteta barve, vsebnost fenolnih spojin, metanola, etil acetata in izoamil acetata. Na variabilnost značilno vplivajo še vsebnosti žveplovega dioksida, reducirajočih sladkorjev, titrabilnih kislin in acetaldehida.
- Vino, ki nosi največ variabilnosti med vzorci, je vino, pridelano po tehnologiji zorenja na drožeh. Temu sledi vino, pridelano s hladno maceracijo drozge.
- Parametri, ki najbolj značilno vplivajo na variabilnost vina, so povezani z načinom pridelave in dodatkom mlečnokislinskih bakterij. Preostalo variabilnost med vzorci lahko razložimo z dejstvom, da je pridelano grozdje iz različnih vinogradniških območij in da se tehnologije trgatve in pogoji med vinifikacijo razlikujejo.
- Vina, pridelana z maceracijo drozge, vsebujejo največ skupnih in taninskih fenolov. Takšen način pridelave značilno vpliva na povečano vsebnost metanola in izoamilalkohola. Maceracija grozdja vpliva na kompleksnejši vonj in okus vina. Ocenjevalci so opisali aromo v maceriranih vinih kot zrelo aromo po suhih sadežih.
- Vina, pridelana z zorenjem na drožeh, so senzorično najboljše ocenjena. Opisana so kot harmonična z izrazno sortno sadno, cvetlično aromo. Najboljše ocenjeno vino je pridelano s kombinacijo predelave drozge s hladno maceracijo in zorenjem vina na drožeh.
- Vina, pridelana po klasičnem postopku takojšnje pridelave, vsebujejo relativno manj višjih alkoholov in njihovih estrov ter so senzorično slabše ocenjena.
- Iz povedanega lahko zaključimo, da je za optimalno kemijsko sestavo in senzorično kakovost vina sorte malvazija hladna maceracija drozge in zorenje vina na drožeh najboljši tehnološki način, s predpostavko, da je grozdje zdravo in potrgano v polni zrelosti.

9 VIRI

Darias-Martin J.J., Rodriguez O., Diaz E., Lamuela-Raventos R.M. 2000. Effect on skin contact on the antioxidant phenolics in white wine. Food Chemistry, 71: 483 – 487.

Falgue E., Darriet P., Fernandez E., Dubourdieu D. 2008. Volatile profile and differentiation between Albarino wines from defferent origins. International Journal of Food Science and Technology, 43: 464 – 475.

Gomez-Miquez M.J., Gonzales-Miret M.L., Hernanz d., Fernandez M.A., Vicario I.M., Heredia F.J. 2005. Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. Journal of Food Engineering 78: 238 – 245.

Hrček L., Korošec-Koruza Z. 1996. Sorte in podlage vinske trte: Ilustrirani prikaz trsnega izbora za Slovenijo. Ptuj, SVA Veritas: 79 – 81.

Košmerl T. 2005. Hladna maceracija in biološki razkis vina. Sodobno kmetijstvo, 38,4: 10 – 12.

Košmerl T. Kač M. 2007. Osnovne kemijske analize mošta in vina: laboratorijske vaje za predmet Tehnologija vina. 3. izd. Ljubljana, Biotehniška fakulteta, Oddelek za živilstvo: 106 str.

Matlab Software. Version 7.0.1.24704.2004. Natick, The Math Works, Inc.: software

Nemanič J., 2006 Ali razumemo vino., Ljubljana, Kmečki glas: 279 strani

Radeka S., Herjavec S., Peršurić Đ., Lukić I., Sladonja B. 2007. Effect of different maceration treatments on free and bound varietal aroma compounds in wine of Vitis vinifera L. cv. Malvazija istarska bijela. Food Technology and Biotechnology, 46, 1: 86 – 92.

Ribéreau-Gayon P., Dubourdieu D., Doneche B., Lonvaud A. 2006 Handbook of enology. Vol. 1: The microbiology of wine and vinifications. 2 nd ed. Chichester, John Wiley & Sons, Ltd: 79 – 113, 414 – 417.

Vrščaj-Vodošek T. 2004. Vpliv vinifikacije na vsebnost dušikovih spojin v vinih malvazija in chardonnay. Magistrsko delo. Ljubljana, Biotehniška fakulteta, Oddelek za živilstvo: 18 – 29.

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Alternativni načini zatiranja strun (Coleoptera, Elateridae) na njivah

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IZVLEČEK

Strune, ličinke hroščev pokalic (Coleoptera: Elateridae), imajo velik gospodarski pomen v pridelavi živeža in krme v Sloveniji, drugih državah Evrope in na drugih celinah. Zaradi dokazanih negativnih učinkov sintetičnih insekticidov, se za zatiranje strun vse bolj ne le raziskujejo, ampak tudi uporabljajo alternativne metode . Med njimi je vse več v uporabi biofumigacija, ki je največkrat predstavljena kot uspešna alternativa sintetičnim fumigantom. V tej zvezi so križnice lahko v tla vnesene na več načinov. Med najbolj učinkovite spada uporaba moke iz semena, saj je prav v semenu koncentracija glukozinolatov največja. S kolobarjem in privabilnimi posevki izkoriščamo rastlinske izločke, ki z alelopatijo ali na kakšen drug način zmanjšujejo vitalnost strun ali jih na ta način odvračajo od glavnih posevkov. Privabilni in varovalni posevki pa varujejo tudi tla pred erozijo in zmanjšujejo gospodarski pomen plevelov. Poplavljanje njiv je zaradi potrebe po dostopu vode zahtevno, pri tem postopku pa imata velik pomen dolžina poplavljanja in temperatura vode. Medtem ko je v drugih evropskih državah dostopnost sintetičnih insekticidov za zatiranje strun nekoliko večja, pa je v Sloveniji registriran le sintetični insekticid teflutrin in entomopatogena gliva *Beauveria bassiana*. Našim bralcem in pridelovalcem želimo predstaviti alternativne možnosti zatiranja strun. V prispevku je predstavljeno 10 takšnih načinov, ki so se v raziskavah na različnih delih sveta pokazali kot učinkoviti in bi lahko našli mesto tudi na njivah v Sloveniji.

Ključne besede: strune, Elateridae, *Agriotes* spp., zatiranje, njive, privabilni posevki, varovalni posevki, biofumigacija, odporne sorte, kolobar, biotično varstvo rastlin, mineralna gnojila, poplavljanje, tretirano seme, mehanična obdelava, semiokemikalije

ABSTRACT

ALTERNATIVE METHODS FOR CONTROLLING WIREWORMS (Coleoptera, Elateridae) IN THE FIELDS

Wireworms, the larvae of the click beetles (Coleoptera: Elateridae), have a large economic impact in food and fodder production in Slovenia, other European countries, as well as in other continents. Because of proved negative effects of synthetic insecticides, alternative methods for cotrolling wireworms are not only studied but also used in common practice. Among the methods mentioned biofumigation is more and more commonly used as alternative to synthetic fumigants. In the process of biofumigation the Brassicas can be incorporated into the soil in different ways; one of the most effective way is the use of Brassicaceous seed meals, since the concentration of the glucosinolates are the highest in the seeds. With the use of crop rotation and trap crops we exploit the plant secretions, which have the ability (allelopatic etc.) to diminish the vitality of the wireworms or to repel them from the main crops. Trap crops and cover crops protect the soil against erosion and they also diminish the economic impact of weeds. Flooding of the fields is often pretentious measure, owing to necessity of water access; time interval of flooding and water temperature are important factors of this alternative method. In other European countries the number of registered synthetic insecticides against the wireworms is higher compared to Slovenia, where only tefluthrin and entomopathogenic fungus *Beauveria bassiana* are registered for the same purpose, therefore we would like to present different alternatives to insecticies to our readers and also to food and fodder producers. In this article 10 alternative methods, which showed the efficacy in diminishing the economic importance of wireworms in different parts of the world, are presented. At least some of them have a potential to become a part of sustainable strategies for controlling wireworms in Slovenia.

Key words: wireworms, Elateridae, *Agriotes* spp., control, fields, trap crops, cover crops, biofumigation, resistant varieties, rotation, biological control, mineral fertilizers, flooding, seed treatment, mechanical cultivation, semiochemicals

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1 UVOD

V Sloveniji je bilo doslej ugotovljenih 140 vrst pokalic (Coleoptera: Elateridae), medtem ko podatki za Evropo potrjujejo zastopanost 176 vrst (Milevoj et al., 2005). Pokalice rodu *Agriotes* predstavljajo eno od gospodarsko pomembnejših skupin škodljivcev v Evropi (Furlan et al., 2010) in Severni Ameriki (Milonas et al., 2010). V Evropi se pojavlja okrog 40 vrst tega rodu, med seboj pa se precej razlikujejo tako po bionomiji kot škodljivosti. V domači raziskavi v obdobju 2002-2004 je bilo z uporabo feromonskih vab ugotovljeno, da se v Sloveniji najštevilčneje pojavlja poljska pokalica (*A. lineatus* [L.]), sledi ji vrsta *A. brevis* Candèze, manj številčne pa so solatna pokalica (*A. sputator* [L.]), motna pokalica (*A. obscurus* [L.]) in žitna pokalica (*A. ustulatus* [Schaller] (Milevoj et al., 2005). V Avstriji so na kmetijskih zemljiščih ugotovili največjo razširjenost strun *A. obscurus*, *A. sputator*, *A. brevis*, *A. ustulatus*, *A. lineatus* in *A. proximus* (Schwarz). Tretja in četrta vrsta se v največjem obsegu pojavljata na toplejših (sušnejših) lokacijah in v alkalnih tleh, prva, peta in šesta vrsta pa v višjih legah, kjer je več padavin in v bolj kislih tleh, bogatih s humusom. Druga vrsta je razširjena po vsej Avstriji (Landl et al., 2010; Staudacher et al., 2012). Strune treh vrst, *A. obscurus*, *A. sputator* in *A. lineatus*, so v Veliki Britaniji zelo pomembni škodljivci krompirja (Parker in Howard, 2001).

Strune povzročajo izpad pridelka na njivah, kjer so posejana žita in druge poljščine, krmne rastline in zelenjava. Strune žita lahko poškodujejo že takoj po setvi, njihovo prehranjevanje pa traja vse do razraščanja. Zavrtajo se v nabrekla semena in izjedo kalčke. Lahko tudi prežvečijo in izsesajo koreninski vrat (Vrabl, 1992). Posebno škodljive so na krompirju (*Solanum tuberosum* L.) in koruzi (*Zea mays* L.); pri prvem povzročajo »kozmetične poškodbe« na gomoljih (izvrtine vplivajo na tržno manj zanimiv pridelek) (Parker in Howard, 2001; Johnson et al., 2008), pri drugi pa izjedo kaleča zrnja ali se zavrtajo v mlade rastline, kar posledično vpliva na manjšo gostoto rastlin (Waliwitiya et al., 2005; Noronha, 2011; Hermann et al., 2012). Omenjeni polifagi so škodljivi tudi na korenju, jagodah in nekaterih drugih poljščinah in vrtninah (Vernon et al., 2008).

Kadar strune iščejo hrano, se gibljejo vodoravno, glede na koncentracijo ogljikovega dioksida in rastlinske izločke. Ličinke se prehranjujejo le s tekočo hrano, ki jo dobijo tako, da zmečkajo in prežvečijo precej rastlinskega tkiva. Zato kažejo posebno preferenco do mladih, nežnih tkiv, z veliko vsebnostjo vode. Značilno za strune je, da rastline vedno napadajo v večjih ali manjših otokih na posameznih parcelah in da niso nikoli povsem enakomerno razporejene (Vrabl, 1992).

Strune so škodljivci z zelo dolgim razvojnim krogom, kar otežuje njihovo zatiranje (Noronha, 2011). Na njivah s krompirjem se začnejo strune pojavljati v poznem poletju in se z gomolji prehranjujejo do spravila pridelka. Med najuspešnejše načine zatiranja strun v preteklosti so uvrščali uporabo insekticidov z dolgotrajnim delovanjem, ki so bili navadno vdelani v tla pred sajenjem (Piqué et al., 1998; Kuhar in Alvarez, 2008). Prepoved uporabe učinkovitih insekticidov iz skupin kloriranih ogljikovodikov, organskih fosforjevih estrov in karbamatov je vplivala na iskanje novih aktivnih snovi, ki bi lahko uspešno nadomestile omenjene insekticide (van Herk et al., 2008; Van Herk in Vernon, 2011). Med učinkovitimi nadomestki so bili neonikotinoidi (Van Herk et al., 2008; van Herk in Vernon, 2011), za katere pa je bilo ugotovljeno, da imajo neželeno neciljno delovanje na čebele (Vidau et al., 2008; Gentz et al., 2010).

V Sloveniji se je število aktivnih snovi, registriranih za zatiranje strun, v zadnjih 17 letih zmanjšalo za sedemkrat. Leta 1995 (Priročnik o fitofarmacevtskih sredstvih…, 1995) je bilo v ta namen registriranih 13 aktivnih snovi, v letih 1999 (Priročnik o fitofarmacevtskih sredstvih…, 1999) in 2002 (Priročnik o fitofarmacevtskih sredstvih…, 2002) sedem aktivnih snovi, trenutno (Seznam registriranih fitofarmacevtskih sredstev…, 2012) pa sta za zatiranje strun na voljo le še piretroid teflutrin in entomopatogena gliva *Beauveria bassiana*.

V pričujočem članku želimo predstaviti alternativne načine zatiranja strun, saj je zaradi vse manjšega števila insekticidov, ki so registrirani za njihovo zatiranje, gospodarski pomen strun vse

večji, posledično pa je vse bolj izražena potreba po novih – alternativnih – načinih njihovega zatiranja.

2 ALTERNATIVNI NAČINI ZATIRANJA STRUN

2.1 Privabilni posevki (trap crops) in varovalni posevki (cover crops)

Različne izvedbe metod privabilnih posevkov so se doslej izkazale za učinkovite pri zmanjševanju številčnosti in/ali škodljivosti različnih vrst ali skupin škodljivcev na rastlinah glavnih posevkov - tudi pri strunah. V enem od poskusov so intenzivnost prehranjevanja strun v nasadih jagod uspešno zmanjšali s setvijo pšenice (*Triticum aestivum* L.) in ovsa (*Avena sativa* L.) med gredice z jagodami. Ugotovili so namreč, da kaleče seme omenjenih vrst žit privablja strune in jih s tem posledično odvrača od jagod (Vernon, 2005). Ker obstaja bojazen, da bi se z omenjeno metodo število strun in njihova številčnost v tleh le še povečevala, so del semena, uporabljenega v poskusu, tretirali z insekticidom, ki je kot aktivno snov vseboval 3 % teflutrina. Ugotovili so, da je bila smrtnost strun v obravnavanjih s tretiranim semenom zelo visoka, medtem ko je bilo netretirano seme žit zelo močno poškodovano (Vernon, 2005).

Tudi z grahom (*Pisum sativum* L.) kot privabilnim posevkom za strune se lahko poškodovanost gomoljev krompirja zaradi napada teh škodljivcev občutno zmanjša. Pri preizkušanju pšenice, oljne redkve (*Raphanus sativus* var. *oleiformis* Pers.) in graha, se je prav slednja rastlinska vrsta izkazala za najbolj učinkovito pri privabljanju strun. Privabilne posevke so v omenjenem primeru posejali tri mesece po sajenju krompirja. Avtorji sklepajo, da je večja afiniteta strun do korenin graha povezana z njihovim izločanjem lahko dostopnih sladkorjev, ki so za škodljivce bistveno bolj privlačni od glikoalkaloidov in klorogene kisline, ki se pojavljajo v zunanji plasti krompirjevih gomoljev. Ker pa je privabljanje strun s strani izločkov graha časovno omejeno, avtorji predlagajo setev te poljščine približno dva tedna pred pobiranjem krompirja, ko gomolji krompirja že prenehajo z rastjo in izločanjem CO_2, v njih pa se zmanjša tudi vsebnost sladkorjev (Landl in Glauniger, 2011).

V intenzivni pridelavi krompirja je možna tudi uporaba varovalnih posevkov. Bistvena naloga teh je varovanje tal pred erozijo, zmanjšanje zapleveljenosti njive, lahko pa služijo tudi kot podor (Crow et al., 2001). V enem od poskusov so na njivi s krompirjem takoj po spravilu pridelka kot varovalni posevek posejali hibrid sirka *Sorghum bicolor* (L.) Moench in sudanske trave *S. arundinaceum* (Desv.) Stapf var. *sudanense* (Stapf) Hitchc. Z omenjeno setvijo so vplivali na manjšo škodljivost strun na gomoljih krompirja v naslednjem letu (Jansson in LeCrone, 1991).

2.2 Biofumigacija

Biofumigacija je novejši okoljsko sprejemljivejši način zatiranja strun, uporabna pa je tudi proti drugim škodljivim talnim organizmom. Prve poglobljene raziskave o uporabnosti te metode v varstvu rastlin datirajo v konec 90-ih let prejšnjega stoletja (Sarwar in Kirkegaard, 1998), ko je bil predstavljen postopek razgradnje rastlinske gmote križnic v tleh. Biofumigacija temelji na uporabi rastlin iz družine križnic (Brassicaceae) za potrebe zatiranja talnih škodljivcev (De Nicola et al., 2013), talnih patogenov in plevelov (Mattner et al., 2008). Talna razgradnja (hidroliza) glukozinolatov, kot tipičnih sekundarnih metabolitov v križnicah, omogoča uspešno izrabo omenjenih rastlin kot alternativo metil bromidu (Lazzeri et al., 2004). Produkti, ki nastanejo pri razgradnji glukozinolatov - izotiocianati in v manjši meri nitrili in epitionitrili - imajo namreč izrazito insekticidno delovanje. Biofumigacija temelji na vnosu (inkorporaciji) rastlinske gmote križnic z različnimi načini – podor, vnašanje posušene rastlinske gmote oziroma moke iz semena križnic (ta zaradi predhodnih postopkov ne vsebuje olja) – v tla (De Nicola et al., 2013).

S postopkom biofumigacije se poveča vnos rastlinske gmote v tla, kar pripomore tudi k večji rodovitnosti tal (De Nicola et al., 2013). V zadnjih letih je bilo več raziskav usmerjenih v delovanje vodne suspenzije semenske moke abesinske ogrščice (*B. carinata* Braun). Ugotovljeno je bilo, da je mogoče omenjeno raztopino uporabljati tudi med rastno dobo, možen pa je večkratni vnos ali kombinirana uporaba z nekaterimi talnimi insekticidi (De Nicola et al., 2013).

Furlan je s sodelavci (2004) poročal o visoki smrtnosti strun zaradi vnosa 18 ton posušene rastlinske gmote križnic na hektar. Učinek je bil enak delovanju 3-6 ton moke semena/ha. Insekticidno delovanje semenske moke se je po 72 h občutno zmanjšalo. Želena smrtnost strun je bila z uporabo semenske moke dosežena tedaj, ko so v tla vnesli 160 µmol glukozinolata/ liter tal. Da bi z uporabo rastlinske gmote križnic na hektar vnesli isto množino glukozinolatov kot z vnosom moke semen, je potrebna večja količina rastlin (Furlan et al., 2010). Kot ozadje omenjeni trditvi izpostavljamo predvsem dejstvo, da je koncentracija glukozinolatov največja prav v semenih (Hoagland et al., 2008). Za namen biofumigacije se največkrat uporabljata rjava (*Brassica juncea* [L.] Czem.) in abesinska ogrščica (*Brassica carinata*) (Noronha, 2011). Priporočilo, da z vnosom rastlin, ki imajo visoko vsebnost glukozinolatov, dosežemo tudi višje vrednosti izotiocianatov v tleh, velja upoštevati. V času vnosa rastlinske gmote v tla (zaoravanje) pa je potrebno velikokrat tla še dodatno zaliti (Morra in Kirkegaard, 2002).

S pomočjo biofumigacije lahko uspešno zatremo tudi plevele (zmanjšana kalitev semen) (Hoagland et al., 2008) in talne glive v tleh (Mattner et al., 2008), kot so vrste *Fusarium* spp., *Pythium* spp., *Phytophthora* spp. (Cheah et al., 2008) ter *Rhizoctonia solani* (Cohen et al., 2005).

2.3 Odporne sorte

Poleg različne dovzetnosti različnih rastlinskih vrst na napad strun (Willis et al., 2010) je bilo ugotovljeno, da dovzetnost rastlin za omenjene škodljivce niha tudi znotraj rastlinske vrste (Parker in Howard, 2001; Johnson et al., 2008). Raziskava, ki sta jo v letu 1995 izvedla Olsson in Jonasson, je kot vir odpornosti krompirja na napad strun navajala vsebnost glikoalkaloidov (Johnson et al., 2008). Glikoalkaloidi so v omenjeni raziskavi obravnavani kot rastlinski toksini z antimikrobnim, insekticidnim in fungicidnim delovanjem (Nema et al., 2008). Sorte krompirja, ki vsebujejo nizke vrednosti glikoalkaloidov, naj bi bile bolj dovzetne za napad strun. Na prehranjevanje strun naj bi delovala stimulativno tudi vsebnost sladkorjev, vendar za omenjeno še ni trdnih dokazov (Johnson et al., 2008). Večji pojav strun v tleh pa lahko vpliva tudi na zmanjšanje pridelka drugih rastlinskih vrst; v eni od raziskav je bil ta pri sladkornem trsu – v odvisnosti od kultivarja - zmanjšan za 40-60 % (Larsen et al., 2012).

2.4 Kolobar

Teorija, da na zmanjšanje populacij strun v tleh vpliva tudi travinje kot večletni predhodni posevek, se vse bolj kaže za zmotno (van Herk in Vernon, 2006; Hermann et al., 2012). To je bilo ustrezno upoštevano tudi v tehnoloških navodilih za integrirano pridelavo zelenjave in poljščin za leto 2012 (Tehnološka navodila …, 2012). V eni od raziskav v ZDA so preučevali vpliv predhodnih rastlinskih vrst na številčnost strun v posevku ovsa (*Avena sativa* L.). Kot predhodne posevke so uporabili bombaž (*Gossypium* spp.), sladki krompir (*Ipomoea batatas* [L.] Lam.), tobak (*Nicotiana* spp.), koruzo in sojo (*Glycine max* (L.) Merr.). Ugotovili so, da slednji rastlinski vrsti vplivata stimulativno na število strun v tleh, medtem ko med vplivom bombaža, sladkega krompirja in tobaka kot predhodnih posevkov ni bilo signifikantnih razlik (Willis et al., 2010).

Uporaba kolobarja je dolgo časa veljala za uspešen ukrep zatiranja strun; predvsem kot alternativa intenzivni rabi insekticidov oziroma pojavu rezistence škodljivcev nanje (Noronha, 2011). Kljub malo spodbudnim podatkom o vplivu kolobarja na zmanjševanje pojava strun v tleh, lahko z uporabo različnih vrst gorjušic, rjave gorjušice in črne gorjušice (*Brassica nigra* L.), lucerne (*Medicago sativa* L.) ter navadna ajde (*Fagopyrum esculentum* Moench) vplivamo na manjše število teh talnih organizmov. Lucerna je zaradi sposobnosti, da iz tal absorbira odvečno vlago, obravnavana kot rastlinska vrsta, ki vpliva na manjši pojav strun v tleh. Delovanje navadne ajde na strune je bilo doslej preučevano predvsem z vidika alelopatskega delovanja, rezultati nekaterih raziskav pa kažejo na uspešno insekticidno delovanje rjave gorjušice in navadne ajde, če sta bili vključeni v kolobar pred sajenjem krompirja; obseg poškodb zaradi strun na gomoljih krompirja je bil namreč v takšnih tleh precej manjši (Noronha, 2011). Alelopatsko delovanje navadne ajde na rastline, posajene blizu nje, je pogojeno predvsem z delovanjem alkaloidov, zlasti fagomina, 4-piperidona in 2 piperidinemetanola (Iqbal et al., 2002).

2.5 Biotično varstvo

Uporaba entomopatogene glive *Metarhizium anisopliae* (Metsc.) Sorokin (Hypocereales: Clavicipitaceae) predstavlja eno od uspešnih alternativ v varstvu rastlin pred napadom strun (Kabaluk in Ericsson, 2007), kajti nanos mikrobiotičnega insekticida z omenjeno glivo kot aktivno snovjo, je mogoč tudi na njivi. Ugotovljeno je bilo, da na delovanje omenjenega agensa pomembno vplivajo abiotični in biotični dejavniki, kot so temperatura, vlaga v tleh ter dostopnost njihovih žrtev v tleh. Rezultati enega od poljskih poskusov kažejo na najboljše delovanje glive pri temperaturah višjih od 18 °C, izpostavljenost glivi vsaj 48 ur pri 18 °C pa vpliva na 100 % smrtnost strun (Kabaluk in Ericsson, 2007).

Rezultati preučevanja delovanja entomopatogene glive *Beauveria bassiana* (Bals.-Criv.) Vuill. na ličinke vrste *Agriotes lineatus* niso pokazali znakov patogeneze. Na drugi strani je delovanje glive *Metarhizium anisopliae*, sevov V1002 in LRC181A, povzročilo 90 in 100 % smrtnost 3 tedne po inokulaciji. Prav tako se uporaba entomopatogene glive *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm. ni izkazala za učinkovito pri zatiranju vrste *Agriotes lineatus* (Ansari et al., 2009). Med entomopatogenimi ogorčicami je pri zatiranju ličink vrste *Agriotes lineatus* najboljšo učinkovitost pokazala vrsta *Heterorhabditis bacteriophora* (sev UWS1) s povzročeno 67 % smrtnostjo po treh tednih (Ansari et al., 2009), medtem ko je bil vpliv vrst *Steinernema affine* in *S. kraussei* na strune zanemarljiv (Puza et al., 2010).

Insekticidni učinek glive *Metarhizium anisopliae* (sev F52) pa je možno še izboljšati. Z željo po preučitvi potencialnega sinergizma so seme koruze pred sajenjem tretirali z omenjeno glivo, kateri so dodali insekticid spinosad (80 % aktivne snovi). Omenjeni ukrep je pripomogel k manjši škodljivosti strun, pri čemer zadovoljivega delovanja samostojne uporabe spinosada niso ugotovili (Kabaluk in Ericsson, 2007a). Sinergistično delovanje glive *M. anisopliae* in spinosada so preučevali ob tretiranju tal. Spinosad so aplicirali v treh različnih koncentracijah (0, 45 in 90 ppm), medtem ko je bila gliva na seme nanesena v dveh različnih koncentracijah (0 in 1,42 x 10^5 konidijev/ml). Ugotovili so, da je bila v obravnavanjih z obema snovema smrtnost strun najvišja, pri čemer podrobnejša razlaga omenjene interakcije zaenkrat še ostaja neznanka (Ericsson et al., 2007).

2.6 Mineralna gnojila

V poljski raziskavi v Nemčiji so ugotovili, da ima na strune določeno insekticidno delovanje tudi mineralno gnojilo kalcijev cianamid ali apneni dušik (Ritter et al., 2011; Apneni dušik…, 2012). Podatki o načinu delovanja apnenega dušika na strune in druge talne škodljivce so sicer skopi, največkrat pa je njegova aktivnost pojasnjena kot insekticidna oziroma repelentna. Ob predpostavki, da na insekticidno delovanje kalcijeva cianamida vpliva vlaga v tleh, so delovanje omenjenega mineralnega gnojila preizkušali v standardnih razmerah in z vnosom dveh različnih množin vode v tla. Gnojilni učinek 100 mg kalcijevega cianamida na 100 g suhih tal je bil enak učinku 150 kg N/ha, rezultati omenjenega poskusa pa ne beležijo vpliva kalcijevega cianamida na smrtnost strun (Ritter et al., 2011).

Na drugi strani pa je bilo doslej ugotovljeno fungicidno delovanje apnenega dušika proti glivam *Rhizoctonia solani* J.G. Kühn, *Fusarium oxysporum* E.F. Sm. & Swingle in *Verticillium dahliae* Kleb. v nasadih jagod. Višja vlažnost v tleh je vplivala na boljše delovanje omenjenega gnojila (Lijing et al., 2007), ki se je izkazalo za ustrezno pri gojenju cvetače, z namenom zatiranja povzročiteljice golšavosti kapusnic (*Plasmodiophora brassicae* Woronin). Fungicidno delovanje kalcijevega cianamida so v slednjem primeru pripisali predvsem dvigovanja pH reakcije tal (Tremblay et al., 2005), torej posrednemu delovanju na glivo.

2.7 Poplavljanje

Začasno poplavljanje njiv velja za učinkovit ukrep zatiranja dveh vrst talnih škodljivcev, gosenic vrste *Chrysoteuchia topiaria* (Zeller) in ogrcev vrste *Tomarus subtropicus* Blatchley, vpliv poplavljanja na strune pa je odvisen predvsem od tipa tal, temperature tal med poplavljanjem in vrste strun. Poplavljanje v poletnem času naj ne bi bilo učinkovito pri zatiranju ličink vrst *Melanotus communis* (Gyllenhal), *Limonius agonus* (Say) *in Lacon* spp. Poplavljanje v jesenskem in poletnem času ima zaradi višje temperature boljši učinek pri

zatiranju strun kot poplavljanje v zimskem času. Glede na rezultate omenjenega poskusa pa naj bi bil eden od glavnih dejavnikov učinkovitosti poplavljanja tudi slanost tal; namreč višja kot je slanost tal, krajši časovni interval poplavljanja je potreben (van Herk in Vernon, 2006).

Metoda poplavljanja njiv se je pri zatiranju strun izkazala za uspešno tudi pri pridelavi sladkorne pese. Zadovoljivo delovanje na te škodljivce so ugotovili pri vodi, katere temperatura je bila višja od 22 °C, obdobje poplavljanja pa je bilo daljše od 6 tednov (Larsen et al., 2012). Če število strun v tleh pred sajenjem sladkorne pese ustreza preseženemu gospodarskemu pragu škode, pa lahko s kratkotrajnim poplavljanjem v omenjenem časovnem intervalu dosežemo podobno učinkovitost kot z uporabo nekaterih insekticidov iz skupine organskih fosforjevih estrov (Glaz in Cherry, 2003; Larsen et al., 2012).

2.8Tretiranje semena ali predhodnega posevka z insekticidi

Gre za metodo, ki sicer ne spada med klasične alternativne načine zatiranja talnih škodljivcev, spada pa med precej učinkovite in gospodarne, saj je količina uporabljenega insekticida na površinsko enoto precej manjša kot pri klasičnem vnosu granuliranih insekticidov v tla. Za uspešen način varovanja žitnih posevkov pred napadom strun se je izkazalo tretiranje semena z lindanom (Vernon et al., 2009, 2011); ob uporabi lindana se je namreč v tleh zmanjšala številčnost strun. Njegova uporaba pa je na številnih območjih že prepovedana, eden od najpomembnejših vzrokov za to je njegova fitotoksičnost.

Tudi fipronil velja za učinkovito kemično sredstvo za zatiranje strun. Omenjeno sredstvo je še posebno učinkovito, ob nanosu na površje semena (strune se rade prehranjujejo s semenom), s tem pa je relativno majhna tudi poraba insekticida na površinsko enoto (Chaton et al., 2008). Količina fipronila, s katerim je obdano seme, se skozi rastno dobo zmanjšuje. Pri sončničnem semenu, ki je bilo tretirano s 437 µg/posamezno seme, je bilo ugotovljeno, da se med rastno dobo količina snovi (v 4 mesecih) zmanjšuje za 0,3 µg/dan. Zaradi slabe mobilnosti te snovi v tleh, pa je velika večina ostane v istem talnem sloju, v katerem je bilo seme (Raveton et al., 2007) .

Z dodajanjem fipronila v 0,5 % koncentraciji talnim vabam lahko uspešno vplivamo na večjo smrtnost vrste *Melanotus sakishimensis* Ohira na njivah s sladkornim trsom. Fipronil se je izkazal za učinkovit insekticid tako v laboratorijskih kot v poljskih razmerah, rezultati poskusa pa kažejo, da fipronil deluje na strune privabilno in insekticidno. Posebno uspešno delovanje fipronila (v 1 % koncentraciji v obliki pelet) je bilo potrjeno v laboratorijskih razmerah, medtem ko je fipronil v poljskem poskusu dosegel najvišjo učinkovitost v odmerku 6 kg/0,1 ha. V slednjem je uporaba fipronila vplivala na višji pridelek sladkornega trsa (Tarora et al., 2007). V Sloveniji fipronil nima več registracije, kot aktivna snov (80 %) pa je bil dostopen v pripravku Regent 80 WG (), ki je bil namenjen za zatiranje koloradskega hrošča (*Leptinotarsa decemlineata* [Say]) na krompirju. Pomembno je bilo, da smo rastline krompirja z omenjenim pripravkom tretirali takrat, ko se je večina ličink izlegla iz jajčec (Priročnik o fitofarmacevtskih sredstvih…, 2002).

Krompir, katerega so ob sajenju tretirali z imidaklopridom (70 g/t), se je izkazal za zelo odpornega na napad strun (Huiting in Ester, 2009). Da pa bi kmetje zmanjšali količino uporabljenih insekticidov, lahko uporabijo tudi metodo tretiranja predhodnega posevka z insekticidom (Parker in Howard, 2001)). V praksi to največkrat pomeni, da je na primer ozimna pšenica ali druga vrsta žita tretirana z insekticidom, pozneje pa na istem zemljišču posadijo krompir (Parker in Howard, 2001).

2.9 Večkratna mehanična obdelava tal

Mehanična obdelava tal v več ponovitvah naj bi pripomogla k zmanjšanju populacije strun v tleh in posledično manj napadenim gomoljem krompirja (Landl in Glauniger, 2011). Tudi oranje omogoča, da so strune veliko bolj izpostavljene naravnim sovražnikom (Larsen et al., 2012).

2.10 Semiokemikalije

Med relativno novejše metode detekcije hroščev pokalic spada uporaba feromonskih vab (Vernon in Tóth, 2007). Med letoma 1999 in 2001 je v več evropskih državah potekala raziskava spremljanja populacijske dinamike osmih gospodarsko pomembnih vrst pokalic: *Agriotes ustulatus, A. litigiosus* Rossi, *A. sputator, A. obscurus, A.*

lineatus, A. rufipalpis Brulle, *A. sordidus* Illiger in *A. brevis* (Vernon in Tóth, 2007), že nekaj let prej pa so bile feromonske vabe v enak namen uporabljene v Rusiji in še nekaterih državah bivše Sovjetske zveze (Vernon in Tóth, 2007).

Triletni poljski poskus (1986-89) izpostavlja dejstvo, da so lahko feromonske vabe izredno učinkovite pri masovnem lovljenju samcev, s čimer se zaradi zmanjšanja kopulacije zmanjša tudi številčnost potomcev – strun. Izkazalo se je, da pri nizki zastopanosti strun v tleh (vrsta *Agriotes sputator*) lahko uporabimo 30 vab/ha. Ugotovili so, da se lahko s pomočjo omenjene metode številčnost strun v visoko intenzivnem kolobarju zmanjša za 86 % (Balkov, 1991). Ena od možnosti uporabe feromonov je tudi uporaba v namene konfuzije samcev. Ivashchenko in Chernova (1995) poročata o pomembnem aplikativnem odkritju, ko sta 120 g feromona uporabila na 1 ha velikem zemljišču; s tem sta povzročila, da je bilo več kot 70 % samic neoplojenih.

Uporaba feromonov v namen spremljanja samcev pokalic (*Agriotes* spp.), da bi na ta način ugotovili njihovo povezavo s strunami v tleh in posledično z obsegom poškodb na gomoljih krompirja, pridobiva na pomenu tudi v praksi. Največjo težavo pri uporabnosti feromonov za potrebe predvidevanja poškodb zaradi strun na gomoljih krompirja, je predvsem v tem, da se odrasli osebki med posameznimi vrstami pokalic razlikujejo, ličinke pa je težko razlikovati in navadno lahko zabeležimo le njihovo skupno število (Blackshaw in Vernon, 2007).

3 SKLEPI

Res je, da je uporaba sintetičnih insekticidov doprinesla k učinkovitemu zatiranju talnih škodljivcev, vendar je vse več podatkov o njihovem negativnem vplivu na okolje (Gentz et al., 2010), tudi na neciljne organizme (Peck et al., 2009). S tem namenom so informacije o alternativnih metodah zatiranja strun vse bolj dobrodošle (Hermann et al., 2012). Medtem ko z uporabo privabilnih posevkov največkrat vplivamo na zmanjšanje števila strun v bližini glavnega posevka, njihove številčnosti pa v tleh ne zmanjšamo, pa biofumigacijo največkrat omenjajo kot najbolj uspešno alternativo sintetičnim fumigantom (metilbromidu) (Mattner et al., 2008; Furlan et al., 2010). Razgradnja rastlinske gmote križnic v tleh namreč pozitivno pripomore tudi k zatiranju drugih škodljivih organizmov v tleh (Mattner et al., 2008). Ker vemo, da vsebnost glukozinolatov variira med posameznimi rastlinskimi vrstami (Bohinc et al., 2012), se za potrebe biofumigacije ponavadi odločamo za tisto rastlinsko vrsto, ki vsebuje največ glukozinolatov. Pri tem pa je pomemben tudi način aplikacije, zlasti čim hitrejša zadelava zmulčene rastlinske gmote v tla. Poleg križnic, ki so vse bolj pomemben sestavni del kolobarja (Morra in Kirkegaard, 2002), pa je v zvezi z zmanjševanjem številčnosti in posledične škodljivosti strun v tleh, pomembno tudi vključevanje ajde v kolobar (Valenzuela in Smith, 2002; Noronha, 2011).

Uporaba biotičnih agensov pri zatiranju strun obeta veliko, vendar pa bo potrebno še veliko raziskav na omenjeno temo, da bo natančno ovrednotena učinkovitost in gospodarnost uporabe različnih vrst in sevov (Hermann et al., 2012). Zaradi največkrat manjše učinkovitosti samostojne uporabe biotičnih agensov pri zatiranju strun, v primerjavi s sintetičnimi insekticidi, pa velja v prihodnje več časa nameniti raziskavam potencialnih sinergizmov med dvema ali več okoljsko sprejemljivimi načini njihovega zatiranja (Ericsson et al., 2007).

Ker je seznam registriranih insekticidov za zatiranje strun v številnih državah, tudi v Sloveniji (Seznam registriranih…, 2012), kratek, v nekaterih državah za njihovo zatiranja uporabljajo še druge alternativne načine. Eden od takšnih načinov je poplavljanje njiv, vendar v številnih primerih način pridelovanja rastlin pridelovalcem tega ne dovoljuje (Cherry in Nuessly, 2010). Glede na slabe izkušnje z uporabo neonikotinoidov pri tretiranju semenskega materiala (Gentz et al., 2010), pa lahko fipronil v insekticidni oblogi semena učinkovito vpliva na večjo smrtnost strun (Raveton et al., 2007). V Italiji in na Hrvaškem je bil vnos fipronila v tla dovoljen za zatiranje ličink koruznega hrošča (Modic et al., 2009). V Sloveniji je danes uporaba tega insekticida prepovedana, predvsem zaradi škodljivih vplivov na koristne

organizme (Seznam registriranih …, 2012). Kljub temu pa sta lindan in fipronil kot aktivni snovi še vedno zastopani na seznamu aktivnih snovi, katerih uporaba v Evropi ni prepovedana (EU Pesticide Database, 2012).

Znano je, da je številčnost strun v tleh pogojena z različnimi dejavniki okolja (Hermann et al., 2012), vse bolj pa ugotavljamo, da se njihovo število v zadnjih letih povečuje tudi zaradi vse bolj omejenega števila registriranih talnih insekticidov. Ugotavljamo, da je trenutno med alternativnimi metodami za zatiranje strun najbolj raziskana biofumigacija, zadovoljivo učinkovitost pa v nekaterih delih sveta, kjer jim namenjajo pozornost, predstavljata tudi uporaba privabilnih posevkov in odpornih sort. Uporabo kratkotrajnega namakanja zemljišča, gnojenja z mineralnimi gnojili in uporabe biotičnih agensov pa bi bilo v prihodnje še bolj raziskati, saj je podatkov o njihovem delovanju še premalo, čeprav nakazujejo za dobro učinkovitost. Zlasti uporaba biotičnih agensov spada trenutno med dražje načine zatiranja strun (pa tudi drugih talnih škodljivcev), menimo pa, da bi bilo mogoče njihovo učinkovitost - ta bi zagotovo vplivala na širšo uporabo pri zatiranju strun – povečati s hkratno uporabo dveh ali večih okoljsko sprejemljivih načinov zatiranja (feromonske vabe x naravni sovražnik, privabilni posevki x naravni sovražnik idr.).

4 ZAHVALA

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5 VIRI

Ansari, M.A., Evans, M., Butt, T.M. 2009. Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. Crop Protection. 28: 269-272.

Apneni dušik. 2012. http://www.apneni-dusik.com/index.html (21.9.2012)

Balkov, V.I. 1991. Attractant traps for control of wireworms. Zashchita Rastenii. 5: 24-25.

Blackshaw, R.P., Vernon, S. 2008. Spatial relationships between two *Agriotes* click-beetle species and wireworms in agricultural fields. Agricultural and Forest Entomology. 10: 1-11.

Bohinc, T., Goreta Ban, S., Ban, D., Trdan, S. 2012. Glucosinolates in plant protection strategies: a review. Archives of Biological Sciences. 64, 3: 821-828.

Chaton, P.F., Lempérière, G., Tissut, M., Ravanel, P. 2008. Biological traits and feeding capacity of *Agriotes* larvae (Coleoptera: Elateridae): A trial of seed coating to control larval populations with the insecticide fipronil. Pesticide Biochemistry and Physiology. 90,2: 97-105.

Cheah, L.H., Marsh, A.T., Hedderley, D., Falloon, R.E. (2008). Crop rotation with Brassicas reduces *Phytophthora* populations in potato soil. New Zealand Plant Protection. 61: 256-260.

Cherry, R., Nuessly, G. 2010. Repellency of the biopesticide, azadirachtin, to wireworms (Coleoptera: Elateridae). Florida Entomologist. 93, 1: 52-55

Cohen, M.F., Yamasaki, H., Mazzola, M. 2005. *Brassica napus* seed meal soil amendment modifies microbiological community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. Soil Biology & Biochemistry. 37: 1215-1227.

Crow, W.T., Weingartner, D.P., Dickson, D.W., McSorley, R. 2001. Effect of sorghum-sudangrass and velvetbean cover crops on plant-parasitic nematodes associated with potato production in Florida. Supplement to the Journal of Nematology. 33, 4S: 285-288.

De Nicola, G.R., D'Avino, L., Curto, G., Malaguti, L., Ugolini, L., Cinti, S., Patalano, G., Lazzeri, L. 2013. A new biobased liquid formulation with biofumigant and fertilising properties for drip irrigation distribution. Industrial Crops and Products 42: 113-118.

Ericsson, J.D., Kabaluk, J.D., Goettel, M.S., Myers, J.H. 2007. Spinosad interacts synergistically with the insect pathogen *Metarhizium anisopliae* against the exotic wireworms *Agriotes lineatus* and *Agriotes*

obscurus (Coleoptera: Elateriadae). Journal of Economic Entomology. 100, 1: 31-38.

EU Pesticides Database. 2012. European Commission. Health and Consumers. Plants. http://ec.europa.eu/food/plant/plant_protection_products/pesticides_database/index_en.htm (23.9.2012)

Gentz, C.M., Murdoch, G., King, G.F. 2010. Tandem use of selective insecticides and natural enemies for effective reduced-risk pest management. Biological Control 52: 208-215.

Glaz, B., Cherry, R. 2003. Wireworm (Coleoptera: Elateridae) effects on sugarcane emergence after short-duration flood applied at planting. Journal of Entomological Science. 38, 3: 449-456.

Furlan, L., Bonetto, C., Palatano, G., Lazzeri, L. 2004. Potencial of biocidal meals to control wireworm populations. Agroindustria. 3, 3: 313-316.

Furlan, L., Bonetto, C., Finotto, A., Lazzeri, L., Malaguti, L., Patalano, G., Parker, W. 2010. The efficacy of biofumigant meals and plants to control wireworms populations. Industrial Crops and Products. 31: 245-254.

Huiting, H.F., Ester, A. 2009. Neonicotinoids as seed potato treatments to control wireworms. Commun Agric Appl Biol Sci. 74, 1: 207-216.

Iqbal, Z., Hiradate, S., Noda, A., Isojima, S.I., Fujii, Y. 2002. Allelopathy of buckwheat: Assessment of allelopathic potencial of extract of aerial parts of buckwheat and identification of fagomine and other related alkaloids as allelochemicals. Weed Biology and Management. 2: 110-115.

Ivashchenko, I.I., Chernova, S.V. 1995. Biologically active substances against click beetle. Zashchita rastenij. 9: 16-17.

Jansson, R.K., LeCrone, S.H. 1991. Effects of summer cover crop management on wireworm (Coleoptera, Elateridae) abundance and damage to potato. Journal of Economic Entomology. 84, 2: 581-586.

Johnson, S.N., Anderson, E.A.; Dawson, G., Griffiths, D.W. 2008. Varietal suscepbility of potatoes to wireworm herbivory. Agricultural and Forest Entomology. 10: 167-174.

Kabaluk, J.T., Ericsson, J.D. 2007. Environmental and behavioral constrains on the infection of wireworms by *Metarhizium anisopliae*. Environmental Entomology. 36, 6: 1415-1420.

Kabaluk, J.T., Ericsson, J.D. 2007. *Metarhizium anisopliae* seed treatment increases yield of field corn when applied for wireworm control. Agronomy Journal. 99: 1377-1381.

Kirkegaard, J.A.; Sarwar, M. 1998. Biofumigation potencial of brassicas. I. Variation in glucosinolate profiles of diverse field-grown brassicas. Plant and Soil. 201: 71-89.

Kuhar, T.P.; Alvarez, J.M. 2008. Timing of injury and efficacy of soil-applied insecticides against wireworms on potato in Virginia. Crop Protection. 27: 792-798.

Landl, M., Furlan, L., Glauninger, J. 2010. Seasonal fluctuations in *Agriotes* spp. (Coleoptera: Elateridae) at two sites in Austria and the efficiency of bait trap designs for monitoring wireworm populations in the soil. Journal of Plant Diseases and Protection, 117, 6: 268-272.

Landl, M., Glauniger, J. 2011. Preliminary investigations into the use of trap crops to control *Agriotes* spp. (Coleoptera: Elateridae) in potato crops. Journal of Pest Science Doi: 10.1007/s10340-011-0348-3

Larsen, N.A., Nuessly, G.S., Cherry, R.H., Glaz, B. 2012. Varietal susceptibility to the corn wireworms *Melanotus communis* (Coleoptera: Elaterideae) in sugarcane. Journal of Pest Science. doi: 10.1007/s10340-012-0435-0

Lazzeri, L.; Leoni, O.; Macini, L.M. 2004. Biocidal plant dried pellets for biofumigation. Industrial crops and products. 20: 59-65.

Lijing, W., Tongle, H., Lijing, J.I., Keqiang, C.A.O. 2007. Inhibitory efficacy of calcium cynamide on the pathogens of replant diseased in strawberry. Front. Agric. China. 1, 2: 183-187.

Mattner, S.W., Porter, I.J., Gouder, R.K., Shanks, A.L., Wren, D.J., Allen, D. 2008. Factors that impact on the ability of biofumigants to suppress fungal pathogens and weeds on strawberry. Crop Protection. 27: 1165-1173.

Morra, M.J., Kirkegaard, J.A. 2002. Isothiocyanate release from soil incorporated *Brassica* tissues. Soil Biology & Biochemistry. 34: 1683-1690.

Hoagland, L., Carpenter-Boggs, L., Reganold, J.P., Mazzola, M. 2008. Role of native soil biology in Brassicaceous seed meal-induced weed suppression. Soil Biology & Biochemistry. 40: 1689-1697.

Herman, A., Brunner, N., Hann, P., Wrbka, T., Kromp, B. 2012. Correlations between wireworm damages in potato fields and landscape structure at different scales. Journal of Pest Science. Doi: 10.1007/s10340-012-0444-z

Milevoj, L., Gomboc, S., Bobnar, A., Smodiš, T., Valič, N., Mikuš, T. 2005. Spremljanje aktivnosti pet vrst pokalic rodu *Agriotes* (Coleoptera: Elateridae) s

feromonskimi vabami v okolici Ljubljane. Zbornik predavanj in referatov s 7. slovenskega posvetovanja o varstvu rastlin (ur. Vajs, S., Lešnik, M.), Zreče, 8.-10. marec 2005. Ljubljana, Društvo za varstvo rastlin Slovenije: 254-262.

Milevoj, L., Gomboc, S., Bobnar, A., Mikuš, T., Gril, T. 2005. Učinkovitost različnega števila feromonskih vab na nalet poljske pokalice (*Agriotes lineatus* L.). Acta Agriculturae Slovenia. 85, 2: 375-384.

Milonas, P.G., Kontodimas, D.C., Michaelakis, A., Raptopoulus, D.G., Konstantopoulou, M.A. 2010. Optimization of pheromone trapping method for click beetles (*Agriotes* spp.) in Greece. Phytoparasitica. 38: 429-434.

Modic, Š., Urek, G., Milevoj, L., Barbarič, M., Verbič, J., Poje, T., Knapič, M., Knapič, V., Orešek, E. 2009. Varstvo koruze pred koruznim hroščem (*Diabrotica virgifera virgifera* LeConte). Republika Slovenija. Ministrstvo za kmetijstvo, gozdarstvo in prehrano. Fitosanitarna uprava RS: 68 str.

Nema, P.K., Ramayya, N., Duncan, E., Niranjan, K. 2008. Potato glycoalkaloides: formation and stategies for mitigation. Journal of the Science of Food and Agriculture. 88: 1869-1881.

Noronha, C. 2011. Crop rotation as a management tool for wireworms in potatoes. V: Insect pathogens and entomopathogenic nematodes. Biological Control in IPM Systems. IOBC/wprs Bulletin 66: 467-471

Parker, W.E., Howard, J.J. 2001. The biology and management of wireworms (*Agriotes* spp.) on potato with particular reference to the U.K. Agricultural and Forest Entomology 3:85-98.

Peck, D. C. 2009. Comparative impacts of white grub (Coleoptera: Scarabaeidae) control products on the abundance of non-target soil-active arthropods in turfgrass. Pedobiologia, 52, 5: 287-299

Piqué, J., Eizaguirre, M., Pons, X. 1998. Soil insecticide treatments against maize soil pests and corn borers in Catalonia under tradicional crop conditions. Crop Protection. 17: 557-561.

Puza, V., Mracek, Z. 2010. Does scavenging extend the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)? Journal of Invetebrate Pathology. 104, 1: 1-3.

Priročnik o fitofarmacevtskih sredstvih v Republiki Sloveniji. 1995. 1. izdaja. RS – Ministrstvo za kmetijstvo, gozdarstvo in prehrano. Ljubljana: 552 str.

Priročnik o fitofarmacevtskih sredstvih v Republiki Sloveniji. 1999. 2. izdaja. Društvo za varstvo rastlin Slovenije in RS – Ministrstvo za kmetijstvo, gozdarstvo in prehrano. Ljubljana: 550 str.

Priročnik o fitofarmacevtskih sredstvih v Republiki Sloveniji. 2002. 3. izdaja. Društvo za varstvo rastlin Slovenije in RS – Ministrstvo za kmetijstvo, gozdarstvo in prehrano. Ljubljana: 814 str.

Raveton, M., Aajoud, A., Willison, J., Cherifi, M., Tissut, M., Ravanel, P. 2007. Soil distribution of fipronil and its metabolites originating from seed-coated formulation. Chemosphere. 69: 1124-1129.

Ritter, C., Katroschan, K.U., Richter, E. 2011. Alternative methods to control wireworms (*Agriotes* spp., Coleoptera: Elateridae) in vegetable production – potencial of calcium cynamide and *Metarhizium anisopliae*. In: Insect pathogens and entomopathogenic nematodes. Biological Control in IPM Systems. IOBC/wprs Bulletin 66: 521-524.

Seznam registriranih fitofarmacevtskih sredstev na dan 22.9.2012. Republika Slovenija. Ministrstvo za kmetijstvo in okolje. Fitosanitarna uprava Republike Slovenije. http://spletni2.furs.gov.si/FFS/REGSR/index.htm (23.9.2012)

Staudacher, K., Schallhart, N., Pitterl, P., Wallinger, C., Brunner, N., Landl, M., Kromp, B., Glauninger, J., Traugott, M. 2012. Occurrence of *Agriotes* wireworms in Austrian agricultural land. Journal of Pest Science: 7 str. (v tisku), DOI 10.1007/s10340-011-0393-y

Tehnološka navodila za integrirano pridelavo zelenjave 2012. Ministrstvo za kmetijstvo in okolje. http://www.mko.gov.si/fileadmin/mko.gov.si/pageuploads/podrocja/Kmetijstvo/Integrirana_pridelava/TN_IPP_2012.pdf

Tehnološka navodila za integrirano pridelavo zelenjave 2012. Ministrstvo za kmetijstvo in okolje. http://www.mko.gov.si/fileadmin/mko.gov.si/pageuploads/podrocja/Kmetijstvo/Integrirana_pridelava/TN_IPZ_2012.pdf (18.9.2012)

Tarora, K., Arakaki, N., Uehara, K., Ishimine, M., Kobayashi, A., Nagayama, A. 2007. Control of the sugarcane wireworm *Melanotus sakishimensis* (Coleoptera: Elateridae) by a fipronil bait. Japanese Journal of Applied Entomology and Zoology. 51, 2: 129-133.

Tremblay, N., Bélec, C., Coulombe, J., Godin, C. 2005. Evaluation of calcium cyanamide and liming for control of clubroot disease in cauliflower. Crop Protection. 24, 9: 798-803.

Valenzuela, H., Smith, J. 2002. Green manure crops: Buckwheat. SA-GM-4. CTAHR: 1-3 str.

Van Herk, W.G., Vernon, R.S. 2006. Effect of temperature and soil of the control of a wireworm, *Agriotes obscurus* L. (Coleoptera: Elateriae) by flooding. Crop Protection 25: 1057-1061.

Van Herk, WG., Vernon, R.S. 2011. Categorization and numerical assessment of wireworm mobility over time following exposure to bifenthrin. Journal of Pest Science. Doi: 10.10007/s10340-011-0381-2.

Van Herk, W.G., Vernon, R.S., Tolman, J.H., Ortiz Saavedra, H. 2008. Mortality of a wireworm, *Agriotes obscurus* (Coleoptera: Elateriadae) after topical application of various insecticides. Journal of Economic Entomology. 101, 2: 375-383.

Vernon, R.S. 2005. Aggregation and mortality of *Agriotes obscurus* (Coleoptera: Elateriadae) at insecticide-treated trap crops of wheat. Journal of Economic Entomology. 98, 6: 1999-2005.

Vernon, R.S., Tóth, M. 2007. Evaluation of pheromones and a new trap for monitoring *Agriotes obscurus* in the Fraser valley of British Columbia. Journal of Chemical Ecolology. 33: 345-351.

Vernon, R.S., van Herk, W., Tolman, J., Ortiz Saavedra, H., Clodius, M., Gage, B. 2008. Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). Journal of Economic Entomology. 101, 2: 365-374.

Vernon, R.S., van Herk, W.G., Clodius, M., Harding, C. 2009. Wireworm management I: Stand protection versus wireworm mortality with wheat seed treatments. Journal of Economic Entomology. 102(6): 2126-2136.

Vernon, R.S., Van Herk, W.G., Clodius, M., Harding, C. 2011. Crop protection and mortality of *Agriotes obscurus* wireworms with blended insecticidal wheat seed treatments. Journal of Pest Science. Doi: 10.1007/s10340-011-0392-z

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Viguès, B., Brunet, J.C., Texier, C., Biron, D.G., Blot, N., El Alaoui, H., Belzunces, L.P., Delbac, F. 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previusly infected by *Nosema ceranae*. Plos One 6, 6: e21550. doi: 10.1371/journal.pone.0021550

Vrabl, S. 1992. Škodljivci poljščin. ČZP Kmečki glas. Ljubljana: 142 str.

Waliwitiya, R., Isman, M.B., Vernon, R.S., Riseman, A. 2005. Insecticidal activity of selected monoterpenoids and rosemary oil to *Agriotes obscurus* (Coleoptera: Elateriade). Journal of Economic Entomology. 98, 5: 1560-1565.

Willis, R.B., Abney, M.R., Holmes, G.J., Schulteheis, J.R., Kennedy, G.G. 2010. Influence of preceding crop on wireworms (Coleoptera: Elateriade): abundance in the coastal plain of North Carolina. Journal of Economic Entomology. 103, 6: 2087-2093.

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Pol-naravno travinje kot vir semena za obnovo ruše velike naravne vrednosti

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IZVLEČEK

V zadnjih letih pol-naravno travinje vedno bolj pridobiva večnamensko vlogo v prostoru. Poleg zagotavljanja voluminozne krme domačim živalim postajajo pomembne tudi številne druge funkcije pol-naravnega travinja, med katerimi se vse bolj izpostavlja tudi biotska pestrost ruše. S tega vidika so posebej cenjene ruše velike naravne vrednosti. Skozi zgodovino se je biotska pestrost ohranjala predvsem z ekstenzivno pašno rabo in košnjo rastlin v obdobju dozorevanja semena. Z intenzifikacijo gospodarjenja na travinju v dvajsetem stoletju in uporabo komercialnih semenskih mešanic se je na pol-naravnem travinju biotska pestrost ruše na splošno pričela zmanjševati. V zadnjem obdobju smo se začeli zavedati negativnih posledic upadanja biotske pestrosti in začeli razvijati ukrepe za zaustavitev tega procesa. Med temi ukrepi ima pomembno vlogo setev oz. obnova ruše velike naravne vrednosti. Vir semen za zasnovo take ruše je lahko le biotsko pestro pol-naravno travinje. V svetu so se v zadnjih letih razvile različne metode pridobivanja semena in načini setve, ki omogočajo uspešno vzpostavitev izvorne biotsko pestre ruše prilagojene lokalnim rastnim razmeram.

Ključne besede: pol-naravno travinje, biotska pestrost, pridelava semena, setev

ABSTRACT

SEMI-NATURAL GRASSLAND AS SOURCE OF SEEDING MATERIAL FOR SWARD RESTORATION IN HIGH NATURE VALUE AREAS

In recent years semi-natural grassland has gained a multifunctional role in the environment. In addition to providing forage for farm animals, many other functions of semi-natural grassland, including biodiversity, are even so important. From this point of view, grasslands of high natural value are of high significance. Throughout the history, biodiversity has been maintained primarily by the extensive grazing and harvesting hay during seed maturation. With the intensification of grassland management in the 20th century and the use of commercial seed mixtures on the semi- natural grasslands, biodiversity of the sward in general began to decline. We have recently become aware of the negative consequences of declining biodiversity and begun to develop measures to stop this process. Among these measures, establishment and restoration of semi-natural grassland with high natural value play an important role. Seed material for the establishment and restoration of such sward can be obtained only from species rich semi-natural grassland. In recent years a variety of methods have been developed all over the world for seed production and sowing on semi-natural grasslands, allowing successful reestablishment of original biodiversity, adapted to local growing conditions.

Key words: semi-natural grassland, biodiversity, seed production, seeding

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1 UVOD

Travinje v Sloveniji, kot tudi na območju Evropske unije (EU), pokriva pomemben delež kmetijskih zemljišč v uporabi (57,2 % v Sloveniji oziroma 33 % v EU; SURS, 2011; Eurostat 2010). Večinoma je nastalo kot samoniklo po krčenju gozdov. Tako nastalo trajno travinje je pogosto biotsko izredno pestro, saj je poleg mnogih rastlinskih vrst tudi pomemben gostitelj številnih vretenčarjev in nevretenčarjev (Hopkins in Holz, 2006). Kljub njegovemu pomembnemu proizvodnemu potencialu za voluminozno krmo in okoljskemu pomenu pa se je delež trajnega travinja med katero sodi tudi pol-naravno travinje v Evropi v zadnjih 50 letih zmanjšal za 15 % in več. Na drugi strani pa so se njive posejane s koruzo več kot podvojile (Peeters, 2012). Danes biotsko pestremu pol-naravnemu travinju pripisujemo večnamensko vlogo v prostoru. Poleg pridelave voluminozne krme za domače živali v ospredje vse bolj prihajajo neproizvodne funkcije travinja, ki so predvsem okoljevarstvene in vključujejo varovanje habitatov, tal, virov pitne vode, vezavo ogljika, ter vzdrževanje biotske pestrosti in genskih virov.

Pol-naravno travinje je relikt evropske tradicionalne kulturne krajine in spada med vrstno najbogatejše habitate v zahodnih, severnih in osrednjih predelih Evrope (Poschlod in sod., 1998; Kaligarič in sod., 2006). Skozi zgodovino se je biotsko pestro pol-naravno travinje ohranjalo z dokaj ekstenzivno pašo, in s košnjo v času, ko je seme mnogih travniških rastlin že dozorevalo. V Sloveniji se delež travinja od površine kmetijskih zemljišč v uporabi sicer povečuje, vendar se absolutna površina ekstenzivnega biotsko pestrega travinja v zadnjih desetletjih zmanjšuje v korist urbanizacije, intenzifikacije rabe in zaraščanja (Cunder, 1998). Zaradi urbanizacije in opuščanja omenjenih tradicionalnih načinov rabe se spreminja tudi podoba tradicionalne krajine, kar posledično pomeni, da prihaja do razdrobljenosti ekstenzivnega travinja, izolacije ter upadanja biotske pestrosti okolja.

Negativnih posledic upadanja biotske pestrosti pol-naravnega travinja in problemov, povezanih z varovanjem okolja in tradicionalne kulturne krajine, so se v Evropi pričeli zavedati že pred nekaj desetletji in jih na območju EU pričeli reševati tudi s sprejetjem direktive o habitatih leta 1992 (Hopkins, 2009).

Pomembno vlogo pri ohranjanju biotsko pestrega travinja ima obnova le tega ter vzpostavitev nove biotsko pestre trajne ruše. Znotraj varovanih in degradiranih območjih se pri obnovi take ruše srečujemo z večplastno problematiko. Semenarske hiše sicer za obnovo travinja ponujajo komercialno uveljavljene mešanice, vendar imajo požlahtnjene sorte travniških rastlinskih vrst v primerjavi z nepožlahtnjenimi rastlinami praviloma večje potrebe po oskrbi z vodo in hranili, vprašljiva pa je tudi njihova trpežnost. Velikokrat razvoj botanične pestrosti preprečujejo prav požlahtnjene komercialne sorte trav, ki so v primerjavi z nepožlatnjenimi sortami v travni ruši veliko bolj konkurenčne (Mitchley in sod., 2012). Poleg tega komercialno uveljavljene požlahtnjene sorte trav in metuljnic rastišču ne dajejo izvorne rastlinske raznolikosti. To lahko pridobimo le z avtohtonim semenom rastlin določenega rastišča. K ohranjanju in obnovi biotsko pestrega ekstenzivnega travinja bi torej lahko veliko pripomogla talna semenska banka, vendar ta pogosto vsebuje tudi seme številnih neželenih (plevelnih) rastlinskih vrst, predvsem v nekoliko globljem delu tal, kjer semena teh vrst izgubijo kalivost šele po mnogih letih (Willems in sod., 1993; Willems, 1995; Bekker in sod., 1998). Za uspešno in trajnostno obnovo je torej potrebno lokalno pridelano seme. Do nedavnega je obnovo biotske pestrosti travinja s takim semenom omejeval Zakon o semenskem materialu kmetijskih rastlin, ki je dovoljeval le prodajo preizkušenih požlahtnjenih sort. Šele s sprejetjem direktive 2010/60/EU o določitvi nekaterih odstopanj pri trženju semenskih mešanic krmnih rastlin, namenjenih za uporabo pri ohranjanju naravnega okolja, so podane smernice za zakonsko urejeno pridelavo t. i. ohranjevalnih semenskih mešanic. Te so lahko izredno pomembne pri obnovi ali vzdrževanju biotsko pestrega travinja s posebej dragocenimi in ogroženimi rastlinskimi vrstami. Takemu travinju pravimo tudi travinje velike naravne vrednosti (Krautzer in Pötsch, 2009).

V nadaljevanju prispevka so podana najpomembnejša dognanja o novi vlogi pol-naravnega travinja v prostoru, možnostih pridelave

ohranjevalnih semenskih mešanic in obnove biotsko pestrega pol-naravnega travinja.

2 POL-NARAVNO TRAVINJE IN NJEGOVE FUNKCIJE

Uporaba krme s pol-naravnega travinja za prehrano prežvekovalcev je še vedno osnovni način rabe, vendar se v zadnjem obdobju ta pomen travinja v nekaterih okoljih nekoliko zmanjšuje. Röscheva in sod., (2009) navajajo, da v Nemčiji že skoraj četrtina pol-naravnega travinja ne opravlja več svoje prvotne funkcije, še več, opuščeno pol-naravno travinje se zarašča oz. pogozduje ali pa spreminja v orno kmetijsko zemljo. Oboje, z vidika ohranjanja biotske pestrosti naravnih habitatov ni ugodno. Gledano nekoliko širše, nova vloga travinja ni omejena samo na ohranjanje biotske pestrosti in na njegovo vizualno vrednost v kulturni krajini. Pol-naravno travinje lahko obravnavamo kot biološki filter, ki preprečuje spiranje hranil v nadzemne in podzemne vire pitne vode (Carrow, 2005). Vendar travinje ne preprečuje samo spiranje lahko topnih hranil kot je npr. nitratna oblika dušika, ampak veže tudi pline in težke kovine (svinec, stroncij, kadmij) ter jih pomaga zadrževati v neaktivni obliki v plasti korenin (Starczewski in sod., 2009). Pomembna je tudi vloga travinja kot blažilca hrupa v okolici urbanih predelov. Bolund in Hunhammar (1999) navajata, da zatravljene površine zmanjšujejo hrup v okolju za 3 dB. Tako so travniki in pašniki ob avtocestah in prometnicah pomemben blažilec onesnaževanja in hrupa (Pauwels in Gulinck, 2000). Pol-naravno travinje proizvede velike količine kisika: okrog 10 do 15 t ha^{-1} letno, obenem pa je tudi vir različnih organskih kislin, estrov in eteričnih olj (Krzymowska-Kostrowicka, 1991; cit. po Starczewski in sod., 2009). V obdobju prizadevanj zmanjševanja izpustov toplogrednih plinov ima izreden pomen tudi zmožnost travinja, da veže izdatne količine atmosferskega ogljika (Vidrih, 2006).

V poletnem času je zelo izrazita mikroklimatska funkcija travinja. Temperatura nad travno rušo je v primerjavi z urbanimi površinami nižja za 5 do 7 °C, medtem ko je vlaga višja za 6 do 13 %, kar je posledica relativno velike transpiracije. Vpliv travinja na temperaturo in transpiracijo je večji, v kolikor so rastline višje in travna ruša bolj zgoščena (Starczewski in sod., 2009).

Višina in zgoščenost travne ruše vplivata na manjšo hitrost vetra (za pribl. 10 %) v primerjavi s urbanim okoljem. Na ta način travinje zmanjšuje vetrno erozijo tal in prispeva k čistejšemu ozračju, saj ovira prenos različnih aerosolov.

Tradicionalna ekstenzivna raba travinja edina omogoča gnezdenje nekaterim pticam. (Pärt in Söderström, 1999). Tak primer je barjansko travinje, ki omogoča gnezdenje številnim ogroženim vrstam ptic med drugim tudi koscu (*Crex crex* L.), kot edini globalno ogroženi vrsti, ki v večjem številu gnezdi tudi v Sloveniji (Tome in sod., 2005). Pol-naravno travinje lahko povežemo tudi z dejavnostmi, ki niso neposredno povezane s kmetijstvom. Na ta način lahko veliko prispeva k turistični atraktivnosti pokrajine in obenem služi različnim oblikam rekreacije, predvsem pohodništvu. V Francoskih Alpah in Pirenejih kar 65 % pašnikov in travnikov prečkajo pohodne poti z označenimi počivališči, pozimi pa se iste površine spremenijo v smučišča (Warda in sod., 2004; cit. po Starczewski in sod., 2009). Ekstenzivna reja pašnih živali na travnikih in pašnikih samo še poveča atraktivnost oz. privlačnost travinja. Z ustreznim pristopom bi torej morali ohraniti precejšen delež biotsko pestrega travinja, vendar je nujno potrebno povezati neproizvodne in proizvodne funkcije pol-naravnega travinja na način, da ohrani ali izboljša ekonomski položaj kmetijskih gospodarstev na ruralnih območjih.

V zadnjih 10 do 15 letih so bile razvite številne nove možnosti izrabe travinja v energetske namene, vendar njihova racionalna raba ni vedno skladna s smernicami in pravilniki za ohranitev biotske raznovrstnosti naravnih habitatov (Prochnow in sod., 2009). Slovenija je država z največjim deležem kmetijskih zemljišč velike naravne vrednosti v Evropi (60 do 80 % od vse kmetijske zemlje v uporabi, povprečje EU je 15 do 25 %; ARSO, 2008). Obenem je 25,1 % kmetijskih površin vključenih v različne oblike varovanja (Natura 2000, parki). Zaradi velike stopnje ohranjenosti biotske raznovrstnosti, razdrobljenosti in reliefnih značilnosti pol-naravno travinje na

omenjenih območjih ni najbolj primerno za ekonomsko rentabilno energetsko izrabo biomase.

Prav botanično pestro pol-naravno travinje pa lahko predstavlja tudi vir semena, ali prostor za pridelavo ohranjevalnih semenskih mešanic, ki jih lahko uporabimo za obnovo oziroma vzpostavitev botanično pestre združbe na že obstoječih travnikih ali sanacijo degradiranih površin ob večjih zemeljskih delih. Pri zbiranju in pridelavi ohranjevalnih semenskih mešanic je potrebno upoštevati različne smernice in direktive EU (92/43/EGS; 2009/147/ES; 2010/60/EU), ki predpisujejo zahteve o ohranjanju habitatov, varovanju ptic ter postopkih, označevanju in količinskih omejitvah pridelave ohranjevalnih semenskih mešanic.

3 TRAVINJE VELIKE NARAVNE VREDNOSTI IN OHRANJEVALNE SEMENSKE MEŠANICE

V slovenski strokovni literaturi iz 19. stoletja zasledimo, da so kmetje v preteklosti že obnavljali rušo na načine, s katerimi danes v tujini uspešno obnavljajo biotsko pestro travinje. Povše (1876) namreč navaja: »*Povsod, kjer zapazimo, da je travnik revno obraščen ali ima slabe rastline, treba je napraviti in zboljšati trato. To se vrši po raznih načinih. Nekteri naberejo drugje na kakem drugem zemljišči potrebne ruše, da jo na travnik pokladajo. To stane dovolj dela in tudi stroškov, in vspeh vendar ni kaj poseben. Ali vsakemu hočemo svetovati napravo trate s setvijo. Potrebujemo semena raznih trav in zelišč, dobivamo in odgojujemo jih na travnih semeniščih*.« Vendar že v nekoliko kasnejši slovenski literaturi (Turk, 1924) zasledimo priporočila o pridelovanju in setvi travnih mešanic na način, ki je prevladoval v dvajsetem stoletju in je v uporabi večinoma še danes. Vsesplošna intenzifikacija kmetijstva je namreč v ospredje postavila samo nekatere požlahtnjene sorte trav in metuljnic, ki so pomembne predvsem za pridelavo velikih količin kakovostne krme. Zavedajoč se pomembnosti botanično pestrega pol-naravnega travinja pri zaščiti habitatov, biotske raznovrstnosti ter varovanja floristične in genetske identitete regije, so se šele v zadnjih letih izboljšale ter razvile nekatere nove metode pridobivanja semen, ki zadovoljujejo potrebe pri obnovi in ohranjevanju travinja velike naravne vrednosti. Govorimo o t. i. ohranjevalnih semenskih mešanicah. Te so že dokaj poznane v angleško govorečih in alpskih državah. Ohranjevalne semenske mešanice morajo biti sestavljene iz rastlinskih vrst, značilnih za želeno rastlinsko združbo in regijo izvora (Jongepierová in sod., 2007). Pomembno je, da pri izbiri območja pridelave ohranjevalne semenske mešanice izberemo pol-naravno travinje, katerih tla imajo enako založenost hranil in podoben hidrološki status ter podnebne razmere kot rastišča, na katerih bomo mešanico uporabili za obnovo ruše. S tem zagotovimo optimalno prilagojenost sestave mešanice lokalnim podnebnim in talnim razmeram. V nekaterih državah (Nemčija, Avstrija in Švica) so zato določili območja oz. fitogeografske regije znotraj katerih se lahko pridelana ohranjevalna mešanica uporabi, oziroma trži (VWW, 2011; REWISA, 2011; CPS, 2009). Velja pa poudariti, da se meje fitogeografskih regij ne pokrivajo z mejami zveznih dežel ali pokrajin.

V Nemčiji so v zvezni deželi Saška-Anhalt ustanovili register travnikov (ww.spenderflaechenkataster.de), primernih za pridelavo ohranjevalnih semenskih mešanic. Register ustreznih travnikov je predvsem baza podatkov, ki pridelovalcem ohranjevalnih semenskih mešanic omogoča učinkovito iskanje, načrtovanje in pridelavo ohranjevalnih semenskih mešanic v skladu z naravovarstvenimi načeli. Določeni travnik se uvrsti v register travnikov, primernih za pridelavo ohranjevalnih semenskih mešanic, če izpolnjuje poleg prej omenjenih še naslednje posebne pogoje: predstavlja reprezentativno rastlinsko sestavo za regijo in rastlinsko združbo, vsebuje zelo majhno število tujerodnih vrst (predvsem zaradi njihove velike tekmovalne sposobnosti), ni načrtovana sprememba rabe zemljišča, pomemben pa je tudi ustrezen način gospodarjenja s pol-naravnim travinjem (Krautzer in sod., 2011). Za katerikoli način pridobivanja semen je potrebno dovoljenje naravovarstvenih inštitucij in odobritev lastnikov zemljišč. Pridelovalci ohranjevalnih semenskih mešanic lahko požanjejo seme samo pod posebnimi pogoji, kot je upoštevanje primernega

termina za spravilo s čim manjšim škodljivim vplivom na habitate.

Za kmetovalce in ostale uporabnike ohranjevalnih semenskih mešanic so poleg deklariranega regionalnega porekla pomembne še informacije o kakovosti semenskega materiala v prodaji, kar omogočata le ustrezen nadzor pridelave in certifikacija. Sestava ohranjevalnih semenskih mešanic je zelo različna med fitogeografskimi regijami, do razlik pa prihaja tudi znotraj posameznih fitogeografskih regij. Količina semena posameznih rastlinskih vrst v mešanici je namreč odvisna od številnih dejavnikov kot so: botanična sestava, termin in način žetve ter tudi leto pridelave. Število rastlinskih vrst in njihovo razmerje v mešanici je v prvi vrsti odvisno od rastlinske združbe, pomemben dejavnik pa je tudi čas žetve. Kasnejše žetve namreč zmanjšujejo delež trav, povečujejo pa delež zeli oz. dvokaličnic. V ohranjevalnih semenskih mešanicah je lahko precejšen delež primesi (plev, zemlje idr.). Pomembna parametra pri nadzoru kakovosti ohranjevalnih semenskih mešanic sta še čistota in kalivost semenskega materiala (Krautzer in sod., 2011).

4 PRIDOBIVANJE SEMENA OHRANJEVALNIH SEMENSKIH MEŠANIC

Pridobivanja semen ohranjevalnih semenskih mešanic se lahko lotimo na različne načine; semena posameznih rastlinskih vrst lahko pridelujemo na polju, ali jih zbiramo na različne načine na botanično pestrem pol-naravnem travinju**.** V primerjavi s semensko pridelavo klasičnih požlahtnjenih sort trav in metuljnic je pridelava semen posameznih rastlinskih vrst na polju in priprava ohranjevalnih semenskih mešanic bistveno zahtevnejša (Krautzer in sod., 2004; Krautzer in sod., 2010b). Od pridelovalca se poleg znanja o tehnologiji pridelave in dodelave semena zahteva tudi dobro poznavanje rastlinskih vrst, njihovega razvoja in medsebojnih vplivov. V prvi vrsti zahtevajo vse rastlinske vrste dobro pripravljeni setveno plast tal. Za številne vrste v ohranjevalnih semenskih mešanicah je namreč značilna nekonkurenčnost z njivskimi pleveli oz. zelo počasen mladostni razvoj rastlin. Da se izognemo morebitnim izgubam pridelka morajo biti ukrepi za zatiranje plevelov in varstvo pred boleznimi opravljeni čim bolj zgodaj oz. pravočasno (Krautzer in sod., 2004). Kakovostni parametri, kot sta čistota in kalivost semena, so prav tako v veliki meri odvisni od pridelovalca oz. termina in načina žetve. Po žetvi je pomemben hiter transport in čim hitrejši pričetek sušenja semenskega materiala (Krautzer in sod., 2010a). Zaradi velike vsebnosti vlage se namreč lahko požeto seme začne hitro segrevati. Pri sušenju je pomembno, da temperatura ne preseže 42 °C, saj pri višjih temperaturah seme izgubi sposobnost kalitve (Krautzer in sod., 2004). Večina slovenskih kmetij ne razpolaga z velikimi zmogljivostmi za dodelavo, sušenje in skladiščenje, zato menimo, da bodo za slovenske pridelovalce v začetnih letih pridelave ohranjevalnih semenskih mešanic zanimivejše v nadaljevanju predstavljene metode ali njihove kombinacije.

Ročno zbiranje semena posamezne rastlinske vrste na biotsko pestrem travinju je eden od načinov pridobivanja kakovostnega semena, saj lahko vsakokrat naberemo seme v optimalnem času zrelosti. Ta način dela je primeren pri obnovi manjših degradiranih površin. Metodo lahko kombiniramo še z drugimi metodami. Tako nabrano seme lahko uporabimo tudi za obogatitev ohranjevalnih semenskih mešanic, ki smo jih pridobili na druge načine (Krautzer in Pötsch, 2009). Običajno se ročno nabrano seme uporablja za nadaljnje razmnoževanje, ki ga potem naknadno vključujemo v ohranjevalno semensko mešanico. Slabost te metode je, da za ta način zbiranja semen potrebujemo veliko časa in poznavanje rastlinskih vrst (Stevenson in sod., 1995; Scotton in sod., 2003).

Zbiranje semena s posebnim strojem ali t. i. krtačenje je metoda, ki se najpogosteje uporablja v Severni Ameriki in Angliji (Krautzer in Pötsch, 2009). S pomočjo vrtečega se valja z različno dolgimi krtačami otresejo seme, ki pada v zbiralnik (Jongepierová in Mitchley, 2009). Tako zbrani material se lahko uporabi za takojšnjo setev ali pa se ga dosuši, očisti primesi ter ustrezno skladišči do primernega časa setve (Edwards in sod., 2007). Pridelki tako zbranega semena znašajo med 20 in

80 kg ha^{-1} (Haslgrübler, 2010). Ker gre za t. i. nedestruktivno metodo, saj ruše ne pokosimo, lahko na isti lokaciji zbiramo seme ob zrelosti različnih rastlinskih vrst.

Zelo učinkovito metodo predstavlja **žetev biotsko pestrega trajnega travinja s kombajnom** (Krautzer in Pötsch, 2009; Krautzer in sod., 2010b). Pri tem načinu površine požanjemo v optimalnem roku zrelosti semen želenih rastlinskih vrst. Po žetvi sledi sušenje semenskega materiala. Z žetvijo manjših površin v različnih terminih, prilagojenih zrelosti različnim rastlinskim vrstam, pridobimo vrstno bogat semenski material, ki ga posušenega lahko skladiščimo tudi več let. Pridelek semenskega materiala lahko znaša med 150 in 200 kg čistega semena ha^{-1}, odvisno od razvojnega stadija rastlinske združbe. Kombajn že ob žetvi izloči vsa večja stebla in bili, zato lahko glede na stopnjo čistosti seme uporabimo za ročno setev, setev s sejalnico ali hidrosetev. Razmerje med požeto in posejano površino je lahko različno, npr. od 1:1 do 1:2 (Krautzer in Pötsch, 2009). Žetev s kombajnom je otežena na nagnjenih in neravnih terenih (Jongepierová in Mitchley, 2009).

Pri odločitvi katera izmed metod pridelave ohranjevalnih semenskih mešanic je najprimernejša v danih razmerah, je potrebno pretehtati številne dejavnike. Glede na reliefno razgibanost in fitocenološko raznolikost slovenskega trajnega travinja ne moremo priporočiti enotne metode za zbiranje oz. pridelavo ohranjevalnih semenskih mešanic.

5 METODE OBNOVE RUŠE VELIKE NARAVNE VREDNOSTI

Glavna cilja obnove pol-naravnega travinja z veliko naravno vrednostjo sta želena botanična sestava, stabilnost in trpežnost travne ruše. Za uspešno obnovo pol-naravnega travinja z veliko naravno vrednostjo obstajajo različne metode. V tujini se kmetovalci in lastniki zemljišč skupaj s strokovnjaki odločajo za ustrezno metodo na podlagi stroškov povezanih s tehnologijo obnove, naravovarstvenih zahtev ter želene sestave rastlinske združbe na izbrani lokaciji (Krautzer in sod., 2004). V ravnini je pri obnovi degradiranih površin bogatih s hranili velikokrat potrebno odstraniti vrhnjo plast tal oz. je potrebna vsaj obdelava tal s krožno brano ali globokim oranjem (Török in sod., 2011). Ustrezna obdelava in predsetvena priprava tal omogočata bistveno uspešnejši razvoj in vzpostavitev želene rastlinske združbe v primerjavi z vsejavanjem (Kiehl in sod., 2010). Osiromašenje tal za vzpostavitev nizko produktivne botanično pestre rastlinske združbe bi samo s košno ali pašno rabo in brez vračanja hranil trajalo več desetletij (Verhagen in sod., 2001). Namen odstranitve vrhnje plasti je hitro osiromašenje globokih tal s bogatih s hranili in semenskim materialom neželenih rastlin, predvsem zelo konkurenčnih trav (Pywell in sod., 2002). Uspešnost vzpostavitve botanično pestrega travinja je v veliki meri odvisna od odstranjenega deleža vrhnje plasti tal (van Diggelen, 2009). V zgornjih 15 cm tal je namreč prisoten predvsem dušik vezan v organski snovi, medtem, ko sta fosfor in kalij vezana na mineralni del tal tudi v nižjih plasteh tal. Van Diggelen (2009) navaja, da je potrebno za uspešno vzpostavitev nizko produktivne biotsko pestre združbe odstraniti vrhnjih 30 cm tal ali več. Odstranjevanje vrhnje plasti tal v kombinaciji s drugimi intenzivno raziskujejo v zahodno evropskih državah (Nizozemska, Velika Britanija, Nemčija), kjer imajo zaradi desetletij intenzivne pridelave krme in rabe mineralnih gnojil velike težave s vzpostavljanjem prvotne biotske pestrosti travinja.

V primeru, da odstranitev vrhnje plasti tal ni potrebna je najpreprostejši način obnove manjših degradiranih površin in pol-naravnega travinja ročna setev ohranjevalnih semenskih mešanic. V kolikor nam reliefne in talne razmere dopuščajo, pa lahko uporabimo različne sejalnice in trosilnike mineralnih gnojil, vendar je pri setvi ali vsejavanju potrebno paziti, da seme vseh rastlinskih vrst enakomerno porazdelimo po površini. Ohranjevalne semenske mešanice običajno vsebujejo od 20 do 30 rastlinskih vrst. Jongepierová in Mitchley (2009) sta ugotovila uspešno obnovo trajnega travinja pri uporabi majhne količine (17 do 20 kg ha^{-1}) ohranjevalnih semenskih mešanic. Kljub temu Krautzer in sod. (2010b) priporočajo za setev nekoliko večje količine semena (do 50 kg ha^{-1}). Obnovo degradiranih površin lahko uspešno izvedemo s spomladansko setvijo in uporabo t. i. varovalnih

posevkov žit (60 kg ha^{-1} ovsa ali jarega ječmena), vendar daje najboljše rezultate pozno poletna ali zgodnje jesenska setev (od sredine avgusta do sredine septembra). Slednja je priporočljivejša predvsem za suhe travnike pa tudi zaradi dormantnosti semen nekaterih zeli (npr. *Plantago major* L., *Heracleum sphondylium* L., *Galium aparine* L.), ki jo prekine stratifikacija semen pri nizkih temperaturah (Gselman in Kramberger, 2008). Krautzer in sod. (2010b) navajajo, da je veliko lažje vzpostaviti botanično pestro združbo na vlažnih travnikih, saj je vsejavanje možno skozi celotno obdobje vegetacije. Za zagotovitev enakomernega vznika je po setvi priporočljivo valjanje. Uporabimo lahko tudi druge metode, ki dopolnjujejo uporabo ohranjevalnih semenskih mešanic pri obnovi pol-naravnega travinja z veliko naravno vrednostjo ali jih v celoti nadomestijo. Prednost v nadaljevanju opisanih metod je predvsem v tem, da omogočajo varovanje tal pred različnimi oblikami erozije in jih priporočamo predvsem na hribovitih in nagnjenih terenih.

Skozi stoletja je bila najbolj običajna metoda obnove travnate ruše setev **senenega drobirja** bogatega s semeni trav, metuljnic in zeli, ki so se nabrala na senikih (Krautzer in Wittmann, 2006). Kot navaja Turk (1924), je bila ta metoda običajna tudi na Slovenskem, vendar so v tistem času na podlagi takratnega znanja in drugačnih pogledov pri gospodarjenju na travinju, to ocenjevali kot nesmisel. Težnja takratne stroke je bilo upravičeno povečanje pridelkov in izboljšanje kakovosti takrat prevladujočega ekstenzivnega in biotsko pestrega travinja.

Danes strokovna in znanstvena literatura navaja, da je seneni drobir lahko bogat vir semen travniških rastlin in odličen material za ozelenitev površin ali obnovo travne ruše. Za kakovosten seneni drobir mora biti košnja dovolj pozna, da lahko travniške rastline dozorijo; priporočajo pa tudi uporabo senenega drobirja mlajšega od dveh let. (Krautzer in Wittmann, 2006). Pogosto se priporoča čiščenje drobirja, da pridobimo večji delež čistega semena. Seneni drobir sejemo v količini od 0,5 do 2 kg na m^2, debelina pa naj ne preseže 2 cm. Da preprečimo razpihovanje semena drugam je priporočljiva setev omočenega materiala z vodo ali na vlažna tla. Pri slabi kalivosti lahko manjkajoče rastlinske vrste kasneje še dodatno vsejemo.

V ravninskih predelih lahko uspešno obnovo pol-naravnega travinja dosežemo z ustrezno pripravo zemljišča in strojno setvijo. Na zelo strmih pobočjih je strojna setev velikokrat otežena. V takem primeru dosežemo najboljše rezultate pri setvi z vodno tehniko oziroma t. i. hidrosetvijo (Cook in sod., 1997). Pri hidrosetvi se v cisterni pripravi mešanico semen, vode, hranil, ter glutena, ki jo enakomerno razpršimo po površini. Hidrosetev lahko kombiniramo z uporabo zastirke, ki omogoča hitrejšo kalitev in zmanjšujejo erozijo (Barnet in sod., 1967; Sidle in sod., 1993). Pri setvi na manj nagnjenih pobočjih in zavetrnih legah je dovolj, če tla oziroma seme zaščitimo z organskimi zastirkami, kot je seno ali slama. Vendar je pri nanosu potrebno paziti na primerno razporeditev in debelino zastirke. Pri zastirki s senom debelina materiala ne sme biti večja od 2 cm, pri zastirki iz slame pa je lahko največ 3 do 4 cm ali 300 do 600 g suhe snovi na m^2, hkrati pa mora biti še prepustna za svetlobo (Krautzer in Wittmann, 2006; Krautzer in Pötsch, 2009). Na bolj nagnjenih terenih se za preprečevanje vetrne in vodne erozije uporabljajo številne komercialno dostopne sintetične ter organske tekstilne prekrivke iz jute ali kokosovih vlaken. Slednje so primernejše, ker so biološko razgradljive (Krautzer in Wittmann, 2006). V uporabi pa so tudi metode, kjer se pri obnovi degradiranih območij uporablja na različne načine vzgojene sadike ogroženih rastlinskih vrst v kombinaciji s katero izmed prej omenjenih metod obnove degradiranih območij. (Krautzer in Pötsch, 2009). Kakovostna izvedba omenjenih metod zahteva veliko znanja in opremljenost s prilagojenimi stroji, kar je pogosto povezano s višjimi stroški izvedbe obnove pol-naravnega travinja.

V praksi ima vsaka od metod obnove svoje prednosti in slabosti. Prednost uporabe ohranjevalnih semenskih mešanic v primerjavi z drugimi metodami je hitro osnovanje in trpežnost obnovljene ruše, saj ni težav s pretiranim širjenjem kakovostnih trav, ki bi izpodrivale zelnate dvokaličnice. Krautzer in sod. (2011) na podlagi izkušenj z obnavljanjem steljnikov, vlažnih in pol-suhih travnikov navajajo, da prvo leto po vsejavanju ohranjevalnih semenskih mešanic lahko opazimo 30 do 50 % rastlinskih vrst enakih izvornemu rastišču. Pri čisti setvi pa lahko uspešnost prenosa v prvem letu po setvi doseže tudi 60 % rastlinskih vrst enakih izvornemu

rastišču, ob tem da se lahko v 10 do 20% deležu pojavijo tudi enoletni pleveli, ki že v drugem letu po setvi izginejo ali je njihov delež zanemarljivo majhen (Jongepierová in sod., 2007). Uspešnosti obnove pol-naravnega travinja z ohranjevalnimi semenskimi mešanicami je velika, kljub temu pa je še vedno odvisna od številnih dejavnikov: kakovosti semena, semenske banke v tleh, načina setve, priprave in založenosti tal s hranili ter vremenskih razmer po setvi. Čeprav je obnova pol-naravnega travinja z ohranjevalnimi semenskimi mešanicami eden izmed dražjih načinov obnove, tovrstno travinje ne izpolnjuje samo sekundarnih funkcij, ampak omogoča tudi proizvodnjo zadostne količine kakovostne krme za živinorejo (Jongepierová in sod., 2007).

6 ZAKLJUČKI

Še pred nekaj leti nam je bila obnova botanično pestrega travinja in vzpostavitev avtohtone rastlinske združbe na degradiranih območij popolnoma neznana. Danes na podlagi znanstvene literature in primerov dobre prakse iz tujine vidimo, da je izvedljiva z ustreznimi metodami zbiranja, dodelave in setve semena. Posebej perspektivne so ohranjevalne semenske mešanice, ki se bodo lahko uporabljale pri obnovi degradirane travnate ruše z namenom ustvarjanja ruše velike naravne vrednosti in za zasnovo nove travnate ruše po večjih gradbenih posegih v naravi, predvsem na zavarovanih območjih in območjih Nature 2000. Ohranjevalne semenske mešanice zagotavljajo trajnostno rešitev za tovrstno rušo, saj so rastlinske vrste teh mešanic prilagojene lokalnim razmeram. Ker je pridelava ohranjevalnih semenskih mešanic in obnova travnate ruše z njimi, zahtevnejša od pridelave in setve požlahtnjenih sort, bo ob uveljavitvi v prakso zagotovo potrebna strokovna podpora pridelovalcem in finančna spodbuda s strani države.

7 ZAHVALA

Zahvaljujemo se Ministrstvu za kmetijstvo in okolje za financiranje projekta CRP - Ohranjanje biotske raznovrstnosti travinja z vzpostavitvijo sistema pridelovanja ohranjevalnih semenskih mešanic (V4-1128), v okviru katerega je nastal ta prispevek.

8 VIRI

ARSO 2008. Kazalci okolja v Sloveniji. Kmetijska območja visoke naravne vrendosti. (T. Cunder) http://kazalci.arso.gov.si/?data=indicator&ind_id=57 (20. dec. 2012)

Barnett A.P., Diseker E.G., Richardson E.C. 1967. Evaluation of mulching methods for erosion control on newly prepared and seeded highway black-slopes. Agronomy Journal 59: 83-85.

Bekker R.M., Bakker J.P., Grandin U., Kalamees R., Milberg P., Poschlod P., Thompson K., Willems J.H. 1998. Seed size, shape and vertical distribution in the soil: indicators of seed longevity. Functional Ecology 12: 834-842.

Bolund P., Hunhammar S. 1999. Ecosystem services in urban areas. Ecological Economics 29, 293-301.

Carrow R.N. 2005. Can we maintain turf to customers satisfaction with less water? Agricultural Water Management 80:117-131.

Cook A., Baker S.W., Canaway P.M., Hunt J.A., 1997. Evaluation of turf established using 'liquid sod' as compared with establishment using seed and turf. Journal of Turfgrass Science 73: 73-83.

Cunder T. 1998. Razširjenost travinja v Sloveniji. Sodobno kmetijstvo 31(4): 173-175.

CPS 2009. Švicarska komisija za ohranitev prostoživčih rastlinskih vrst. Swiss Commission for Wild Plant Conservation. www.cps-skew.ch (2. dec. 2012).

Direktiva 2009/147/ES Evropskega parlamenta in sveta z dne 30. Novembra 2009 o ohranjanju prosto živečih ptic (kodificirana različica). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:020:0007:0025:SL:PDF (19. dec. 2012).

Direktiva komisije z dne 30. avgusta 2010 o določitvi nekaterih odstopanj pri trženju semenskih mešanic krmnih rastlin, namenjenih za uporabo pri ohranjanju naravnega okolja. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:228:0010:0014:SL:PDF (16. dec. 2012).

Direktiva sveta 92/43/EGS z dne 21. maja 1992 o ohranjanju naravnih habitatov ter prosto živečih živalskih in rastlinskih vrst. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1992L0043:20070101:SL:PDF (18. dec. 2012).

Edwards A.R., Mortimer S.R., Lawson C.S., Westbury D.B., Harris S.J., Woodcock B.A., Brown V.K. 2007. Hay strewing, brush harvesting of seed and soil disturbance as tools for the enhacement of botanical diversity in grasslands. Biological conservation 134: 372-382.

Eurostat 2010. Agri-environmental statistics. http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Agri-environmental_statistics (20. dec. 2012).

Gselman A., Kramberger B. 2008. Življenjska doba in alokacija semen v travniških tleh. Znanstvena monografija, Fakulteta za kmetijstvo Univerze v Mariboru. 78 str.

Halsgrübler P. 2010. Nutzung des Samenpotentials naturnaher Grunlandflächen. Das Europäische Projekt Salvere, Bericht zur Tagung: Gewinnung, Produktion und Verwendung von Wildpflanzen und Saatgut, LFZ Raumberg-Gumpenstein, 27-30.

Hopkins A. 2009. Relevance and functionallity of semi natural grassland in Europe- status qou and future prospective. International Workshop of the Salvere – Project 2009, 9-14.

Hopkins A., Holz B. 2006. Grassland for agriculture and nature conservation: production, quality and multifunctionality. Agronomy Research 4(1): 3-20.

Jongepierová I., Mitchley J., Tzanopoulus J. 2007. A field experiment to recreate species rich hay meadows using regional seed mixtures. Biological Conservation, 139: 297-305.

Jongepierová I., Mitchley J. 2009. Ecological principles for the re-creation of species rich grasslands in agricultural landscapes. Ur. Cagaš B., Macháč R., Nedělnik J. Alternative Functions of Grassland, Brno, Czech Republik, 7-9 september 2009. 469-477.

Kaligarič M., Culiberg M., Kramberger B. 2006. Recent vegetation history of the North Adriatic grasslands : expansion and decay of an anthropogenic habitat. *Folia geobotamica* 41(3): 241-258.

Kiehl K., Kirmer A., Donath T.W., Rasran L., Hölzel N. 2010. Species intorduction in restoration projects – Evaluatuion od different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. Basoc amd Applied Ecology 11: 285-299.

Krautzer B., Bartel A., Kirmer A., Tischew S., Feucht B., Wieden M., Haslgrübler P., Rieger E., Pötsch E.M. 2011. Establishment and use of High Nature Value Farmland. Ur. Pötsch E.M., Krautzer B., Hopkins A. Grassland Farming and Land Management Systems in Montainous Regions, Raumberg-Gumpenstein, Austria, 457-469.

Krautzer B., Graiss W., Blaschka A. 2010a. Seed production of site-specific grasses and herbs in Austria. Proceedings 7th European Conference on Ecological Restoration, Avignon, France, 23-27 august 2010, 1-4. http://ser.semico.be/ser-pdf/EA_SER2010_271.pdf (2. jan. 2013).

Krautzer B., Graiss W., Haslgrübler P. 2010b. Die Kulturlandschaft als Quelle für naturschutzfachlich wertvolles Saat- und Pflanzgut. Bericht zur Tagung, Gewinung, Produktion und Verwendung von Wildpflanzen und Saatgut 20. Mai 2010, 1-8.

Krautzer B., Peratoner G., Bozzo F. 2004. Site specific Grasses and Herbs: Seed Production and use for restoration of mountain environments. 111 s.

Krautzer B., Pötsch E.M. 2009. The use of semi-natural grassland as donor sites for restoration of high nature value areas. Ur. Cagaš B., Macháč R., Nedělnik J. Alternative Functions of Grassland, Brno, Czech Republik, 7-9 september 2009. 478-492.

Krautzer B., Wittmann H. 2006. Restoration of alpine ecosystems. Ur.: van Andel J. in Aronson J. Restoration Ecology, The New Frontier. Blackwell Publishing, 208-220.

Mitchley J., Jongepierová I., Fajmon K. 2012. Regional seed mixtures for the re-creation of species-rich meadows in the White Carpatian Mountains: results of a 10-yr experiment. Applied Vegetation Science 15, 253-263.

Pauwels G., Gulinck H. 2000. Changing minor rural road networks in relation to landscape sustainability and farming practices in West Europe. Agriculture, Ecosystems & Environment 77: 95-99.

Pärt T., Söderström B. 1999. The effects of management regimes and location in landscape on the conservation of farmland birds breeding in semi-natural pastures. Biological Conservation 90: 113–123.

Peeters A. 2012. Past and future of European grasslands. The challenge of the CAP towards 2020. Ur. Golinski P., Warda M., Stypiński P. Grassland – a European Resource? Lublin, Poland, 17-32.

Poschlod P., Kiefer S., Traenkle U., Fischer S. Bonn S. 1998. Species richness in calcareous grasslands in affected by disprsability in space and time. Applied Vegetation Science 1: 75-90.

Povše F. 1876. Umni kmetovalec ali splošni poduk, kako obdelovati in zboljšati polje, travnike, vrtove in gozde. Družba Sv. Mohora, 270 str.

Prochnow A., Heiermann M., Plöchl M., Linke B., Idler C., Amon T., Hobbs, P.J. 2009. Bioenergy from permanent grassland – A review: 1. Biogas. Bioresource Technology 100 (21): 4931-4944.

Pywell R.F., Bullock J.M., Hopkins A., Walker K.J., Sparks T.M., Burke J.W.M., Peel S. 2002. Restoration of species-rich grassland on arable land: assessing the limiting processes using multi-site experiment. Journal of Applied Ecology 39: 294-309.

REWISA 2012. Regionale Wildpflanzen und Samen www.rewisa.at (4. dec. 2012)

Rösch C., Skarka J., Raab K., Stelzer V. 2009. Energy production from grassland – Assessing the sustainability of different process chains under German conditions. Biomass and Bioenergy, 33 (4): 689-700.

Scotton M., Vescovo L., Carbonari A. 2003. Grassland seed harvesting for ecological restoration. Ur.: Kirilov A., Todorov N., Katerov I. Optimal forage systems for animal production and the environment. Proceedings of the 12th Symposium of the European Grassland Federation, Pleven, Bulgaria, 26-28 Maj 2003, 588-591.

Siedle R.C., Brown R.W., Williams B.D. 1993. Erosion processes on arid minespoil slopes. Soil Science Society of America Journal 57: 1341-1347.

Starczewski K., Affek-Starczewska A., Jankowski K. 2009. Non-marketable functions of grasslands. Ur.: Cagaš B, Machač R., Nedelnik J. Alternative functions of grasslands. Proceedings of the 15th of the European Grassland Federation Symposium. Brno, Czech Republic, 7-9 September 2009, 37-45.

Stevenson M.J., Bullock J.M., Ward L.K. 1995. Recreating semi-natural communities, effects of sowing rate on establishment of calcareous grasslands. Restoration Ecology 3:279-289.

SURS 2011. Struktura kmetijskih zemljišč v uporabi po zemljiških kategorijah. http://www.stat.si/letopis/2011/16_11/16-06-11.htm (20. dec. 2012).

Tome D., Sovinc A., Trontelj P. 2005. Ptice Ljubljanskega barja. DOPPS. 138 str.

Török P., Vida E., Deák B., Lengyel S., Tóthmérész B. 2011. Grassland restoration on former croplands in Europe: an assessment of applicability of techniques and costs. Biodiversity and conservation 20: 2377-2332.

Turk J. 1924. Travništvo. Slovenskim živinorejcem v pouk. Družba Sv. Mohorja, 270 str.

van Diggelen R. 2009. Ecological restoration of semi-natural grassland from agricultural fields Status qou and future prospects. International Workshop of Salvere project, Raumberg-Gumpenstein, 23-26.

Verhagen R., Klooker J., Bakker J.P., van Diggelen R. 2001. Restoration success of low-production plant on former agricultural soils after top-soil removal. Applied Vegetation Science 4: 75-82.

Vidrih M. 2006. Vezava ogljika v pašeni ruši visokega krasa. Doktorska disertacija. 102 str.

VWW 2012. Združenje nemških pridelovalcev ohranjevalnih semenskih mešanic in prostoživečih rastlin www.natur-im-vww.de (6.12.2012).

Willems J.H. 1995. Soil seed bank, seedling recruitment and actual species composition in an old and isolated chalk grassland site. Folia Geobotanica eg Phytotaxonomica 30:141-156.

Willems J.H., Peet R.K., Bik L.P.M. 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. Journal of Vegetation Science 4: 203-212.

CONTENT ANALYSIS OF THE PAPERS IN THE ACTA AGRICULTURAE SLOVENICA

VSEBINSKA OBDELAVA PRISPEVKOV V ACTA AGRICULTURAE SLOVENICA let. 101 št. 1

Tomaž BARTOL[a], Karmen STOPAR[b],

SUBJECT INDEX BY AGROVOC DESCRIPTORS
PREDMETNO KAZALO PO DESKRIPTORJIH AGROVOC

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VSEBINSKO KAZALO PO SKUPINAH ZNANJA (PREDMETNIH KATEGORIJAH)

POPRAVEK/CORRIGENDUM

Za zvezek 99-3 pravilen zapis v članku Genska banka koruze v Sloveniji je »Zadnji hibrid, vpisan v sortno listo leta 2011, je hibrid Lj-220w z zelo kakovostnim poltrdim belim zrnjem, namenjen predvsem za ljudsko prehrano« (na strani 325).

In issue 99-3 correct spelling in the article The Slovenian maize gene bank »The latest hybrid, entered in the EU Common catalogue in 2011, is a hybrid Lj-220w with high-quality semi-flint grain type, with white endosperm and primarily for purpose of human consumption« (on page 325).

NAVODILA AVTORJEM

(letniki z liho številko - rastlinska proizvodnja)

Prispevki

Sprejemamo izvirne znanstvene članke s področja agronomije, hortikulture, rastlinske biotehnologije, raziskave živil rastlinskega izvora, agrarne ekonomike in informatike ter s sorodnih področij - **letniki z liho številko** (npr. 97, 99) - v slovenskem in angleškem jeziku; pregledne znanstvene članke samo po poprejšnjem dogovoru. Objavljamo tudi izbrane razširjene znanstvene prispevke s posvetovanj, vendar morajo taki prispevki zajeti najmanj 30 % dodatnih originalnih vsebin, ki še niso bile objavljene. O tovrstni predhodni objavi mora avtor obvestiti uredniški odbor. Če je prispevek del diplomske naloge, magistrskega ali doktorskega dela, navedemo to in tudi mentorja na dnu prve strani. Navedbe morajo biti v slovenskem in angleškem jeziku.
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The Journal *accepts original scientific* articles from the fields of agronomy, horticulture, plant biotechnology, plant-related food-and-nutrition research, agricultural economics, information-science, and related research - odd-numbered volumes (for example: 97, 99) - in Slovenian or English language. Review articles are published in advance agreement with the editorial board. Extended versions of selected proceedings-papers can also be considered for acceptance, provided they include at least 30% of new original content, but the editorial board must be notified beforehand. If the article is based on a thesis or dissertation, the thesis-type must be indicated (BSc, MSc, PhD...), along with the role of the candidate and advisor, at the bottom of the first article page.
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