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Effects of seed size and aging on field performance of lentil (*Lens culinaris* Medik.) under different irrigation treatments

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ABSTRACT

A sub-sample of lentil (*Lens culinaris* 'Kimia') seeds was kept as bulk (S_1) and another sample was separated to large (S_2) and small (S_3) seeds. A sub-sample of each size was kept as control or high vigor seed lot (A_1) and the two other sub-samples were artificially aged for 2 and 4 days (A_2 and A_3, respectively). Field performance of these seeds was evaluated during 2011 and 2012. Yield components and grain yield of lentil decreased with decreasing water availability. The highest yield components (except 1000 grain weight) and grain yield per unit area were obtained by plants from large seeds. The superiority of plants from large seeds in grain yield was more evident under limited irrigations than under well watering. Seed aging resulted in poor stand establishment and consequently low grain yield per unit area. Plants from aged large seeds showed the lowest reduction in grain yield per unit area, compared with those from aged small and bulk seeds. It seems that cultivation of large seeds somehow can reduce the deleterious effects of drought stress and seed aging on grain yield per unit area of lentil.

Key words: grain yield, lentil, seed aging, seed size, drought stress

IZVLEČEK

VPLIV VELIKOSTI IN STARANJA SEMENA NAVADNE LEČE (*Lens culinaris* Medik.) NA NJENO USPEVANJE V POLJSKEM POSKUSU PRI RAZLIČNIH REŽIMIH NAMAKANJA

Semena navadne leče (*Lens culinaris* 'Kimia') so bila pred setvijo razdeljena na večji podvzorec (S_1) in manjšega, ki je bil razdeljen na velika (S_2) in mala (S_3) semena. Podvzorec semen vseh velikost je bil vzet kot kontrola, oziroma kot vitalna semena (A_1), ostala dva podvzorca semen sta bila umetno starana 2 in 4 dni (A_2 in A_3, vzorca). Uspevanje semen je bilo v poljskem poskusu ovrednoteno v sezonah 2011 in 2012. Komponente pridelka in pridelka zrnja leče so upadale z zmanjševanjem dostopnosti vode. Največje vrednosti komponent pridelka (razen mase 1000 zrn) in pridelka zrnja na enoto površine so bile dosežene z rastlinami iz velikih semen. Superiornost rastlin iz velikih semen v pridelku zrnja je bila še bolj očitna v razmereh omejenega namakanja v primerjavi z dobro zalitimi rastlinami. Staranje semen je povzročilo slabšo vzpostavitev sestoja posevka in posledično manjši pridelek zrnja na enoto površine. Rastline iz velikih semen so imele manjše zmanjšanje pridelka na enoto površine v primerjavi s tistimi iz staranih manjših semen in semen enotnega vzorca. Izgleda, da setev velikih semen pri navadni leči nekako zmanjša škodljive učinke sušnega stresa in staranja semen na pridelku zrnja na enoto površine.

Ključne besede: pridelek zrnja, navadna leča, staranje semen, velikost semen, sušni stres

1 INTRODUCTION

Drought stress is known to limit plant productivity in many regions of the world. The onset of stress may initially cause a loss of cell turgor which in turn reduces gas exchange and leaf elongation since both are turgor-dependent processes. The result is a decrease in growth rate since this is a function of transpiration rate and leaf area (Chartzoulakis *et al*., 1993). Some studies have

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shown that growth rates of several plants are directly proportional to the availability of water in the soil (Kamel and Loser, 1995; Ghassemi-Golezani *et al*., 2009). The reduction in growth, yield and quality by water stress has been well documented (Kriedemann and Barrs, 1981; Ghassemi-Golezani *et al*., 2009). Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hu and Schmidhalter, 1998). Water stress may range from moderate and of short duration to extremely severe and prolonged summer drought that has strongly influenced evolution and plant life (Pereira and Chaves, 1995). The physiological responses of plants to water stress and their relative importance for crop productivity vary with species, soil type, nutrients and climate.

High and rapid field emergence and early establishment are essential to obtain an adequate stand and to gain an advantage of the growing season before the onset of the severe drought stress late in the season (TeKrony and Egli, 1991). This can be achieved by cultivating high vigor seeds (Ghassemi-Golezani *et al*., 2010). Seed vigor comprises those properties that determine the potential for rapid and uniform emergence under a wide range of field conditions (ASPB, 2003). Seed vigor could be reduced by aging. Reducing seed vigor following aging has been attributed to a series of metabolic defects that accumulate in embryonic and non-embryonic structures (Osborne, 1983). A large number of reactive oxygen species are generated in the seed during aging which causes lipid peroxidation (McDonald, 1999). This free radical induced non-enzymatic peroxidation, which has the potential to damage membrane, is the major cause of seed deterioration. The deleterious effects of aging on seed vigor and viability are associated with the damage occurring at the membrane, nucleic acid and protein levels (Fujikura and Karssen, 1995). Natural and artificial aging have been reported to reduce seed vigor of many species (Vieira *et al*., 1999; Ghassemi-Golezani *et al*., 2010, 2011, 2012) and were associated with field emergence, growth and yield of barley (Kim *et al*., 1989), winter oilseed rape (Ghassemi-Golezani *et al*., 2010), maize (Ghassemi-Golezani *et al*., 2011) and chickpea (Ghassemi-Golezani *et al*., 2012).

Another factor which may influence seed vigor is seed size, since it is generally proportional to the amount of food reserves that will be destined to the embryo (Lloret *et al*., 1999). Seed size reflects maternal environment, since it is influenced by resource availability (Krannitz *et al*., 1999; Wulff *et al*., 1999) and the environmental conditions to which the mother plant was exposed during seed formation (Vaughton and Ramsey, 1998). Large seeds have a better performance than small seeds, especially under competitive conditions (Eriksson, 1999; Moles and Westoby, 2004). Better performance can be seen in higher emergence (Seiwa, 2000), increased seedling growth (Osunkoja *et al*., 1994), reduced mortality (Seiwa, 2000), increased root/shoot ratio (Lloret *et al*., 1999) and higher grain yields (Rao, 1981).

Larger seeds with well-developed root systems of seedlings may gain an advantage by reaching soil moisture at deeper levels (Leishman and Westoby, 1994). Schimpf (1977) argued that moisture availability was more important than length of the growing season, due to the increased capacity of seedlings from large seeds to establish roots in deeper soil horizons. Royo *et al*. (2006) found that larger seeds resulted in high biomass, green area index, number of spikes per m^2 and heavier kernels. Seed size was also found to be significantly influencing early vigor, tiller number, plant height and dry-matter production in pearl millet (Manga and Yadav, 1995). However, the interaction of seed size and aging on field performance of crops is not clear. Thus, this research was carried out to investigate the effects of seed size and aging on seedling establishment and yield of lentil under different irrigation treatments in two years.

2 MATERIALS AND METHODS

Seeds of lentil (*Lens culinaris* 'Kimia') were obtained from Research Center of Dry-land, Kermanshah, Iran. A sub-sample of the seeds was kept as bulk (S_1) with 1000 grain weight of 42 g. The other seeds were separated by a sieve with four millimeters diameter. The seeds that remained on the sieve were considered as large (S_2) with 1000 grain weight of 50 g and those passed the sieve were considered as small (S_3) seeds with 1000 grain weight of 35 g. Seeds of each size were divided into three sub-samples. A sub-sample was kept as control or high vigor seed lot with 97.2% normal germination (A_1). The two other sub-samples with about 20% moisture content were artificially aged, using controlled deterioration test (ISTA, 2010) at 40°C for 2 and 4 days reducing germination to 90.8% and 82.3% (A_2 and A_3, respectively). So, three seed lots from each size with different levels of aging were provided for field experiment.

The field experiment was conducted at the Research Farm of the University of Tabriz (Latitude 38˚05' N, Longitude 46˚17' E, Altitude 1360 m above sea level) in 2011 and 2012. All the seeds were treated with Benomyl at a rate of 2 g kg^{-1} before sowing. Seeds were hand sown in about 5 cm depth with a density of 100 seeds m^{-2} on 5th May 2011 and 14th May 2012. Each plot consisted of 6 rows with 4 m length in 2011 and with 3 m length in 2012, spaced 25 cm apart. The experiments were arranged as split plot factorial, based on RCB design with three replications. All plots were irrigated immediately after sowing and subsequent irrigations were carried out after 70 (I_1), 120 (I_2) and 170 (I_3) mm evaporation from class A pan. Weeds were controlled by hand during crop growth and development.

Seedling emergence was recorded after final establishment in each plot and percentage of emergence was calculated. At maturity, 10 plants were harvested from each plot and grains per plant and grains per unit area and 1000 grain weight were recorded. Finally, plants of 1 m^2 in the middle part of each plot were harvested and grain yields per plant and per unit area were determined. Analyses of variance of the data based on the experimental design and comparison of means at $p \leq 0.05$ were carried out, using MSTATC software. Excel software was used to draw figures.

3 RESULTS

Seed aging had significant effect on mean emergence percentage ($P \leq 0.01$), but this trait was not significantly affected by seed size ($P > 0.05$). The interaction of year × seed aging for seedling emergence percentage was also significant ($P \leq 0.01$). Seedling emergence percentage was significantly decreased with increasing seed aging (Fig. 1). Seedling emergence percentage for non-aged seed lot (A_1) was similar in both years, but for aged seed lots (A_2 and A_3), it was significantly lower in the second year (Fig. 2).

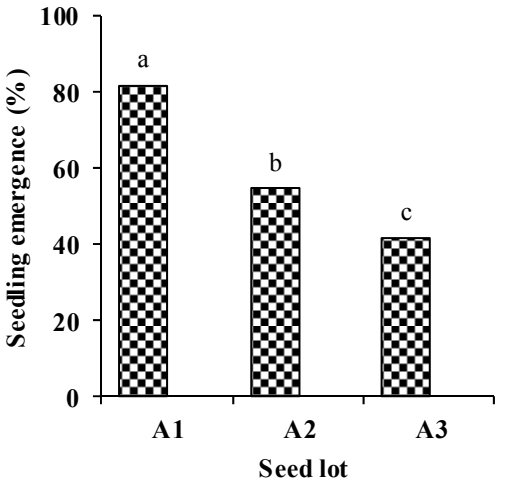

Figure 1: Mean seedling emergence percentage of lentil affected by seed aging
Different letters at each column indicate significant difference at $p \leq 0.05$
A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively

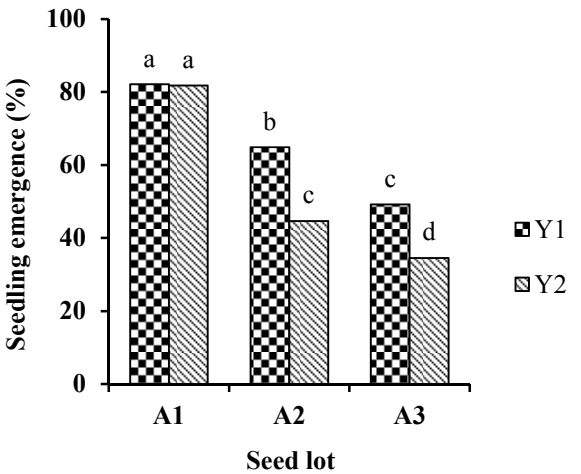

Figure 2: Mean seedling emergence percentage affected by seed aging in two years
Different letters indicate significant difference at $p \leq 0.05$
A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively
Y_1 and Y_2: First and second years, respectively

Combined analyses of variance showed that the effects of irrigation and seed aging on grains per plant, grains per unit area, 1000 grain weight, grain yield per plant and grain yield per unit area were significant. All these traits, except 1000 grain weight, were also significantly affected by seed size. Interaction of irrigation × seed size for grains per plant, grain yield per plant and grain yield per unit area, interaction of irrigation × seed aging for grains per plant, grains per unit area and grain yield per unit area and interaction of seed size × seed aging for grains per plant, 1000 grain weight and grain yield per unit area were significant (Table 1).

Grains per plant, grains per unit area, 1000 grain weight, grain yield per plant and grain yield per unit area significantly decreased with decreasing

water availability. Yield components, except 1000 grain weight, and grain yield per unit area were considerably higher for plants from large seeds (S_2), compared with those from bulk (S_1) and small seeds (S_3) (Table 2). The superiority of plants from large seeds in grain yield per unit area was more evident under limited irrigations than under well watering (Table 3).

Table 1: Combined analyses of variance of the effects of seed size and aging on field performance of lentil under different irrigation treatments

Source of variation	df	MS				
		Grains per plant	Grains per unit area	1000 grain weight (g)	Grain yield (g/plant)	Grain yield (g/m^2)
Year (Y)	1	11.414	5384.914	0.233	0.001	0.001
Error	4	5.975	51568.451	0.924	0.008	159.803
Irrigation (I)	2	4505.796**	16684347.340**	12.364**	5.974**	20425.674**
Y × I	2	1.895	49002.858	0.028	0.006	0.006
Error	8	5.086	21942.423	0.286	0.005	25.996
Seed size (S)	2	338.389**	2571894.525**	0.876	0.863**	2088.630**
Y × S	2	7.895	1489.340	0.007	0.013	0.013
I × S	4	28.046*	128782.552	1.221	0.058*	543.764*
Y × I × S	4	7.960	48940.923	0.095	0.002	0.004
Seed aging (A)	2	307.907**	2972971.062**	225.326**	0.411**	1638.016**
Y × A	2	16.191	8613.432	0.017	0.005	0.007
I × A	4	32.481*	281454.867**	0.137	0.023	460.120*
Y × I × A	4	0.228	49061.627	0.116	0.004	0.018
S × A	4	42.769**	23172.219	9.642**	0.011	529.193*
Y × S × A	4	1.312	3019.664	0.187	0.002	0.006
I × S × A	8	0.968	4568.789	0.453	0.025	244.537
Y × I × S × A	8	0.224	3207.789	0.168	0.006	0.005
Error	96	11.855	72336.390	1.721	0.027	183.939
CV%	-	14.56	17.95	2.91	11.34	19.07

Table 2: Means of yield and yield components of lentil affected by irrigation, seed size and aging

Treatments	**Grains per plant**	**Grains per unit area**	**1000 grain weight (g)**	**Grain yield (g/plant)**	**Grain yield (g/m^2)**
Irrigation					
I_1	31.96 a	2017 a	45.64 a	1.739 a	90.00 a
I_2	25.11 b	1567 b	45.14 b	1.503 b	72.66 b
I_3	13.87 c	912 c	44.68 c	1.082 c	50.72 c
Seed size					
S_1	23.50 b	1489 b	45.63 a	1.433 b	71.50 b
S_2	26.22 a	1721 a	45.13 a	1.571 a	77.15 a
S_3	21.22 c	1285 c	44.67 a	1.319 c	64.73 c
Seed aging					
A_1	21.44 c	1739 a	43.22 c	1.363 b	76.42 a
A_2	23.31 b	1486 b	44.94 b	1.426 b	71.53 a
A_3	26.19 a	1271 c	47.29 a	1.535 a	65.43 b

Table 3: Means of yield and yield components of lentil for interactions of Irrigation × seed size, irrigation × aging and seed size × aging

Traits	Treatment	S_1	S_2	S_3
Grains per plant	I_1	32.94 a	34.72 a	28.22 b
	I_2	23.94 c	28.33 b	23.06 c
	I_3	13.61 de	15.61 d	12.39 e
Grain yield (g/plant)	I_1	1.791 a	1.859 a	1.565 b
	I_2	1.444 c	1.671 b	1.395 c
	I_3	1.064 e	1.184 d	0.997 e
Grain yield (g/m^2)	I_1	92.58 a	90.66 a	86.76 ab
	I_2	73.70 c	81.09 bc	63.19 d
	I_3	48.21 e	59.71 d	44.24 e
		A_1	A_2	A_3
Grains per plant	I_1	29.22 bc	31.28 b	35.39 a
	I_2	22.22 e	24.61 d	28.50 c
	I_3	12.89 f	14.06 f	14.67 f
Grains per unit area	I_1	2411 a	1949 b	1692 cd
	I_2	1786 bc	1556 d	1357 e
	I_3	1022 f	952 f	762 g
Grain yield (g/m^2)	I_1	97.51 a	89.38 ab	83.12 bc
	I_2	78.17 cd	72.34 de	67.46 e
	I_3	53.59 f	52.86 f	45.71 f
		A_1	A_2	A_3
Grains per plant	S_1	19.89 d	23.11 c	27.50 a
	S_2	25.56 ab	26.06 ab	27.06 a
	S_3	18.89 d	20.78 d	24.00 bc
1000 grain weight (g)	S_1	42.79 e	44.94 c	47.90 a
	S_2	43.94 d	44.70 cd	46.36 b
	S_3	42.92 e	45.19 c	47.60 a
Grain yield (g/m^2)	S_1	79.05 ab	71.01 bc	64.44 c
	S_2	79.65 a	77.36 ab	74.45 ab
	S_3	74.07 ab	64.22 c	55.90 d

Different letters indicate significant difference at $p \leq 0.05$

I_1, I_2 and I_3: Irrigation after 70,120 and 170 mm evaporation from class A pan, respectively

S_1, S_2 and S_3: Bulk, large and small seeds of lentil, respectively

A_1, A_2 and A_3: Control and aged seed lots of lentil for 2 and 4 days at 40°C, respectively

Grains per plant, 1000 grain weight and grain yield per plant for plants from the most aged seed lot (A_3) were significantly higher than those from other seed lots (A_1 and A_2), but the highest number of grains per unit area and grain yield per unit area were recorded for plants from non-aged seed lot (A_1) (Table 2). These differences were diminished with increasing water stress. Plants from aged large seeds showed the lowest reduction in grain yield per unit area, compared with those from aged small and bulk seeds (Table 3).

4 DISCUSSION

Seed vigor is declined with aging followed by a loss of germination capacity and viability (Trawatha *et al*., 1995), leading to reductions in seedling emergence percentage (Fig. 1), particularly in the second year (Fig. 2). This could be associated with the physiological changes during seed aging such as increased lipid peroxidation, decreased levels of anti-oxidants and reduced activity of several enzymes involved in

scavenging of free radicals and peroxides (Hsu and Sung, 1997; Bailly *et al*., 1998).

Because of poor stand establishment (Fig. 1), plants from aged seed lots had the opportunity to use the environmental resources more efficiently, which resulted in production of comparatively more and larger grains and grain yield per plant (Table 2). However, this enhancement in grain yield per plant was not enough to compensate for poor stand establishment of plants from aged seed lots. Therefore, reductions in grains per unit area and grain yield per unit area were mainly influenced by large reductions of plant density, due to seed aging (Fig. 1). Decreasing grain yield per unit area due to cultivation of aged seeds were also reported for soybean (Saha and Sultana, 2008), winter oil-seed rape (Ghassemi-Golezani *et al*., 2010), maize (Ghassemi-Golezani *et al*., 2011) and chickpea (Ghassemi-Golezani *et al*., 2012). It seems that cultivation of large seeds somehow can reduce the deleterious effects of seed aging on grain yield per unit area (Table 3) via increasing the number of grains per plant and per unit area (Table 2).

Decreasing grain yield per unit area due to water deficit was attributed to reductions in grains per plant and 1000 grain weight (Table 2). Drought stress can decrease photosynthetic rate and disrupt carbohydrate metabolism in leaves (Kim *et al*., 2000); both may reduce amount of assimilate available for export to the sink organs, thereby increasing flower and pod abortion (Fulai *et al*., 2004), leading to reductions in grains per plant. Reduction in mean grain weight under severe and moderate water stress could be related to decreasing grain filling duration as a consequence of water limitation (Ghassemi-Golezani *et al*., 2009). However, decreasing grain yield under drought stress was mainly influenced by reduction in number of grains per plant rather than 1000 grains weight (Table 2), similar to that reported by Szilagyi (2003) for common bean. High density of plants from non-aged vigorous seeds increased competition of individual plants for water and other resources under limited irrigation conditions. As a result, the superiority of plants from high vigor seeds decreased with decreasing water supply (Table 3).

Al-Karaki (1998) showed that lentil seedlings from large seeds had higher root lengths than those from small seeds at intermediate soil water potential. This may be the reason for better performance of plants from large seeds in grain yield per unit area under limited irrigation conditions (Table 3). Therefore, large seeds may somewhat alleviate the negative effects of drought stress on field performance of lentil.

5 CONCLUSION

It can be concluded that the rate of aging in large seeds is lower than that of small seeds, leading to the production of vigorous plants with high yield in the field. This superiority of large seeds is more evident when plants are subjected to drought stress. Therefore, cultivation of large seeds can reduce deleterious effects of aging and water limitation on field performance of crops such as lentil.

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Effect of Se application on photosynthesis, osmolytes and water relations in two durum wheat (*Triticum durum* L.) genotypes under drought stress

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ABSTRACT

Effect of Se (as Na_2SeO_4 at final concentration of 10 µg l^{-1}) was studied in two durum wheat (*Triticum durum* L.) genotypes in perlite under drought conditions. Se treatment increased slightly biomass of both genotypes under drought but not under control conditions. Photosynthetic rate was depressed by drought while increased by Se treatments in both genotypes up to 2.3 fold. However, transpirational water loss was also enhanced in Se-treated plants under both well-watered and drought conditions. Se application resulted in higher concentrations of soluble proteins and free α-amino acids under drought conditions, but not proline. Our results indicated that Se application improves some physiological parameters such as photosynthesis, accumulation of osmolyes and water use efficiency but did not change significantly plants biomass or water relation parameters.

Key words: organic osmolytes, photosynthetic rate, proline, water use efficiency, water relations

IZVLEČEK

UČINEK UPORABE Se NA FOTOSINTEZO, VSEBNOST OSMOTIKOV IN VODNI REŽIM PRI DVEH GENOTIPIH TRDE PŠENICE (*Triticum durum* L.) V RAZMERAH SUŠNEGA STRESA

Preučevani so bili učinki Se, dodanega kot Na_2SeO_4 v koncentraciji 10 µg l^{-1} na dveh genotipih trde pšenice (*Triticum durum* L.) gojene v perlitu v razmerah sušnega stresa. Tretiranje s Se je neznatno povečalo biomaso obeh genotipov v razmerah sušnega stresa, a ne pri kontroli. Fotosinteza je upadla s sušo, a se je po tretiranju s Se pri obeh genotipih povečala za 2.3 krat. Transpiracijska izguba vode je bila pospešena pri rastlinah tretiranih s Se v razmerah sušnega stresa kot pri dobro zalitih rastlinah. Uporaba Se se je odrazila v večji koncentraciji topnih beljakovin in prostih α-amino kislin v razmerah suše, vendar ne v koncentraciji prolina. Naši rezultati kažejo, da uporaba Se izboljša nekatere fiziološke parameter kot so fotosinteza, akumulacija osmotikov in učinkovitost izrabe vode, vendar ne spremeni značilno biomase rastlin in parametrov vodnega režima rastline.

Ključne besede: organski osmotiki, fotosinteza, prolin, učinkovitost izrabe vode, vodni režim

1 INTRODUCTION

Plants often encounter unfavorable environmental conditions that limit their growth and productivity. Among the various abiotic stresses, drought is the major factor that reduces crop yields worldwide (Farooq et al., 2009). Drought stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Yordanov et al., 2003; Chaves et al., 2009).

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Stomatal closure is one of the earliest responses of plants to water deficit that limits transpirational water loss and helps plants to retain water status under drought. However, closure of stomata in turn, results in reduction of CO_2 availability for photosynthetic carbon metabolism, depresses net CO_2 assimilation rate and inhibits plants ability for dry matter accumulation (Chaves et al., 2009). In addition, declines in the CO_2 availability to the Calvin cycle enzymes result in lower regeneration of $NADP^+$ and production of excess excitation energy that damages photosystems (Hajiboland, 2014).

Synthesis and accumulation of organic osmolytes as compatible solutes is one of the mechanisms for adaptation to water deficit. Accumulation of osmoprotectants including free amino acids, particularly proline and soluble carbohydrates, improves water extraction capacity from dry substrate (Morgan, 1984). There are also numerous evidences on the effect of these compounds in protecting membranes and cell structures from damages caused by dehydration and scavenging of free radicals (Verbruggen and Hermans, 2008).

Selenium is not yet considered an essential nutrient for higher plants and is toxic at higher concentrations (Hartikainen et al., 2000; Germ and Stibilj, 2007). Research works on plants under various environmental conditions showed that Se ameliorates adverse effects of stress (Hajiboland, 2012; Feng et al., 2013). However, the possible mechanisms for the Se-enhanced tolerance of plants to environmental stress have not been fully elucidated (Feng et al., 2013).

Researches on the alleviating effect of Se on environmental stress have mainly focused on the Se-mediated activation of antioxidative defense (Feng et al., 2013). Considering plants biomass, however, a correlation has not been found between the activity of antioxidative defense enzymes and the extent to which Se influenced growth (Hajiboland and Amjad, 2007) and alleviated stress (Yao et al., 2009, Habibi, 2013). Under drought conditions, important parameters such as accumulation of osmotic substances and water uptake capacity as affected by Se are largely unstudied. Se application increased the accumulation of proline (Yao et al., 2009). However, it did not influence water uptake capacity and biomass of plants under drought (Yao et al., 2009; Habibi, 2013). On the other hand, effect of Se on the improvement of plants growth is not restricted to stressful conditions and its application affects positively plants growth and reproduction under optimum growth conditions (Hajiboland and Amjad, 2007; Hajiboland and Keivanfar, 2012). Recently we reported higher CO_2 and NO_3^- assimilation in Se-supplemented wheat plants grown hydroponically under low or adequate nitrogen supply (Hajiboland and Sadeghzadeh, 2014) that evidenced marked alterations in the N and C metabolism by Se.

Research works on the effect of Se on graminaceous crops have been reported for wheat (Yao et al., 2009) and barley (Habibi, 2013), but effect of Se on the amelioration of drought stress in durum wheat has not been studied so far. Durum is mainly grown under rainfed conditions characterized by low and highly variable rainfall and/or extreme temperatures with high probability of terminal drought and heat stress (Nsarellah et al., 2000). In Iran, durum wheat occupies about 10% of total wheat production areas, where drought is main limiting factor for crop production. Durum breeders therefore need to adopt new strategies to alleviate drought stress (Mohammadi et al., 2014). This work was undertaken in order to investigate the effects of Se application on growth, photosynthesis and water relation parameters in two contrastive durum wheat genotypes. This work will provide further information on the mechanisms involved in the Se-improved drought tolerance in graminaceous crops.

2 MATERIALS AND METHODS

Plant culture and treatments

Seeds of two durum wheat genotypes (*Triticum turgidum* ssp. durum L. 'Saji' and 'Erdyt-89') provided by Dryland Agricultural Research Institute (DARI) (Maragheh, Iran) were surface-sterilized and germinated in dark on perlite. According to the instruction of providing institute,

'Saji' is more tolerant genotype to drought stress compared with 'Erdyt-89' (B. Sadeghzadeh, personal communication). After 3 days, seedlings were transferred into light. Five-days-old seedlings were transferred into 1.5 L pots filled with acid-washed perilte irrigated with water or 50% wheat nutrient solution with the following composition (mM): 2.0 Ca $(NO_3)_2$, 1.0 $MgSO_4$, 0.9 K_2SO_4, 0.25 KH_2PO_4, 0.1 KCl and (µM): 2.0 H_3BO_3, 0.4 $MnSO_4$, 1.0 $ZnSO_4$, 0.4 $CuSO_4$, 0.1 FeEDTA and 0.04 $(NH_4)_6MO_7O_{24}$ (Hajiboland et al., 2003). Forty plants were cultured in each pot and each treatment and cultivar was represented by 4 replicate pots. Se treatments at two levels including without (–Se) or with (+Se) Se and irrigation treatments including well-watered (irrigation at field capacity) and drought stress (irrigation at 30 % FC) were started simultaneously 9 days after transplanting. Se and irrigation treatments were assigned randomly to the pots. Application of Se (as Na_2SeO_4) was performed gradually during 4 weeks with the total final amount of 10 µg l^{-1}. Drought stress was imposed by omitting watering and after one week, pots reached the 30% FC. Throughout the experiment, pots were irrigated daily after weighing with nutrient solution or water as interval. Control and water-stressed plants received the same amount of nutrient solution and the respective FC was achieved by different volumes of water.

Plants were grown under greenhouse conditions with a day/night temperature regime of 25-28/15-17 °C, a relative humidity of 70/80% and a photoperiod of 17/7 h at a photon flux density of about 300 µmol $m^{-2}s^{-1}$ provided by natural light supplemented with fluorescent lamps.

Plants harvest

Five weeks after starting Se treatments (4 weeks after reaching the respective FC, 7 weeks after sowing) plants were harvested. Shoot and roots were separated and after determination of fresh weight (FW), washed with distilled water and blotted dry on filter paper. Plants dry weight (DW) was determined after drying in 60 °C for 48 h. Subsamples were taken for biochemical analyses before drying. Before harvest, chlorophyll fluorescence and gas exchange parameters were determined in attached leaves.

Determination of chlorophyll fluorescence and gas exchange parameters

Maximum quantum yield of PSII (F_v/F_m) were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK). CO_2 assimilation and transpiration rates were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 A.M. and 13:00 P.M. at harvest. The measurements were conducted with photosynthetically active radiation (PAR) intensity at the leaf surface of 400 µmol m^{-2} s^{-1}. The net photosynthesis rate by unit of leaf area (*A*, µmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}) and the stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}) were calculated using the values of CO_2 and humidity variation inside the chamber, both measured by the infrared gas analyzer of the photosynthesis system. Instant water use efficiency (*iWUE*) was calculated as the ratio of photosynthesis: transpiration (µmol $mmol^{-1}$).

Assay of leaf pigments and metabolites were performed in fresh samples after grinding at 4 °C using mortar and pestle and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany).105 leaf explants were prepared and for plasmid pART27 2mgfp5-ER 103 explants.

Determinations of leaf pigments

Leaf concentration of Chl a, b and carotenoids (Car) were determined according to Lichtenthaler and Wellburn (1983). Leaves were homogenized in 80% cold acetone in the dark at 4 °C. After 24 h, the absorption of samples was determined at 663 (Chl a), 646 (Chl b) and 470 (Car) nm using spectrophotometer. Determination of anthocyanins was performed using a pH differential method at pH 1 and pH 4.5 in the methanol/HCl (98:2, v/v) extract and was expressed as mg of cyanidine-3-glucoside g^{-1} FW (Giusti and Wrolstad, 2001). Total flavonoids content was determined in the methanol extract of leaves using $AlCl_3$-metanol (2%, w/v) as indicator at 510 nm and quercetin (Sigma) as standard (Grayer, 1989).

Determination of osmotic potential and relative water content

Osmotic potential was determined in the leaf and root samples harvested at 1 h after the lights were

turned on in the growth chamber. Samples were homogenized in pre-chilled mortar and pestle and centrifuged at 4000 g for 20 min at 4 °C. The osmotic pressure of the samples was measured by an osmometer (Heman Roebling Messtechnik, Germany), and the mosmol Kg^{-1} data were recalculated to Mpa using the formula: osmotic potential (MPa) = –c (mosmol Kg^{-1}) $\times 2.5 \times 10^{-3}$ according to the Van't Hoff equation.

Relative water content (RWC%) was measured in the leaves harvested 1 h after starting the light period and calculated according to the formula: (FW-DW)/(TW-DW)×100. For determination of turgid weight (TW), leaf disks (5mm diameter) were submerged for 18 h in distilled water, thereafter, they were blotted dry gently on a paper towel and weighed.

Determinations of organic solutes

For determination of non-structural carbohydrates, samples were homogenized in 100 mM phosphate buffer (pH 7.5) at 4 °C, after centrifugation at 12000 g for 15 min, supernatant was used for determination of total soluble sugars whereas the pellets were kept for starch analysis (Yemm and Willis, 1954). An aliquot of the supernatant was mixed with anthrone–sulfuric acid reagent and incubated for 10 min at 100 °C. After cooling, the absorbance was determined at 625 nm. Standard curve was created using glucose (Merck). For determination of starch, the pellet was resuspended in a 4:1 (v/v) mixture of 8 N HCl/dimethylsulfoxide (Merck). Starch was dissolved for 30 min at 60 °C under agitation. After centrifugation, the supernatant was mixed with iodine–HCl solution and after 15 min at room temperature the absorbance was determined at 600 nm. Starch (Merck) was used for the production of standard curve.

Total soluble proteins were determined using a commercial reagent (Bradford reagent, Sigma) and bovine albumin serum (BSA) as standard. Content of total free α-amino acids was assayed using a ninhydrin colorimetric method (Yemm and Cocking, 1955). Glycine was used for standard curve. For determination of proline, samples were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Proline (Sigma) was used for production of a standard curve (Bates et al., 1973).

Experimental design and statistical analyses

The experiment was undertaken in randomized block design with four replications as four independent pots. Differences between the means were detected according to Tukey's test ($p<0.05$) using Sigma Stat 2.03 software.

3 RESULTS

Shoot fresh weight was decreased by drought stress in –Se plants by about 51% and 57% in 'Saji' and 'Erdyt-89', respectively. Reduction of root fresh weight, however, was lower, e.g. 25% and 28% in 'Saji' and 'Erdyt-89', respectively. Shoot dry weight was not influenced by drought in 'Saji' while decreased up to 37% in 'Erdyt-89'. Root dry weight was not significantly affected by drought in both genotypes (Fig. 1).

Under well-watered conditions, Se treatment did not affect positively shoot fresh or dry weight in 'Saji'. In 'Erdyt-89', it rather decreased shoot dry weight. In drought-stressed plants, fresh and dry weight of Se-treated plants was slightly higher in 'Saji' (Fig. 1).

Leaf and root osmotic potentials were significantly lower under drought conditions in both genotypes. Se treatment, however, did not influence osmotic potentials in the leaves or roots of both genotypes. As expected, RWC was significantly lower in drought-stressed plants in both genotypes, but Se did not affect it. Instant water use efficiency was not changed in response to drought in the absence of Se. In the presence of Se, however, both genotypes showed higher water use efficiency under drought conditions compared with well-watered plants (Table 1).

Table 1: Leaf and root osmotic potential (MPa), leaf relative water content (RWC%) and instant water use efficiency (*iWUE*, the ratio of photosynthesis: transpiration) in two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se) or with Se (+Se) (10 µg l^{-1}). Data of each column within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Treatments		Osmotic potential		RWC	*iWUE*
		Leaves	Roots		
			'Saji'		
Control	–Se	−0.54±0.05 ^{a}	−0.12±0.03 ^{a}	81.09±4.27 ^{a}	4.01±0.47 ^{b}
	+Se	−0.58±0.07 ^{a}	−0.12±0.02 ^{a}	82.24±4.46 ^{a}	3.62±0.17 ^{b}
Drought	–Se	−0.74±0.08 ^{b}	−0.29±0.06 ^{b}	73.61±4.44 ^{b}	3.62±0.17 ^{b}
	+Se	−0.80±0.06 ^{b}	−0.23±0.04 ^{b}	70.08±6.69 ^{b}	5.21±0.71 ^{a}
			'Erdyt-89'		
Control	–Se	−0.51±0.09 ^{a}	−0.12±0.01 ^{a}	89.18±2.76 ^{a}	5.22±0.51 ^{b}
	+Se	−0.58±0.09 ^{a}	−0.18±0.05 ^{ab}	88.10±0.42 ^{a}	5.07±0.20 ^{b}
Drought	–Se	−0.78±0.03 ^{b}	−0.27±0.03 ^{b}	76.47±2.54 ^{b}	4.79±0.93 ^{b}
	+Se	−0.74±0.04 ^{b}	−0.23±0.02 ^{b}	79.96±2.18 ^{b}	6.95±1.12 ^{a}

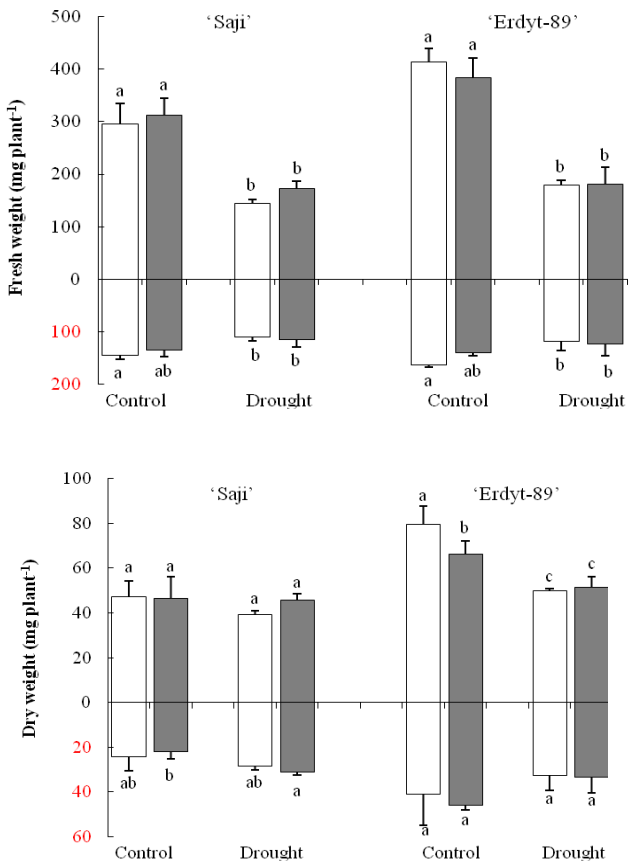

Figure 1: Fresh and dry weight (mg $plant^{-1}$) of shoot (above of the horizontal axis) and roots (below of the horizontal axis) in two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se, open bars) or with Se (+Se, closed bars) (10 µg l^{-1}). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Drought stress did not influence concentration of leaf pigments in 'Erdyt-89' while Chl a and Car concentrations decreased under these conditions in 'Saji'. Se treatment did not change concentrations of Chl a and b in drought-stressed plants but increased these pigments significantly in well-watered ones in 'Saji'. In addition, anthocyanins and flavonoids concentrations were higher in Se-treated plants under well-watered conditions in 'Saji' while this effect in 'Erdyt-89' was observed for anthocyanins under well-watered conditions and for flavonoids in drought-stressed plants (Table 2).

Maximum efficiency of PSII (F_v/F_m) was not influenced by drought or Se in 'Saji', while a slight reduction was observed under drought stress in 'Erdyt-89'. In the latter genotype, Se-treated plants had significantly higher F_v/F_m compared with plants without Se under drought conditions (Fig. 2A). Net photosynthetic rate was lowered significantly by drought stress in both genotypes, Se treatment increased this parameter not only in drought-stressed but also in control plants. This increase was about 37% and 60% in well-watered plants and 126% and 86% in drought-stressed ones, in 'Saji' and 'Erdyt-89', respectively (Fig. 2B). As expected, transpiration rate was lower under drought condition being significant in 'Saji' but slight in 'Erdyt-89'. Se application caused higher transpiration rate under control and drought conditions in both genotypes. However, this effect was not significant in drought-stressed 'Erdyt-89' (Fig. 2C). Similar pattern of changes was observed for stomatal conductance, however, effect of Se under drought conditions was not significant in both genotypes (Fig. 2D).

Table 2: Concentration (mg g^{-1} DW) of chlorophyll (Chl) a, b, carotenoids (Car), anthocyanins and flavonoids in the leaves of two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se) or with Se (+Se) (10 µg l^{-1}). Data of each column within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Treatments		Chl a	Chl b	Car	Anthocyanins	Flavonoids
				'Saji'		
Control	–Se	9.79±1.50 ^{b}	3.49±0.62 ^{b}	2.06±0.31 ^{ab}	51±11 ^{b}	12.0±1.81 ^{b}
	+Se	11.9±1.08 ^{a}	4.91±0.74 ^{a}	2.62±0.20 ^{a}	82±18 ^{a}	20.4±3.70 ^{a}
Drought	–Se	7.22±0.59 ^{c}	2.49±0.59 ^{b}	1.50±0.33 ^{c}	36±13 ^{b}	10.3±1.06 ^{b}
	+Se	7.70±0.27 ^{c}	2.62±0.34 ^{b}	1.63±0.15 ^{bc}	45±11 ^{b}	13.6±1.86 ^{b}
				'Erdyt-89'		
Control	–Se	7.25±0.47 ^{b}	3.28±0.21 ^{ab}	1.93±0.10 ^{ab}	27±15 ^{b}	9.4±2.50 ^{b}
	+Se	8.05±0.64 ^{ab}	3.65±0.23 ^{a}	2.14±0.12 ^{a}	89±23 ^{a}	12.5±2.26 ^{b}
Drought	–Se	7.64±0.54 ^{ab}	3.08±0.18 ^{b}	1.70±0.11 ^{b}	20±4 ^{b}	9.8±1.23 ^{b}
	+Se	8.36±0.35 ^{a}	3.39±0.28 ^{ab}	1.80±0.21 ^{b}	43±4 ^{b}	16.7±1.02 ^{a}

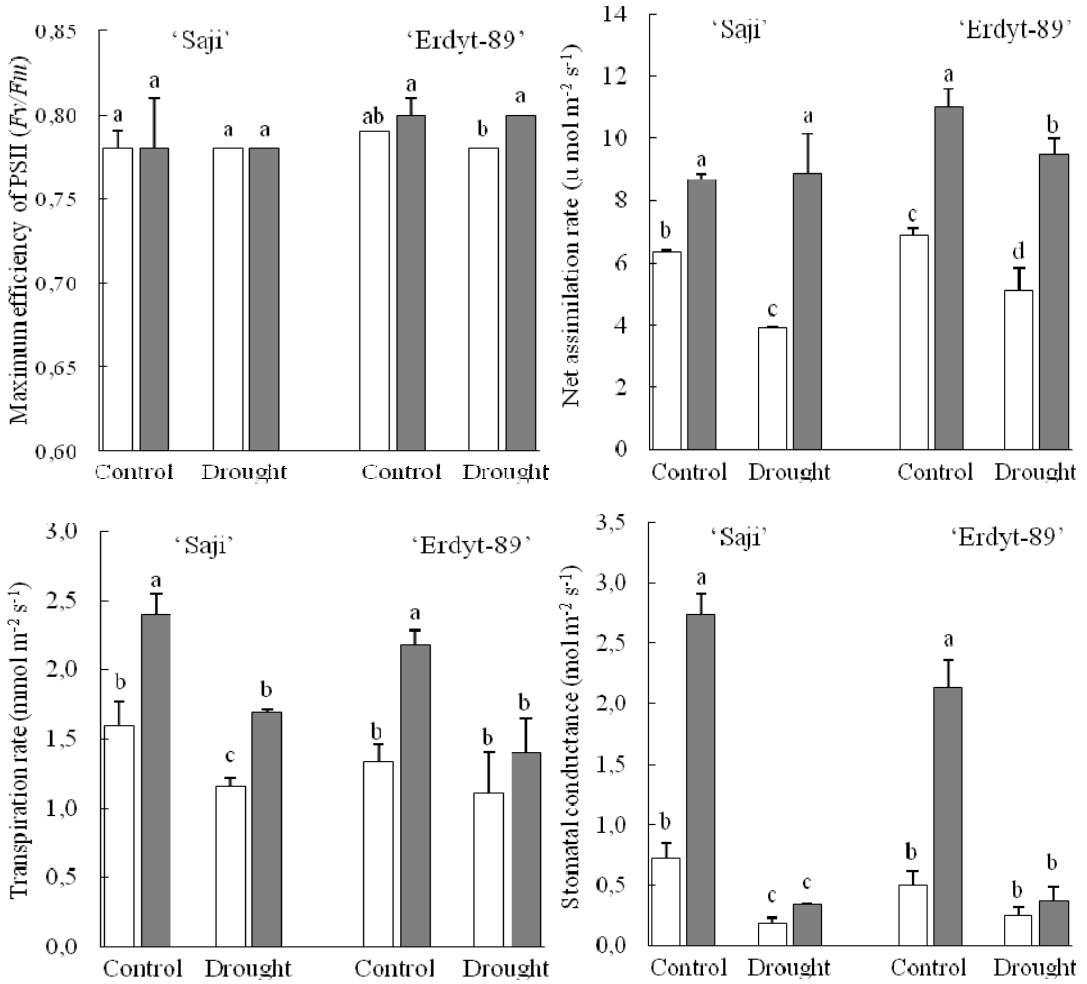

Figure 2: Maximum photochemical efficiency of PSII (*Fv*/*Fm*), net assimilation rate (*A*, μmol m^{-2} s^{-1}), transpiration rate (*E*, mmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) in the leaves of two durum wheat genotypes grown for 4 weeks under control (100% FC), and drought (30% FC) conditions without (–Se, open bars) or with Se (+Se, closed bars) (10 μg l^{-1}). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Concentration of soluble proteins in the leaves and roots was not changed by drought stress in –Se plants. In +Se plants, in contrast, drought stress decreased significantly leaf and root concentrations of soluble proteins in 'Saji'. In 'Erdyt-89', in contrast, an increase was observed in root soluble proteins in +Se plants upon drought stress. Se-treated plants, in general, had higher concentrations of soluble proteins in the leaves and roots. This effect, however, was not significant in the leaves of drought-stressed 'Saji' and in well-watered 'Erdyt-89' (Fig. 3A).

Concentration of free α-amino acids was not affected significantly by water stress conditions in –Se plants. In +Se plants, in contrast, leaf concentration of free amino acids was slightly and significantly higher under drought conditions in 'Saji' and 'Erdyt-89', respectively. In the roots of +Se plants, drought conditions decreased free amino acids concentration in 'Saji' while increased it in 'Erdyt-89'. Se treatment caused a significant decline of leaf free α-amino acids in well-watered 'Saji' while increased it in drought-stressed plants being significant in 'Erdyt-89'. In the roots, however, Se treatment decreased free amino acids in 'Saji' under drought and in 'Erdyt-89' under well-watered conditions (Fig. 3B).

Proline accumulated in the leaves and roots of both genotypes under drought conditions. The increase in proline concentration in response to drought was

much more pronouncedly observed in the leaves of 'Saji'. Se treatment did not affect leaf or root proline concentration in well-watered plants, while caused its reduction in the leaves of both genotypes being significant in 'Erdyt-89'. In the roots, Se-treated 'Erdyt-89' had significantly higher proline concentration under drought conditions compared with –Se plants while it was not affected by Se in 'Saji' (Fig. 3C).

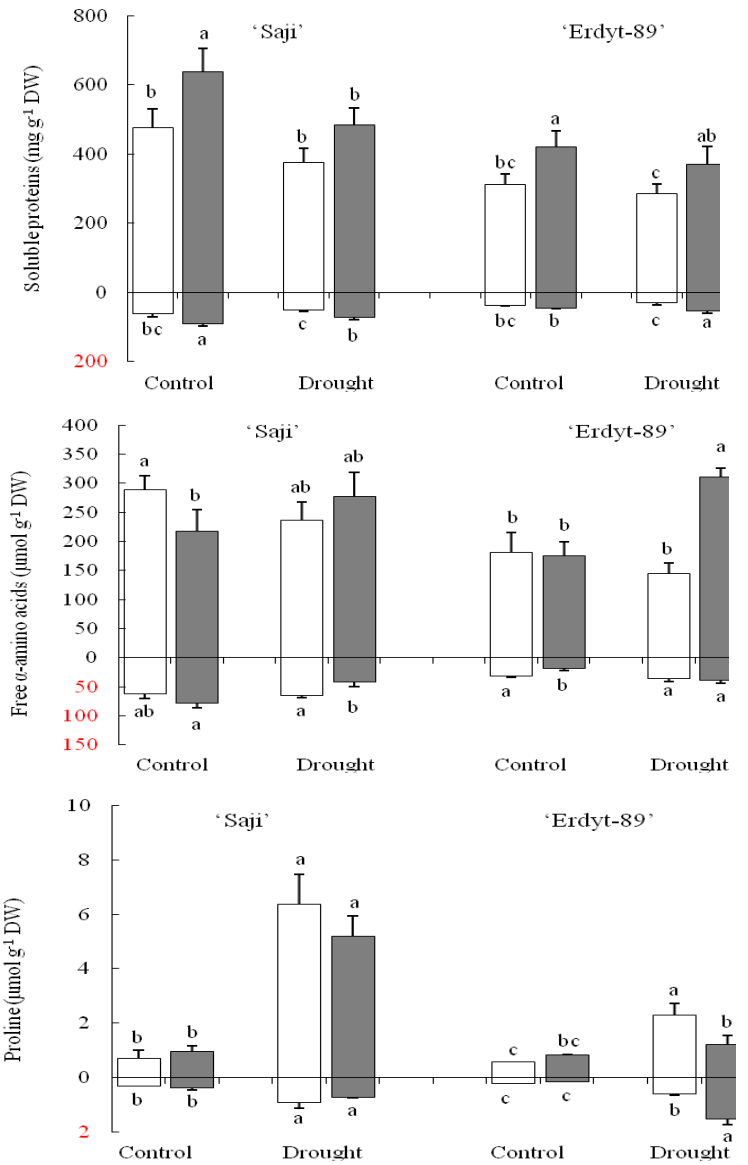

Figure 3: Concentration of soluble proteins (mg g^{-1} DW), total free α-amino acids (µmol g^{-1} DW) and proline (µmol g^{-1} DW) in the leaves (above of the horizontal axis) and roots (below of the horizontal axis) of two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se, open bars) or with Se (+Se, closed bars) (10 µg l^{-1}). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Soluble carbohydrates concentrations increased in response to drought in the leaves and roots. This effect, however, was not significant in the roots of both genotypes in the absence of Se. Se-treated plants had significantly higher soluble carbohydrates in 'Saji' with the exception of leaves

under well-watered conditions. Effect of Se in 'Erdyt-89', however, was significant only in the roots of drought-stressed plants (Fig. 4A).

Starch concentration of leaves decreased by drought stress slightly or significantly in both genotypes regardless of Se treatment. In the roots, in contrast, starch concentration was not affected by water deficit conditions. Se treatment did not affect significantly starch concentration of leaves and roots of both genotypes (Fig. 4B).

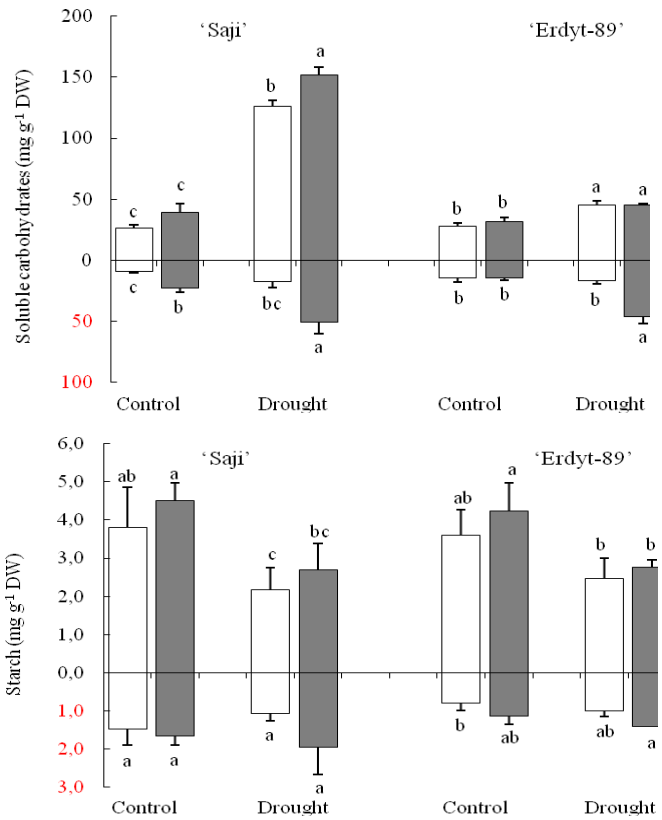

Figure 4: Concentration of soluble carbohydrates and starch (mg g^{-1} DW) in the leaves (above of the horizontal axis) and roots (below of the horizontal axis) of two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se, open bars) or with Se (+Se, closed bars) (10 µg l^{-1}). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

4 DISCUSSION

Fresh biomass of both genotypes was influenced negatively by drought stress, while dry biomass was affected by drought only in 'Erdyt-89'. It indicates higher sensitivity of 'Erdyt-89' to water deficit. This is consistent with the instruction of providing institute, but genotypic differences were higher under field condition (B. Sadeghzadeh, personal communication) compared to our greenhouse experiment.

Root biomass, in general, was much less affected by drought stress than shoot biomass leading to increase of root:shoot dry weight ratio from 0.52 in well-watered to 0.73 and 0.66 in drought-stressed 'Saji' and 'Erdyt-89', respectively. Higher root:shoot ratio is an important factor for water economy of whole-plant under water drought conditions that confer higher water uptake capacity but lower transpiration rate (Farooq et al., 2009). Se application caused only slight improvement of plants dry weight under drought conditions.

Similarly, in wheat (Yao et al., 2009) and barley (Habibi, 2013) Se did not affect dry matter production under drought conditions despite of an improvement in the antioxidative indices.

In contrast to biomass, several physiological parameters were significantly improved by Se in both durum wheat genotypes. Higher leaf concentration of pigments particularly anthocyanins and flavonoids may be important for increasing plant tolerance to various stress conditions. It has been reported that plant tissues containing anthocyanins are usually resistant to drought that is related to superoxide radical scavenging activity and of anthocyanins ability to stabilize the water potential (Chalker-Scott, 1999). Although the widely accepted antioxidant function of flavonoids in plants have been doubted by some authors (Hernández et al., 2009), their contribution to the antioxidant defense capacity of plants and its relevance in plant responses to drought have been widely accepted (Fini et al., 2011). Evidences showed that flavonoids constitute a secondary ROS-scavenging system in plants exposed to severe and prolonged stress conditions (Fini et al., 2011).

Maximum efficiency of PSII (F_v/F_m) was not influenced by drought in 'Saji' while decreased in 'Erdyt-89' that may be another indication for higher drought sensitivity in the latter genotype. Reduction of F_v/F_m revealed damage to photosystems (Hajiboland, 2014) and Se-induced recovery of this ratio implicated that Se may be effective in protection of photosynthetic apparatus from drought-induced damages via enhanced capacity of defense system (Yao et al., 2009; Habibi, 2013) or by increasing CO_2 availability for biochemical reactions i.e. higher stomatal conductance that reduces, in turn, generation of excess excitation energy (Hajiboland, 2014).

Drought conditions, as expected, reduced stomatal opening and in consequence, decreased net transpiration rates. However, 'Saji' was more efficient in the reduction of stomatal conductance and limitation of water loss than 'Erdyt-89' under drought stress. Plants respond to drought primarily by closing stomata for minimizing water loss (Yordanov et al., 2003; Chaves et al., 2009).

As the consequence of stomatal closure, CO_2 diffusion inside leaves decreases and reduction of photosynthesis impairs plants dry matter production under drought (Yordanov et al., 2003). Se treatment here elevated stomatal opening under both control and drought conditions in both genotypes and increased net assimilation rate considerably. Increase in CO_2 fixation upon Se treatment was up to 2.3 and 1.9 fold than –Se plants in 'Saji' and 'Erdyt-89', respectively. Photosynthesis rate of drought-stressed plants supplemented with Se was even exceeded that of well-watered –Si plants (Fig. 2). Sustained or rather higher photosynthetic rate under drought may be of great importance for drought tolerance in Se-treated plants in this work. Maintenance or even elevation of CO_2 fixation under drought not only prevents $NADP^+$ shortage and generation of reactive oxygen species but also provides C skeletons for synthesis of organic osmolytes that are needed particularly under drought conditions.

Higher protein content of Se-treated plants is likely resulted from increased protein synthesis. We observed previously higher nitrate reductase activity that was accompanied by higher amino acids and protein concentrations in Se-treated wheat (Hajiboland and Sadeghzadeh, 2014) and rapeseed plants (Hajiboland and Keivanfar, 2012). Soluble proteins may be involved in protection of tissues against dehydration damages (Verbruggen and Hermans, 2008) particularly in the leaves that was more affected by Se than the roots.

Concentrations of proline were increased by drought conditions in the leaves and roots of both genotypes. Nitrogenous compounds such as free amino acids, proline, glycine betaine and polyamines play important roles in the maintenance of water uptake capacity from dry substrate and protection of tissues from damages caused by dehydration (Morgan, 1984; Verbruggen and Hermans, 2008). Se treatment did not increase proline concentration, in contrast, decreased it in the leaves of both genotypes slightly or significantly. Lower proline content in Se-treated plants, implied likely that they were less affected by osmotic stress and thus, needed lower proline concentrations compared with –Se counterparts. It has been proposed that accumulation of proline in plants grown under osmotic stress is due to hyper-osmosis injury and is not associated necessarily

with higher tolerance (Lutts et al., 1999). Drought-stressed wheat plants (Yao et al., 2009), in contrast, had higher proline concentration when exposed to Se. Here Se-treated 'Erdyt-89', however, had significantly higher proline concentration in the roots under drought.

Leaf and root soluble carbohydrate concentrations were increased significantly by drought that was more pronouncedly observed in 'Saji'. In contrast, leaf starch content was lowered by water deficit. Drought-induced accumulation of soluble sugars is a well-known response in plants that is either the result of increased partitioning of photoassimilates to the synthesis of free sugars and/or enhanced starch degradation (Lee et al., 2008). Here the changes in the concentration patterns of non-structural carbohydrates in the leaves may indicate that under drought conditions soluble sugars accumulated at the expense of starch. It has been also reported that accumulated soluble sugars in white clover under drought were mainly derived from degradation of stored starch rather than a result of *de novo* synthesis (Lee et al., 2008). Se treatment increased concentration of soluble sugars in both leaves and roots of well-watered and drought-stressed plants particularly in 'Saji'. It may be primarily attributed to the higher photosynthetic rate and C assimilation. This suggestion could be confirmed by slightly or significantly higher starch concentration of Se-treated plants. This excludes, in turn, the contribution of starch degradation to the increased concentration of soluble sugars upon Se application in contrast to that observed under drought conditions. Free soluble carbohydrates are effective compounds in osmotic homeostasis, protection of membranes and cell structures against dehydration and have free radicals scavenging activity (Niedzwiedz-Siegien et al., 2004).

Leaf and root osmotic potentials were expectedly lower under drought stress and RWC was lower in both genotypes. Se treatment did not influence these parameters significantly either under well-watered or drought conditions. It implied likely that Se treatment did not influence plants ability for water uptake that contrasted with the report of Kuznetsov (et al., 2003) on soil-grown wheat. Such discrepancy is likely related to species difference, different Se form (selenite versus selenate) and concentrations and/or different growth substrates.

Despite of lower stomatal conductance and transpiration rate in drought-stressed plants, greater reduction of photosynthetic rate caused slightly lower water use efficiency in both genotypes under drought conditions. Se treatment increased stomatal opening being significant under well-watered conditions in both genotypes. Consequently, Se-treated plants had higher transpiration rate that was significant in both well-watered and drought-stressed 'Saji' and well-watered 'Erdyt-89'. Nevertheless, elevation of photosynthesis rate exceeded that of transpiration under these conditions. It resulted in higher instant water use efficiency in drought-stressed plants upon Se treatment in both genotypes.

5 CONCLUSION

Our results indicated that Se application did not change significantly plants biomass under drought conditions but improved some physiological parameters such as photosynthesis, accumulation of osmolyes and water use efficiency but not water relation parameters.

Higher drought sensitivity in 'Erdyt-89' could be attributed to damaged photosynthetic apparatus, failure to limit transpirational water loss and less-efficient accumulation of organic osmolytes under drought conditions compared with 'Saji'. However, two genotypes did not differ in the Se-mediated amelioration of drought stress.

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Simulation of rice yield under different irrigation and nitrogen application managements by CropSyst model

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ABSTRACT

The aim of this study was the calibration and validation of CropSyst model for rice in the city of Rasht. The necessary data were extracted from a field experiment which was carried out during 2005-2007 in a split-plot design. The main plots were irrigation regimes including continuous flooding irrigation and 5-day irrigation intervals. The subplots consisted of four nitrogen levels: zero N application, 45, 60 and 75 kg N ha^{-1}. Normalized Root Mean Squared Error (nRMSE) and Residual Mass Coefficient (Crm) in calibration years were 9.3% and 0.06, respectively. In validation year, nRMSE and Crm were 9.7% and 0.11, respectively. According to other indices to assess irrigation regimes and fertilizer levels, the most suitable treatments regarding environmental aspect were 5-day irrigation regime and 45 kg N ha^{-1}.

Key words: crop model, irrigation, fertilization, rice

IZVLEČEK

SIMULACIJA PRIDELKA RIŽA S CROPSYST MODELOM PRI RAZLIČNIH REŽIMIH NAMAKANJA IN GNOJENJA

Namen te raziskave sta bili kalibracija in validacija CropSyst modela za pridelovanje riža v Rashtu (Iran). Podatki za obdelavo izvirajo iz poljskega split-plot poskusa, ki je potekal v letih 2005-2007. Glavne ploskve so bile namakane, vključno z neprekinjenim poplavnim namakanjem v petdnevnih intervalih. Podploskve so imele štiri režime gnojenja z dušikom in sicer: 0 N –brez dušika, 45, 60 in 75 kg N ha^{-1}. Normalizirana vrednost korena povprečnih kvadriranih napak (Normalized Root Mean Squared Error;nRMSE) in koeficient mase ostankov (Coefficient of Residual Mass; Crm) sta v kalibracijskih letih sta znašala 9.3 % in 0.06 %. V validacijskem letu sta omenjena parametra znašala (nRMSE in Crm) 9.7 % in 0.11 %. Glede na druge kazalnike ocenjevanja namakanja in gnojenja je bil tudi okoljsko najprimernejši tretma petdnevno namakanje in gnojenje s 45 kg N ha^{-1}.

Ključne besede: pridelovalni model, namakanje, gnojenje, riž

1 INTRODUCTION

Nowadays agriculture sector requires production systems with high water productivity. The issue of water scarcity is an important issue from the economical aspect of it due to high irrigation water requirement in the production of rice. Development of new water saving systems is one of increasing rice yield choice with limited water resource. Therefore, multiple ways were approved (Pirmoradian et al. 2004; Kato et al. 2006). The production of rice without constant use of water head above soil surface which is referred to as none-constant-submerging or its alternative intermittent-irrigation is one of those optimum methods. In Asia researchers try to enhance yield under water saving irrigation by using genetic improvement e.g. development of varieties that are resistant to dry condition (Nemoto et al. 1998). Irrigation under limited supply of water decreases photosynthesis, claws, leaf area, biomass, number

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of grain per an ear, thousand kernel weight and grain yield (Zubaer et al. 2007).

Guilan Province with 200 thousands hectares of rice cultivation is one of the important zones of rice production in Iran. Water requirement for agricultural activities is being supplied by Sepidroud dam and its widespread irrigation network. Recently, the decrease of fresh water resources has made a great concern on rice production in Guilan province. Climate changes, degrading of water resources and continuous drought has influenced agriculture in general and farmers' incomes specifically (Rezaei et al. 2013). Besides, local varieties of Iran are resistant to intermittent irrigation conditions (Razavipour et al., 2000). Also, experimental data requires a lot of expenditure and is time consuming in order to receive research aims. Therefore, crop models are offered to save money and time. These models can lead to other advantageous e.g. examining different management scenarios.

The aim of CropSyst development was to simulate cropping systems and crop rotation. So, this model was developed from the early 1990s. CropSyst model is a perfect sample of simulation model that ever used in the world. CropSyst simulates crop system with daily time step that can be in multi-year and multi-crop conditions. In this model, effects of different elements like climate, soil, management and crop characteristics on crop systems and environment has been examined. The significant feature of CropSyst is that it can connect to GIS software. Plant growth is simulated by the model based on arriving time to each phenology stage according to accumulation temperature. CropSyst simulates soil-plant nitrogen balance, crop canopy and crop development, root growth, dry matter production, yield, residue production and decomposition and erosion by water and salinity (Stöckle et al. 1994; Stöckle et al. 2003).

CropSyst model has been applied to estimate soil water balance in rainfed and fallow land and irrigated farm in Southern Africa (Abraha and Savage 2008). Results, showed that the model accuracy was higher in simulation of dry rather than wet soil water balance. This model was evaluated to simulate growth, water- and N-uptake of irrigated winter wheat (*Triticum aestivum* 'Kupava') in Khorezm, in the dry lands of Northwest Uzbekistan, Central Asia (Djumaniyazova et al. 2010).

CropSyst was calibrated and validated by using field experiments. Results of 2005/06 and 2006/07 seasons were used for model calibration and 2007/08 season was used for model validation. The results of modeling were studied while nRMSE of aboveground biomass and grain yield in 2007/08 was 11%.

On first two cropping season and 749 and 869 mm irrigated water, N-leaching was high and ranged from 63 to 106 kg ha^{-1} and was low (7-15 kg ha^{-1}) by 148-395 mm irrigation during 2007/08. In another study, biomass growth of alfalfa (*Medicago sativa* L.) and its influence on soil water dynamic were analyzed under different irrigation treatment in Austria. Simulation results showed satisfactory application by suitable value of RMSE of biomass growth (0.58-3.52 t ha^{-1}) and water content in the soil profile (20.9-50.6 mm) (Raza et al. 2013).

The effect of alternative management options (nitrogen application and soil water content) on maize (*Zea mais* L.) crop was evaluated by CropSyst model at Pisa, Central Italy. Experimental and simulation results were compared. Modeling efficiency (EF) were 0.82 and 0.75 for biomass and soil water content, respectively (Bellocchi et al. 2002). Another study in Southern Italy was done on sorghum and sunflower during 1986-1993. Obtained results based on nRMSE (11 and 25% for simulated yield of sorghum and sunflower, respectively) showed that CropSyst is a suitable model (Ventrella and Rinaldi 1999).

Because of drought in recent years, deficit of water resources and constructing several dams upstream of Sefidroud watershed, the agriculture of Guilan province faced to water deficit (Rezaei 2007). According to water deficit and degrading available water in considered region, more field research as well as introducing suitable tools such as crop models is necessary to test different management scenarios and improving water productivity. So, CropSyst model was calibrated and validated on rice ('Hashemi' variety, a local and a popular variety) under different irrigation and nitrogen

managements to predict yield of this crop under the weather conditions of Rasht.

2 MATERIALS AND METHODS

2.1 Experimental data

This study was conducted at the rice research institute (37˚12′19″N, 49˚38′28″E, 24.6 m a.s.l.) of Iran, located in Rasht, Guilan province. The field experiments were carried out on a silty-clay soil (13% sand, 41% silt, 46% clay). The climate of Rasht was determined to be so humid, based on Köppen method according to 50-year climatic data (1956-2005). Annual mean temperatures range between 13.3 and 17.2 ˚C with an average of 15.9 ˚C; relative humidity: 75 to 87% with an average of 81 %; and mean annual rainfall is 1359 mm. Experimental data were collected during 2005-2007. Some weather and soil characteristics are presented in Table 1 and Table 2. This soil has been always a paddy soil for a long time. About 90% of rainfall in the region falls in autumn and winter. Soil N, P and K were determined according to Kjeldahl method for N, Olsen-sodium bicarbonate extractant method for extractable P and ammonium acetate extractant method for available K. N content in plant (stem and grain) was measured using Kjeldahl method.

Table 1. Weather parameters during rice growth season in Rasht (April-August)

	(crop season of rice)		
Weather parameters	2005	2006	2007
precipitation (mm)	160.9	135	129.9
Average of maximum daily temperature (˚C)	28.3	28.8	28.4
Average of minimum daily temperature (˚C)	18.9	18.5	18.8
Short length radiation (MJ m^{-2} d^{-1})	19.57	20.22	19.87
Average of maximum relative humidity (%)	98.02	96.7	97.28
Average of minimum relative humidity (%)	60.04	55.82	58.84
Average of wind speed (m s^{-1})	3.6	4.57	4.62

Table 2. Some soil characteristics

Saturated water content %	Soil electrical conductivity dS m^{-1}	pH	Total nitrogen mg kg^{-1}	Absorbable phosphorus mg kg^{-1}	Absorbable potash mg kg^{-1}
75	1.12	7.4	1890	17.8	280

The rice variety was ‘Hashemi’ so popular in Guilan province. The research was carried out in a split plot design in a complete randomized block under different regimes of irrigation and nitrogen applications at the Rice Research Institute of Iran, Rasht. There were three blocks as replication. Each block was divided into two main plots and each main plot was divided to four subplots. The main plots consisted of two plots; I_1: continuous submergence irrigation (with 30-50 mm standing water throughout crop growth); and I_2: 5-day irrigation interval.

Subplots with 15 m^2 areas included four levels of nitrogen applications: N_1: zero N application; N_2: total N rate of 45 kg ha^{-1} (applied at basal); N_3: total N rate of 60 kg ha^{-1}; and N_4: total N rate of 75 kg ha^{-1}. To reduce N leaching, half of N_3 and N_4 were applied at basal and the residual of N fertilizer was applied at maximum tillering as a top dressing. The boundaries of plots were covered with nylon to prevent transportation of water, fertilizer and herbicide. For 10 days after transplanting, all plots were under continuous submergence condition. Then irrigation treatments were applied based on their scheduled time. After plant maturity, the yield in different treatment was determined according to harvested samples in a 5 m^2 of middle of each plots.

2.2 Simulation model

To introduce processes of CropSyst simulation, some equations are more important in this study so they are presented here. Crop transpiration-dependent biomass production (Bpt) and LAI are effective on output of CropSyst based on calibration results. Third equation is yield that was one of main evaluated outputs. Therefore, those three equations were presented in this paper, more information can be found in Stockle et al. (1994) and Stöckle et al. (2003).

There is a relationship between crop transpiration and biomass production that is based on carbon and vapor exchange in leaves. So the potential daily biomass production can be calculated as (Tanner and Sinclair 1983):

$$B_{PT} = \frac{K_{BT} T_P}{VPD} \quad (1)$$

where B_{PT} is the crop transpiration-dependent biomass production (kg m^{-2} day^{-1}), T_P is crop potential transpiration (kg m^{-2} day^{-1}), VPD is the daytime mean atmospheric vapor pressure deficit (kPa) and K_{BT} is a biomass-transpiration coefficient (((kg m^{-2}) kPa) m^{-1}).

The increase of leaf area during the vegetative period, expressed as leaf area per unit soil area (leaf area index (LAI)) is calculated as a function of biomass accumulation:

$$LAI = \frac{SLA B}{1 + p^B} \quad (2)$$

where LAI is in m^2 m^{-2}, B is accumulated aboveground biomass (kg m^{-2}), SLA is the specific leaf area (m^2 kg^{-1}) and p is a partition coefficient (m^2 kg^{-1}) controlling the fraction of biomass to leaves (a value of zero apportion all biomass to leaves) (Stockle et al. 2003).

Yield simulation depends on total biomass accumulated at physiological maturity (B_{PM}) and the harvest index (HI= harvestable yield/aboveground biomass):

$$Y = B_{PM} HI \quad (3)$$

where Y is yield (kg m^{-2}) and B_{PM} is also in kg m^{-2}.

In this study CropSyst ver. 4.13.09 was used. Model inputs are entered in database and scenario sectors. Water and fertilizer managements, climate, soil and plant conditions are inputs that are entered in the database which is an important part of the model. Finally running the model is done in scenario sector. In this part some parameters are determined like simulation period, initial features and suitable way of evapotranspiration. The model let user to determine produced outputs while he or she could choose daily or yearly output.

Sensitivity analysis is done by using of different methods like Morris and regression-based method (Confalonieri et al. 2010). In this study Ng and Loomis method was used to calculate sensitivity index (Ng and Loomis 1984):

$$SI = \frac{(\frac{100}{N}) \times \sum_{i=1}^{N} (Xni - Xci)/Xci}{\Delta} \quad (4)$$

where Xni is the new value of the i^{th} data point with a changed value of the input parameter; Xci: the value of output for the i^{th} point in the control simulation run; N: the number of point; Δ is the absolute change in the point parameter; SI in the given form is a measure of the percentage change in the output from that in the control simulation results from a percent change in the value of the point parameter.

2.3 Calibration and validation

After sensitivity analysis, the model should be calibrated. The purpose of calibration is describing experimental station conditions for the model. The inputs include of literature value, measurements and default quantities. Measured values should be used if some sensitive parameters were consisting of them.

Calibration was started from more sensitive parameters that had higher SI. Model primary values were entered by 5 % changing process. To determine the suitable input, simulated and measured values were compared in each stage by statistical indices. In this study three statistical indices were used i.e. Crm, RMSE and nRMSE:

$$Crm = 1 - \frac{\sum_{i=1}^{n}(P_i)}{\sum_{i=1}^{n}(O_i)} \quad (5)$$

$$RMSE = \sqrt{\sum_{i=1}^{n}(P_i - O_i)^2/n} \quad (6)$$

$$nRMSE = \sqrt{\sum_{i=1}^{n}(P_i - O_i)^2/n} \times \frac{100}{\bar{O}} \quad (7)$$

where O_i is observed yield; P_i: simulated yield and n: number of treatments in each year.

If Crm and RMSE are close to zero, then simulations and observations have closer values. nRMSE gives a measure of the relative difference of simulated versus observed data. The simulation is considered excellent with nRMSE less than 10 %, good if nRMSE is greater than 10 and less than 20 %, fair if nRMSE is greater than 20 % and less than 30 % and poor if nRMSE is greater than 30 % (Jamieson et al. 1991; Bannayan and Hoogenboom 2009).

Another issue has been assessed about irrigation water and nitrogen uptake by crop (different simulated parameters by model) with some indices was done. Rice is a sensitive crop to nitrogen so physiological nitrogen use efficiency (PNUE) is defined to analyze the amount of nitrogen uptake (simulated by the model) that is fraction of grain yield (kg ha^{-1}) to nitrogen uptake (kg ha^{-1}) (Montemurro et al. 2006):

$$PNUE = \frac{grain\ yield}{N\ uptake} \quad (8)$$

Both grain yield and N uptake in equation 8 were simulated by the model. Treatment consisted of different irrigations; therefore, an assessment should be done about the amount of irrigated water. Consequently, irrigation water use efficiency (IWUE) was used (Shahnazari et al. 2007). IWUE is the fraction of grain yield (kg ha^{-1}) simulated by the model to irrigation water (m^3 ha^{-1}):

$$IWUE = \frac{grain\ Yield}{irrigatin\ water} \quad (9)$$

Nitrogen harvest index (NHI) was used according to the portion of grain N content (kg ha^{-1}) to N uptake (kg ha^{-1}) simulated by the model (Montemurro et al. 2006) that can be analyzed completely under this study:

$$NHI = \frac{grain\ N\ content}{N\ uptake} \times 100 \quad (10)$$

CropSyst did not simulate grain N content, so observed values were used in NHI because of no significant difference between observed and simulated grain yield and plant N uptake values.

3 RESULTS AND DISCUSSION

3.1 Experimental results

Data of 2005 and 2006 were used for model calibration and 2007 data were considered for model validation. According to measurements and observations during research period, it was determined that maximum daily temperature in all days was lower than cutoff temperature likewise comparison of minimum daily temperature in all days with base temperature showed that only one day in 2007 had lower temperature than base temperature. So air temperature had reasonable fluctuation in the period of three years. Crop parameters that were used are shown in Table 3. Harvest index in 2005, 2006 and 2007 were respectively 0.4, 0.49 and 0.46.

Table 3: Rice crop parameter and references of information

Crop parameter	Crop season			
	2005	2006	Determination method *	Units
Canopy extinction coefficient for total solar radiation		0.5	D	-
ETc		1.05	C	-
Leaf water potential at the onset of stomatal closure		-700	D	J kg^{-1}
Wilting leaf water potential		-1600	D	J kg^{-1}
Maximum water uptake		13	L	mm day^{-1}
Above ground biomass transpiration coefficient (K) for annuals		6	C	kPa kg m^{-3}
Maximum radiation use efficiency		0.003	D	kg MJ^{-1}
Mean daily temperature that limits early growth		11	D	° C
Specific leaf area at optimum temperature		23.1	C	m^2 kg^{-1}
Stem/leaf partition coefficient (SLP)		1.85	C	-
Leaf water potential that begins reduction of canopy expansion		-800	D	J kg^{-1}
Leaf water potential that stops canopy expansion		-1200	D	J kg^{-1}
Emergence	80	80	M	° C-days
Maximum root depth	970	970	M	° C-days
End of vegetative growth	970	970	M	°C-days
Begin flowering	850	850	M	° C-days
Begin filling	900	900	M	° C-days
Physiological maturity	1530	1530	M	° C-days
Leaf area duration	800	800	D	° C-days
Maximum rooting depth		0.35	M	m
Harvest index	0.4	0.49	M	-
Nitrogen demand adjustment	1	0.8	C	-
Base temperature		10	M	° C
Cutoff temperature		42	M	° C

* D: default; M: measured; C: calibrated parameter; L: literature (Saadati 2012)

3.2 Model results

Calibrated and validated values of biomass and yield in three years are shown in Table 4. These two parameters were evaluated by using statistical indices. According to calculated statistical indices (Table 5), Crm and nRMSE in calibration years (2005 & 2006) for biomass and yield were 0.01, 8.3%, 0.06 and 9.3%, respectively. In validation year (2007) nRMSE for biomass and yield were 9.6 and 9.7% and Crm for these parameters were 0.03 and 0.11, respectively. The results of the statistical indices showed that CropSyst is a suitable model for simulating rice yield. A research about rice was done in Northern Italy between 1991 and 2002. Calibration results of CropSyst have shown that nRMSE for biomass ranged from 11 to 29% (Confalonieri and Bocchi 2005). Because of similar climate in Italy and the study zone, it can be said that this model had a better performance in Rasht.

In Figure 1, 1:1 line was drawn between observed and simulated values of biomass and grain yield in calibration and validation years that is consisted of 48 points. Values of nRMSE and Crm were 9.8% and 0.04, respectively. According to Figure 1, the points are distributed properly around the 1:1 line. On the other hand, the points are inclined to underneath of the line so, it shows that the model had slightly underestimated.

Table 4 Observed and simulated values (kg ha^{-1}) in calibration and validation years

	Year	Crop parameter	Number	Observed value	Simulated value
Calibration years	2005	biomass	8	8195	7954
		yield	8	3243	3149
	2006	biomass	8	7793	7153
		yield	8	3857	3538
Validation year	2007	biomass	8	7510	7259
		yield	8	3479	3360

Table 5. Indices of agreement between observed and simulated values

	Crm	RMSE (kg ha^{-1})	nRMSE (%)
Calibration (2005 & 2006)			
Final biomass	0.01	659	8.3
Grain yield	0.06	331	9.3
Validation (2007)			
Final biomass	0.03	722	9.6
Grain yield	0.11	336	9.7

Values of PNUE were determined for N_1, N_2, N_3, N_4 and different irrigation treatments (Figure 2) using simulated values. By comparison of PNUE values in different irrigation it was found that this parameter was higher in continuous submergence than 5-day irrigation intervals. Based on the analysis of variance results (lack of space, results not shown here), grain yield in different irrigation levels had no significant difference thus high value of PNUE in continuous submergence was due to low N crop uptake in 2005 and 2006. But in 2007, it was different, while the difference between PNUE values in all these years were not significant in two treatments. In fact, different N application rates caused a change in the balance of grain yield and N uptake. Therefore, the result had no changing. 5-day irrigation intervals in comparison to continuous submergence caused degradation of PNUE (in 2005 and 2006) but not significant (p>0.05). So, according to PNUE index, 5-day irrigation intervals is recommended as pertinent alternative of continuous submergence to save water.

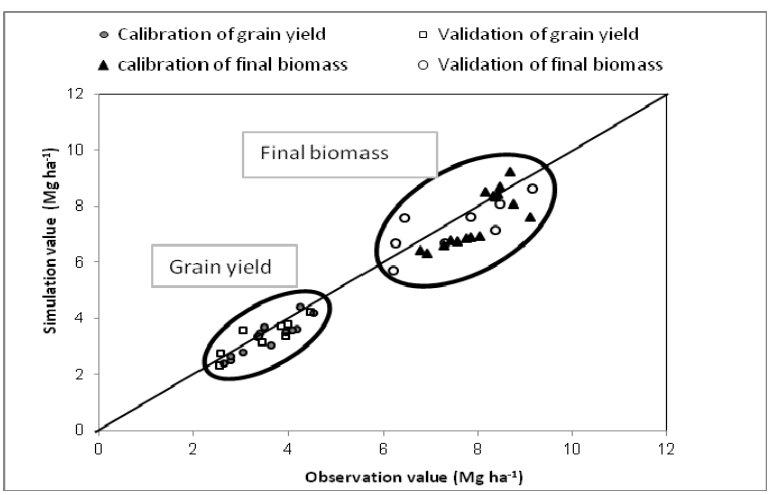

Figure 1: Comparison between observed and simulated value by 1:1 line

According to Figure 3, values of IWUE for 5-day irrigation intervals were the highest in all years, while it was predictable based on less water use in this treatment. IWUE had an ascendant trend from N_1 to N_4. The IWUE value of N_1 had significant difference by others but N_2, N_3 and N_4 did not have significant difference with each other (P>0.05) except of 2007. As a result, the 45 kg N ha^{-1} is recommended based on IWUE.

Irrigation treatments had similar effect on NHI index (Figure 4). By comparison of NHI in three N application treatments (N_2, N_3, N_4) did not see any significant difference, but in $N_1$was so different. By considering all the indices, as the results of N_2, N_3 and N_4 treatments were similar. So, N_2 (45 kg N ha^{-1}) can be recommended based on economical and environmental considerations.

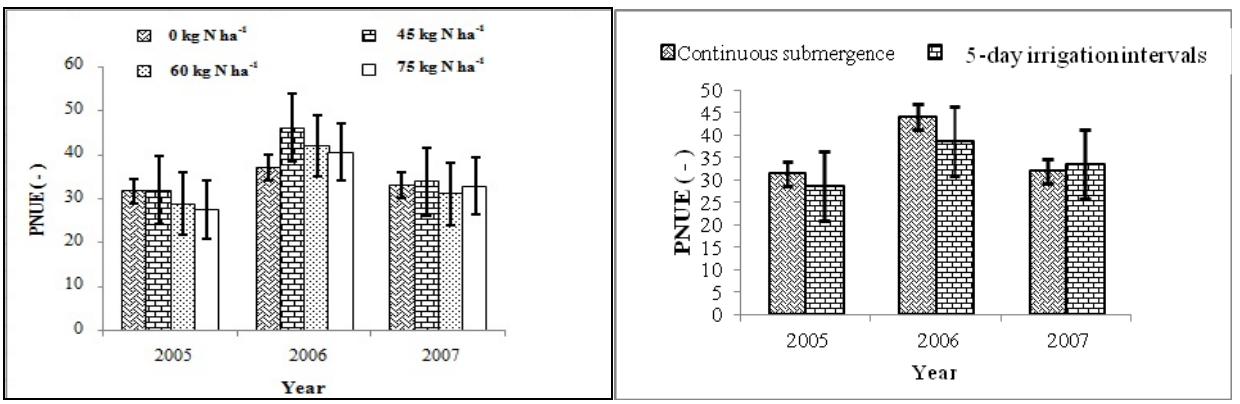

Figure 2: PNUE value in different years under irrigation and N application treatments (error bars represent standard deviation)

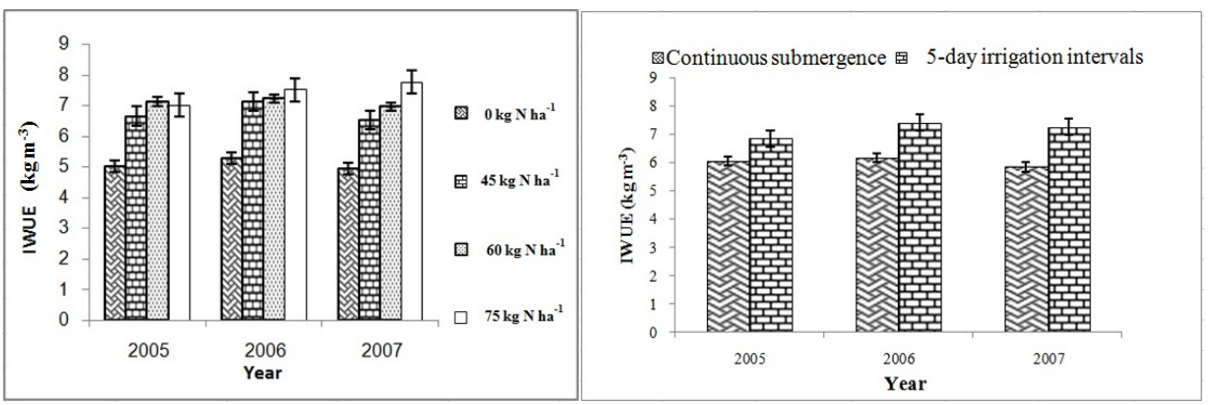

Figure 3. IWUE value in different years under irrigation and N application treatments (kg m^{-3}, error bars represent standard deviation)

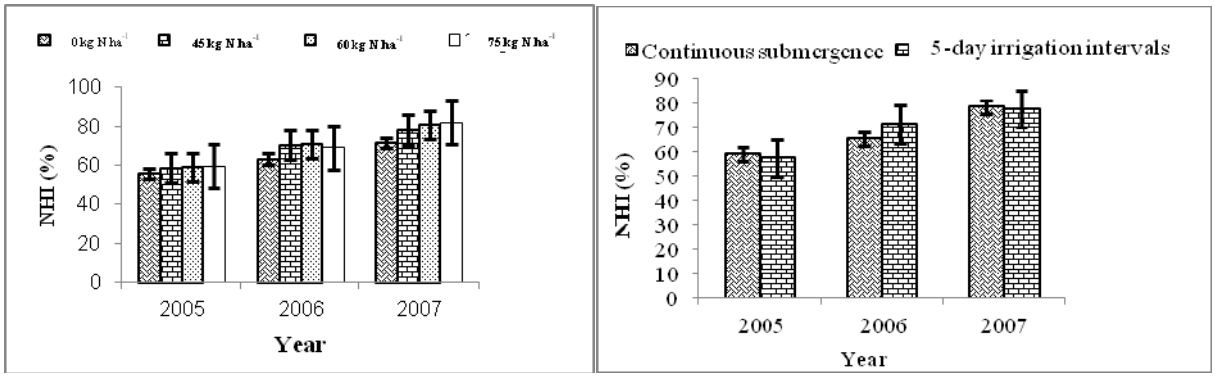

Figure 4. NHI value in different years under irrigation and N application treatments (error bars represent standard deviation)

4 CONCLUSIONS

According to the statistical indices (nRMSE = 9.8% and Crm = 0.04), CropSyst had suitable performance for rice yield simulation in Rasht. Apart from rice, the model could be calibrated and validated for other crops. Because of the importance of water in this region, along with the high cost of N fertilizers and possibility of fertilizer leaching, different management scenarios should be evaluated by crop model, and then optimum management should be recommended to the user regarding economical and environmental aspects. If research studies have been done in a wider scale by connection of CropSyst to GIS, it could be possible to recommend the suitable scenario regarding economical and environmental aspects e.g. whole Guilan province or a wide paddy region.

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Analysis of energy and greenhouse gas balance as indexes for environmental assessment of wheat and maize farming: a case study

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ABSTRACT

In this study, the net balance of greenhouse gas (GHG) emission and energy of wheat and maize production systems in two farms in Khuzestan province of Iran was assessed. The results showed that totally wheat farming is more efficient than maize farming in terms of energy and CO2-eq indexes. The total energy requirement for maize and wheat farming was 92560.24 MJ ha^{-1} and 39624.15 MJ ha^{-1}, which caused the emission of 20191.47 and 7541.04 kg CO_2-equivalent per hectare in maize and wheat farms respectively. Electricity, fertilizers and fuel were the most important pollutants of environment in terms of energy and gas emission in both farms. Theses inputs consumed 55.52, 22.62 and 6.44 % of total energy of maize and 47.32, 21.19 and 9.01 % of total energy of wheat farm and were responsible for the 88.60, 8.79 and 2.03 % of CO_2-equivalent in maize and 86.54, 9.54 and 3.24 % of CO_2-equivalent in wheat farms respectively. The results of this study also showed that the enhancement of 60.74 and 27.02 % in energy ratio and 46.06 and 27.87 % in CO2-eq index in maize and wheat farming can be expected using simple improving scenarios.

Key words: energy indexes, GHG emission, maize, wheat

IZVLEČEK

ANALIZA ENERGETSKE BILANCE IN BILANCE TOPLOGREDNIH PLINOV KOT OCENA OKOLJSKIH KAZALNIKOV PRIDELAVE PŠENICE IN KORUZE NA PRIMERU DVEH KMETIJ

V raziskavi sta bili ocenjeni letna energetska bilanca in bilanca toplogrednih plinov pri pridelavi pšenice in koruze na dveh kmetijah, v provinci Khuzestan, Iran. Rezultati so pokazali, da je pridelovanje pšenice učinkovitejše kot pridelovanje koruze glede na energetsko bilanco in bilanco CO_2. Celokupna poraba energije je za pridelavo koruze in pšenice znašala 92560,24 MJ ha^{-1} in 39624,15 MJ ha^{-1}, kar je povzročilo emisijo 20191,47 in 7541,04 kg CO_2 ha^{-1} pri koruzi, oziroma pšenici. Elektrika, gnojila in goriva so bili najvažnejši povzročitelji onesnaževanja okolja in emisije toplogrednih plinov na obeh kmetijah. Ti vložki so porabili 55,52; 22,62 MJ ha^{-1}, kar je 6,44 % celokupne energije koruze in 47,32; 21,19 MJ ha^{-1}, kar je 9,01 % celokupne energije energije pšenice. Pridelavi sta bili odgovorni za emisiji 88,60; 8,79 kg CO_2 ha^{-1}, kar znaša 2,03 % ekvivalenta CO_2 pri koruzi in 86,54; 9,54 kg CO_2 ha^{-1} in 3,24 % ekvivalenta CO_2 pri pšenici. Rezultati te študije so tudi pokazali izboljšanje ob uporabi izboljšanega scenarija na 60,74 and 27,02 % v energetski bilanci in 46,06 in 27,87 % v bilanci CO_2 pri koruzi in pšenici.

Ključne besede: energetski indeksi, GHG emisije, koruza, pšenica

1 INTRODUCTION

Both scientists and policy makers increasingly recognize that the impact of agriculture on our environment cannot be ignored. However, the issue is complex, because of the many different agricultural activities and actors, the range of environmental pollutants involved, and as a result

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of that, the various environmental effects (Havlikova and Kroeze, 2007). Energy and greenhouse gage (GHG) balance are from most prevalent factors in environmental assessment of agricultural activities. Kok *et al.* (2006) introduced energy as indicator of environmental load because of two main reasons. Firstly, energy use has impact on three environmental issues: resource depletion, local/regional environmental impact (acid rain) and global impact (greenhouse effect). Secondly, the energy statistics are the best available environmental data and provide the possibility to calculate energy requirements in relation to consumption. The rising atmospheric GHG concentration, believed to be the primary cause of global climate change, has encouraged proposals to reduce human-induced GHG emissions. The increasing use of agricultural inputs in modern farming has resulted in an increase in the energy inputs and consequently incensement of agriculture sector share in production of GHG (Pimentel, 1980).

Fossil energy use by agriculture is about 3.0–4.5 % of the total consumption in the developed countries of the world (Enquete Commission, 1995). Agriculture accounts for around 10-12 % of total global GHG emissions but is the main source of non CO_2 GHGs, emitting nearly 60 % of nitrous oxide (N_2O) and nearly 50 % of methane (CH_4) (Smith *et al*., 2007). Bellarby *et al*. (2008) estimate that the production of fertilizers emits between 284 and 575 Tg CO_2-eq yr^{-1}, representing 0.6-1.2 % of total global GHG emissions from all sources. If agricultural production is going to significantly increase while also minimizing its impact on future climate change, it is important to understand both its current contribution to energy and GHG budgets and how agricultural management practices can influence them.

There are many studies that evaluated the environmental effects of agricultural systems using energy in different areas of agriculture including, farming (Asakereh *et al*., 2011; Lorzadeh *et al*., 2011; Abdi *et al*., 2012b), orchards (Sami *et al*., 2011; Loghmanpour *et al*., 2013; Kizilaslan, 2009) animal husbandry (Naghibzadeh *et al.,* 2011; Barber *et al.,* 1989) and etc. Nevertheless, gas emission as result of agricultural in-farm and off-farm activities has not been sufficiently evaluated. Shortages of information about gas emission of agricultural inputs in the production processes may be one of the most important reasons of this ignorance. However, there are some studies that tried to evaluate and analyze the gas emission of agricultural activities (e.g. Eshun *et al*., 2013; Taki *et al*., 2012; Lal, 2004).

Energy and GHG analysis in agricultural production operations results in determining overuse sectors and may act as a platform to improve production processes. The aim of this paper was to analyze and compare the energy and GHG balance in maize and wheat farming in the field level as two most relevant cereals in the world. According to FAO (2012) report, wheat and maize had the biggest shares of cereals production in the world during 2008-2010 with a share of 29.7 % and 36.7 % respectively. Wheat is also the dominant cereal crop in Iran accounting for almost 71.2 % of the aggregate cereal production. Maize is the other dominant cereal in Iran after barley and rice (FAO, 2012).

2 MATERIALS AND METHODS

2.1 Studied farms

The region of study is located in southwest of Iran, in northeast of Khuzestan province (within 29º 58' and 32º58' North latitude and 47º42' and 50º39' East longitude). The region represents semiarid and subtropical climatic conditions with very hot summers and fairly cool winters. Data were collected from two large mechanized farms producing wheat and maize, respectively. Wheat was cultivated in a 500 ha farm during November 2008 – May 2009, and maize was cultivated in a 220 ha farm during July – October 2009. The farms reflected typical large mechanized arable farming systems in the studied region. The both farms had the similar status, and similar management practices were implemented in both of them. Farms soils were mostly silty-clay loam structured from alluvial materials, with less than 1 % soil organic carbon. Topographically, the farms were flat with a slope less than 2% and the average elevation of 25 m above the sea level.

Stubble working was made via disc harrow with a medium working depth and sowing was done using drills. Nitrogen, phosphorous, potassium and other fertilizers were applied at different rates according to crops nutrient need. Irrigation water was scattered in defined periods through flood irrigation system. Weeds were controlled using mechanical distribution of herbicides.

2.2 Energy calculation

Farm inputs and outputs can be expressed in terms of energy equivalent. The total energy use per unit of activity can be expressed in terms of MJ ha^{-1}, indicating overall energy consumption. In this study energy budget was calculated based on a mix of actual on field data from the farms and the energy coefficients. The energy equivalents for different inputs and outputs used in energy budget calculation are shown in Table 1. The energy cost of inputs and practices were adapted from different sources of estimations that best fit Iran conditions. Based on the energy equivalents of the inputs and outputs, the energy balance (MJ output minus MJ input), the energy ratio (MJ output/MJ input), and energy intensity (MJ input per kg of product) were calculated as indexes of energy use efficiency (Singh, 2002; Stout, 1990).

The input energy is also classified into direct and indirect; and renewable and non-renewable forms. The indirect energy included energy embodied in chemicals, manure, machine and equipment; while the direct energy includes human power, fuel and electricity in the production process. On the other hand, non-renewable energy includes diesel, electricity, pesticides, and fertilisers; while renewable energy consists of human and manure fertilisers (Demircan *et al.*, 2006).

Table 1. The energy equivalent of farm inputs and outputs

Item	Unit	Energy equivalent (MJ $unit^{-1}$)	
		Maize	Wheat
Human labor	h	2.2	2.2
Machinery	kg (Average)	132.6	130.8
Fertilizers			
Nitrogen	kg	78.4	78.4
Phosphate	kg	17.4	17.4
Potassium	kg	13.7	13.7
Others	kg	8.8	8.8
Manure	ton	303.0	-
Herbicides	kg or l (Average)	202.9	166.1
Electricity for irrigation	kWh	12.0	12.0
Diesel	l	47.8	47.8
Gasoline	l	46.3	46.3
Seed	kg	14.0	13.0

2.3 GHG emission

Production, storage and application of inputs in agricultural farms invoke combustion of fuels, which results in CO_2 and other GHGs emissions. CO_2 equivalent is an index calculating emission levels of inputs according to their Global Warming Potentials (GWP), assuming a 100-year time horizon. The GWP is expressed in kg CO_2-eq, which is taken to be 1 for CO_2, 296 for N_2O and 23 for CH_4 (IPCC, 2006). Conversion coefficients CO_2-eq are calculated for each farm input based on its GHG emission during its production or/and consumption and can be expressed in kg CO_2-eq per weight of input. In agriculture, GHGs are released by direct use of fuels in farm equipments, (e.g. tillage, sowing, harvesting, water pumping, grain drying), production, transportation and application of farm inputs, burning or other oxidation of biomass and decomposition of crop residues. CO_2-eq index is calculated by the sum of CO_2-eq of all farms inputs in the term of kg ha^{-1}.

In this study, we calculated the GHG balances of wheat and maize production in the field-level. The conversion coefficients used here are presented in Table 2. Using data published by PEPD (2010), we calculated conversion coefficients for different

fuels in Iran and expressed them in terms of kg of CO_2 equivalent. GHG emissions attributable to electricity consumption are based on the fuels used in power generation. Considering the proportion of different fuels in electricity generation, we calculated conversion coefficient for electricity for Iran according to PEPD (2010). N fertilizer has two sources of GHG emission; off-farm emission which involves GHG emission from production, packaging and transporting of fertilizer and on-farm emission which involves emission from soil denitrification and nitrification processes in the field after distribution of fertilizer. Precise measurement of N_2O emission from soil denitrification and nitrification processes is difficult since it depends on many complex interactions taking place in the soil, and can considerably vary depending on temperature, moisture, available N, organic matter, soil aeration, pH and so on (Flynn and Smith, 2010). Nevertheless, direct N_2O emissions have been shown to relate to N inputs. Therefore, amounts of N_2O emissions are often calculated using an emission factor that represents the percentage of any N applied that emit in the form of N_2O (Flynn and Smith, 2010). We used a simple emission factor (percentage of applied N emitted as N_2O) reported by IPCC (2006). The fossil energy and fossil CO_2 contents of manure are considered to be zero. Therefore, we only used an emission factor for GHG emission of manure fertilizer decomposing in the soil. According to IPCC (2006), the amount of C lost via harvested crops is considered to be replaced by C uptake in the following crop and there is no significant long-term accumulation of C in crops or crop products. Therefore, we did not take into account this carbon cycle. For other farm inputs (pesticides and fertilisers), we did not find local reliable data regarding CO_2 equivalent conversion coefficients; therefore we used international consolidated conversion coefficients.

Table 2: The CO2-eq coefficient of farms inputs

Item	Unit ha^{-1}	CO_2-eq coefficient (kg $unit^{-1}$)	References
N fertilizer	kg	6.93 (= 3.97 off-farm + 2.96 on-farm)	Adopted from: Macedo et al. (2008) and IPCC (2006)
P fertilizer	kg	1.30	Macedo et al. (2008)
K fertilizer	kg	0.71	Macedo et al. (2008)
Other fertilizers	kg	0.66	Macedo et al. (2008)
Manure	ton	27.50	Lal, 2004
Gasoline	l	2.94	Calculated according: Macedo et al. (2008) and PEPD (2010)
Diesel	l	3.28	Calculated according: Macedo et al. (2008) and PEPD (2010)
Electricity	kWh	4.18	Calculated according: PEPD (2010)
Human labour	h	0.00	-
Machinery	kg yr^{-1}	0.56	Adopted from: Biswas et al. (2008)
Herbicides	kg or l	29.67	Adopted from: Audsley et al. (2009)

3 RESULTS AND DISCUSSION

3.1 Energy analyses

The amount of physical inputs and outputs and their energy equivalents used per hectare of wheat and maize production are presented in Table 3. Other than seed and gasoline that are higher in wheat production, the amount of other inputs in the maize farm per hectare is higher. Based on the energy equivalents of the inputs and outputs given in Table 1, the average total energy consumed per farms per year were calculated as 92560.24 and 39624.15 MJ ha^{-1} for maize and wheat farming respectively. The energy input of maize farm is higher than most energy reports of maize farming in Iran (e.g. 29307, 26917, 39232 and 34649 MJ ha^{-1} by Lorzadeh *et al*., 2011; Abdi *et al*., 2012a; Taki *et al*., 2012; Lorzadeh *et al*., 2012) and is higher than many reports in other parts of the world (e.g. 12638, 57476, 33976 and 21671 MJ ha^{-1} by Chamsing *et al*., 2006; Shapouri *et al*., 2002; Pimentel and Patzek, 2005 and Lorenz and Morris, 1995). This high energy consumption is due to much higher consumption of water in the studied case and the use of pumping system with high

suction head. Water rate consumption in the studied maize farm is 24562 m^3 ha^{-1} that is again much higher than average water consumption reported by similar studies. For example, Lorzadeh *et al*. (2011), Abdi *et al*., (2012a) and Lorzadeh *et al*. (2012) have reported water usage rates of 7327.13, 5400.00 and 4336.87 m^3 ha^{-1} in maize farms. The total energy input in wheat farm is in the range of energy reports of wheat in Iran (e.g. 37482, 38589 and 43900 MJ ha^{-1} by Shahin *et al*., 2008, Houshyar *et al*., 2010 and Zooleh *et al*., 2011). However higher or smaller numbers have also been reported (e.g. 69373, 83454, 21261 MJ ha^{-1} by Abdi *et al*., 2012b; Beheshtitabar and Keihani, 2007; Sedaghathoseini and Moghaddasi, 2010). This range of energy consumption is also much higher than energy reports of non-mechanized and traditional wheat farming systems such as 12200-15260 MJ ha^{-1} that was reported by Nassiri and Singh (2008) and 4030 MJ ha^{-1} that were reported by Nahatkar and Sharma (2006).

Comparison between the energy patterns of farms reveals that the investigated farms have relatively similar energy consumption pattern, however maize production has the higher operational energy input compared to the wheat production. Irrigation has the highest contribution from total input energy in maize and wheat and contributes to the total energy 55.5 % and 47.3 % for the respective crops. The second highest energy consumer in both farms are fertilizers that consume 22.6 and 21.2 % of total input energy followed by diesel fuel, which consumes 6.4 and 8.7 % of total input energy in the maize and wheat respectively. Other inputs have a relatively small share of input energy. In most of the past studies, electricity, fertilizer and fuel were also reported as the main energy consumers of farming systems (e.g. Taki *et al*., 2012; Lorzadeh *et al*., 2012; Sedaghathoseini and Moghaddasi, 2010; Abdi *et al*., 2012b; Nassiri and Singh, 2008; Lorenz and Morris, 1995). Human labour includes the lowest energy of total input energy in two farms (0.08 and 0.06 % in maize and wheat respectively). Used human labour is considered 32.15 and 11.34 h ha^{-1} for maize and wheat, respectively. This small employment of human labour was expected, since all operations, other than irrigation, were done using mechanical power. The comparatively higher use of human labour for maize production is mainly due to more labour requirement for irrigation operation and greater use of mechanical power, which invokes more operator effort in maize farming (22.1 kg ha^{-1} employment of equipment in maize compared to 14.6 kg ha^{-1} employment of equipment in wheat). The higher employment of machinery in maize also caused higher consumption of diesel fuel in maize farm (5961.4 l ha^{-1}) in comparison to wheat farm (3461.3 l ha^{-1}). The human labour employment per hectare in two farms is relatively smaller than that of semi-mechanized farms of wheat and maize in Iran. For example average human labour employment of 45.9, 99.3, 81.5 and 84.9 h ha^{-1} were reported by Taki *et al*. (2012), Lorzadeh *et al*. (2012) , Abdi *et al*. (2012a) and Lorzadeh *et al*. (2011) in maize and 147.3, 192.0, 42.0 and 94.2 h ha^{-1} were reported by Shahin *et al*. (2008), Abdi *et al*. (2012b), Houshyar *et al*. (2010), Attar (2011) and Beheshtitabar and Keihani (2007) in wheat.

The distribution of inputs used in the production of crops according to the direct, indirect, renewable and non-renewable energy groups, are given in Table 4. In both farms, the share of direct and indirect energy of total input energy is closely similar (62.0 and 63.9 % of direct and 37.9 and 36.0 % of indirect energy in maize and wheat farms respectively). It is also seen that the ratios of renewable energy in both farms are very low. However, this share in the wheat production (7.6 %) is relatively higher than that of maize production (0.8 %) and the rate of renewable energy consumption in wheat farm is higher (715.38 MJ ha^{-1} in maize in comparison to 3013.49 MJ ha^{-1} in wheat). This is mostly because of the high consumption of seed for planting in the wheat farm. Replacing of non-renewable energy whit renewable energy offers a varied range of benefits, including: a decrease in external energy dependence; a boost to local and regional component manufacturing industries; promotion of regional engineering; increased research and development, decrease in impact of electricity production and transformation; increase in the level of services for the rural population; creation of employment, etc. (Míguez *et al.,* 2006).

From Table 3 it is also seen that the total output yield (grain) of maize farm (5294.0 kg ha^{-1}) is higher than wheat farm (3985.0 kg ha^{-1}). Accordingly, the overall energy output of maize farm (74116.0 MJ ha^{-1}) is higher than that of the

wheat (51805.0 MJ ha^{-1}). These results are in the range of average yield of maize and wheat in the Iran (e.g. Abdi *et al*., 2012b; Abdi *et al*., 2012a; Taki *et al*., 2012; Shahin *et al*., 2008; Lorzadeh *et al*., 2012). However, obtained results are generally smaller than the average yields of maize and wheat in mechanized production systems in the world. For example the yields of 6988.7, 7105.2 and 8655.0 kg ha^{-1} were reported by Lorenz and Morris (1995), Shapouri *et al*. (1995) and Pimentel and Patzek (2005), for maize and 4868.0 kg ha^{-1} was reported by Khan *et al*. (2010) for wheat. In addition, some studies in Iran reported higher yields (e.g. 5968.1 - 7272.8 kg ha^{-1} by Sedaghathoseini and Moghaddasi (2010) and 5613.4 kg ha^{-1} by Zooleh *et al*. (2011) for wheat).

Accordingly, the energy indexes of energy ratio, energy balance and energy intensity were estimated 0.80, -18444.24 MJ ha^{-1} and 17.48 MJ kg^{-1} in maize and 1.31, 12180.85 MJ ha^{-1} and 9.94 MJ kg^{-1} in wheat farm respectively (Table 4). These indexes show that mechanized wheat production in the investigated region is more efficient in terms of energy. Energy ratio of maize farm is relatively smaller than that of the other part of Iran. For example, Lorzadeh *et al*. (2011), Abdi *et al*. (2012a), Taki *et al*. (2012) and Lorzadeh *et al*. (2012) reported energy ratios of 1.86, 3.50, 2.60, 2.97 for Iran.

This low energy ratio is mainly because of high energy input, especially water and fertilizer energy, in the maize case study system whereas the energy ratio of wheat is in agreement with the average range of energy ratio index in Iran. Energy ratio in some wheat productions in Iran were reported as 2.72, 2.60, 1.13 - 1.42 and 1.56 by Shahin *et al*. (2008), Houshyar *et al*. (2010), Zooleh *et al*. (2011) and Attar (2011).

Table 3: Input and outputs of farms and their related indexes in the term of energy

Item	Unit	Maize			Wheat		
		Amount of input used per hectare	Input energy (MJ ha^{-1})	%	Amount of input used per hectare	Input energy (MJ ha^{-1})	%
Human labor	h	32.15	70.74	0.08	11.34	24.95	0.06
Machinery	kg	22.12	2932.79	3.17	14.60	1910.15	4.82
Fertilizers			20941.40	22.62		8395.85	21.19
Nitrogen (N)	kg	240.31	18840.43	20.35	96.86	7593.94	19.16
Phosphate (P_2O_5)	kg	20.43	355.45	0.38	11.01	191.51	0.48
Potassium (K_2O)	kg	95.40	1306.98	1.41	36.34	497.90	1.26
Others	kg	22.64	199.26	0.22	12.78	112.50	0.28
Manure	ton	0.79	239.29	0.26	0.00	0.00	0.00
Herbicide	kg or l	2.85	577.29	0.62	1.43	237.92	0.60
Irrigation indirect		-	10278.54	11.10	-	3749.77	9.46
Electricity for irrigation	kWh	4282.72	51392.68	55.52	1562.41	18748.87	47.32
Diesel	l	124.72	5961.45	6.44	72.41	3461.34	8.74
Gasoline	l	0.00	0.00	0.00	2.31	106.76	0.27
Seed	kg	28.95	405.35	0.44	229.89	2988.54	7.54
Total input	-	-	92560.24	100.00	-	39624.15	100.00
Total output	kg	5294.00	74116.00	-	3985.00	51805.00	-

Table 4: Energy indexes of the farms

Energy index	Unit	Maize		Wheat	
		Amount	%	Amount	%
Total output	MJ ha^{-1}	74116.00	-	51805.00	-
Total input	MJ ha^{-1}	92560.24	100.00	39624.15	100.00
Indirect energy	MJ ha^{-1}	35135.37	37.96	14293.69	36.07
Direct energy	MJ ha^{-1}	57424.87	62.04	25330.46	63.93
Renewable energy	MJ ha^{-1}	715.38	0.77	3013.49	7.61
Non renewable energy	MJ ha^{-1}	91844.86	99.23	36610.66	92.39
Energy ratio	-	0.80	-	1.31	-
Energy balance	MJ ha^{-1}	-18444.24	-	12180.85	-
Energy intensity	MJ ha^{-1}	17.48	-	9.94	-

3.2 GHG emission evaluation

Table 5 shows the share and amount of GHG emitted by each input in the farms. Totally, the GHG emission of maize production (20191.47 kg ha^{-1}) is about three times higher than that of wheat production (7541.04 kg ha^{-1}). Considering the share of inputs in the total GHG emission in two farms, the main reason of this difference is about three times higher consumption of electricity (4282.72 compared to 1562.41 kWh ha^{-1}) and N fertiliser (240.31 compared to 96.86 kg ha^{-1}) in maize farm compared to wheat farm (Table 3). Other than electricity, fertilizer and fuel, other inputs have ignorable share of total GHG emission in two farming systems. Electricity in the farms is the dominant source of GHG emissions (88.60 % in maize and 86.54 % in wheat farm). Fertilizers emitted 8.79 % and 9.54 % of CO_2-eq in the maize and wheat farms respectively and are the second important source of Co_2-eq. Nitrogen is the dominant source of GHG emissions among the fertilizers and almost 93.85 and 93.26 % of the total Co_2-eq emissions from fertilizer use and 8.25 % and 8.90 % of total GHG-eq emissions from farming systems associate with nitrogen fertilizers in maize and wheat respectively.

Table 5: The share of inputs from GHG emission of the farms

Item	CO_2-eq			
	Maize		Wheat	
	kg ha^{-1}	%	kg ha^{-1}	%
Human labor	0.00	0.00	0.00	0.00
Machinery	12.39	0.06	8.18	0.11
Fertilizers	1774.54	8.79	719.77	9.54
Nitrogen (N)	1665.36	8.25	671.25	8.90
Phosphate (P_2O5)	26.56	0.13	14.31	0.19
Potassium (K_2O)	67.73	0.34	25.80	0.34
Others	14.89	0.07	8.41	0.11
Manure	21.72	0.11	0.00	0.00
Herbicides	84.42	0.42	42.50	0.56
Irrigation indirect	0.00	0.00	0.00	0.00
Electricity for irrigation	17889.42	88.60	6526.35	86.54
Diesel	408.99	2.03	237.47	3.15
Gasoline	0.00	0.00	6.78	0.09
Seed	0.00	0.00	0.00	0.00
Total	20191.47	100.00	7541.04	100.00

3.3 Opportunities for optimizing greenhouse gases and energy impacts

As it was seen, electricity, fertilizers and fuel are the most important environmental pollutant in the studied farms. Fuel is used in machinery equipments which are inseparable parts of today mechanized agriculture. Nevertheless, employment of certain less fuel consuming systems such as conservation tillage can efficiently help to improve the environmental indexes (Coxworth *et al*., 1999; Entz *et al*., 1995; West and Marland, 2002). In the studied farms, two applicable strategies can be suggested to enhance the environmental impact of farming systems. In the first scenario, using more efficient application of water in the farms, water rate consumption could fall to 12281 m^3 ha^{-1} (50 %) in maize and 6243 m^3 ha^{-1} (30 %) in wheat farms. This is a reasonable scenario since the average water consumption of maize and wheat have been reported 5000-13000 m^3 ha^{-1} and 6000-7000 m^3 ha^{-1} in Iran conditions (Keshavarz and Heidari, 2004). In the second scenario, fertilizer consumption of farms could be decreased up to 20 %. More efficient application of fertilizers such as use of slow and controlled release fertilisers and/or stabilized fertilisers can successfully reduce the application rate of fertilizer without any reduction in the farms yield (Carreres *et al*., 2003; Kochaki *et al*., 2012). The first scenario will enhance the energy ratio of farms by 1.20 and 1.58, and reduce the CO_2-eq by 11246.86 and 5583.13 kg ha^{-1} in maize and wheat farms respectively. The second scenario will enhance the energy ratio of maize and wheat farms by 0.84 and 1.37 and decrease CO_2-eq of farms by 19836.76 and 7397.08 kg ha^{-1} respectively. In addition, if we combine both scenarios, the enhancement of 60.74 and 27.02 % in energy ratio and 46.06 and 27.87 % in CO_2-eq index of maize and wheat farms can be expected (Table 6).

Table 6: The effects of change in some factors on the energy and GHG emission indexes

Item (unit)	Changed factor					
	Water		Fertilizers		Water and fertilizers	
	Maize	Wheat	Maize	Wheat	Maize	Wheat
Factor change (%)	-50 %	-30 %	-20 %	-20 %	-	-
Total input (MJ ha^{-1})	61724.98	32874.56	88420.51	37944.98	57584.56	31195.39
Total input change (%)	-33.31	-17.03	-4.47	-4.24	-37.79	-21.27
Energy balance (MJ ha^{-1})	12391.02	18930.44	-14304.51	13860.02	16531.44	20609.61
Energy balance change (%)	-167.18	55.41	-22.45	13.79	-189.63	69.20
Energy ratio	1.20	1.58	0.84	1.37	1.29	1.66
Energy ratio change (%)	49.96	20.53	4.68	4.43	60.74	27.02
CO_2-eq (kg ha^{-1})	11246.86	5583.13	19836.76	7397.08	10891.95	5439.18
CO_2-eq change (%)	-44.30	-25.96	-1.76	-1.91	-46.06	-27.87

4 CONCLUSION

The present study analyzed the energy and GHG balance as integrative environmental indexes in two production systems of maize and wheat. Although total output and related energy were higher in maize crop than wheat, the indexes showed less environmental impact of wheat farming compared to maize farming. This resulted from the significantly higher inputs entered in maize farming. Electricity used mainly for irrigation pumps was the major energy input among all energy inputs for growing both two crops. Fertilizers played the second dominant role followed by fuel. These three parameters also were the most responsible for GHG emission of the farming systems. The share of renewable energy in two farming systems was negligible. Comparing to semi and non mechanized farming systems, mechanized wheat and maize farming in the studied region were less efficient in terms of energy. The study results also showed that implementation of some reductions in water and fertilizer consumption are able to considerably reduce the environmental effects of both farming systems.

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Impact of fertilization on water resources in karst, example of research field site Sinji Vrh

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ABSTRACT

A research field site (RFS) was established at Sinji Vrh in the western part of Slovenia in order to study flow and solute (particularly pollutant) transport in fractured and karstified rocks, with a focus on the unsaturated zone. RFS consists of surface set-up and a research tunnel, 15 m below the surface. Agrometeorological station was installed on the RFS. A special construction (1.5 m long segments) for collecting water seeping from the ceiling of the research tunnel was developed. At the research field site Sinji Vrh fertilizer application experiments were performed for estimation of impact of fertilization on water resources. Results of the fertiliser application experiments have shown that a thin autochtonous soil cover on karstic rock is insufficient to retain nitrate and prevent pollution of groundwater.

Key words: karstic rock, nitrate pollution, agricultural pollution, unsaturated zone, water resources

IZVLEČEK

VPLIV GNOJENJA NA VODNE VIRE NA KRASU, RAZISKOVALNI POLIGON SINJI VRH

Terenski eksperimentalni poligon na Sinjem vrhu v zahodni Sloveniji je bilo urejeno za raziskave toka vode in prenosa snovi (predvsem onesnažil) v kraško - razpoklinskih kamninah, s poudarkom na nezasičeni coni. Terenski eksperimentalni poligon sestavljata površinski in podzemni del – predor 15 m pod površjem. Nad raziskovalnim rovom je bila nameščena agrometeorološka postaja. V raziskovalnem predoru je bilo nameščeno posebno ogrodje v 1.5 m dolgih segmentih za vzorčenje prenikle vode. Na terenskem eksperimentalnem poligonu je bil izveden poskus z mineralnimi gnojili z namenom ocenit vpliv gnojenja na vodne vire. Rezultati so pokazali, da je tanek sloj avtohotnih tal na površini kraške kamnine nezadostna zaščita pred onesnaževanjem podzemnih voda z nitrati.

Ključne besede: kraška kamnina, onesnaževanje z nitrati, onesnaževanje v kmetijstvu, nezasičena cona, vodni viri

1 INTRODUCTION

Fractured and karstified rocks are very heterogeneous and complex in terms of their geometry and void topology. This results in parameter variability and large uncertainties reflecting complicated hydraulic, mechanical, thermal, and chemical processes. Therefore, detailed studies of these processes have to be performed on a macro scale at instrumented research field sites (Čenčur Curk, 1997, 2001). Such a site was established at Sinji Vrh in the western part of Slovenia (Figure 1). The main goal of the research field site at Sinji Vrh (RFS Sinji Vrh) was the study of flow and solute (particularly pollutant) transport in fractured and karstified rocks, with a focus on the unsaturated zone.

In hydrogeological systems of karstified aquifers, the unsaturated zone above the water table has

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physical and chemical retention properties which provide a potential natural protection zone for karstic groundwater. Simulation of fertilisation on meadow at this research field site was performed in order to study the behaviour of nitrates and their percolation through karstic soil and underlying unsaturated zone of karstified rock.

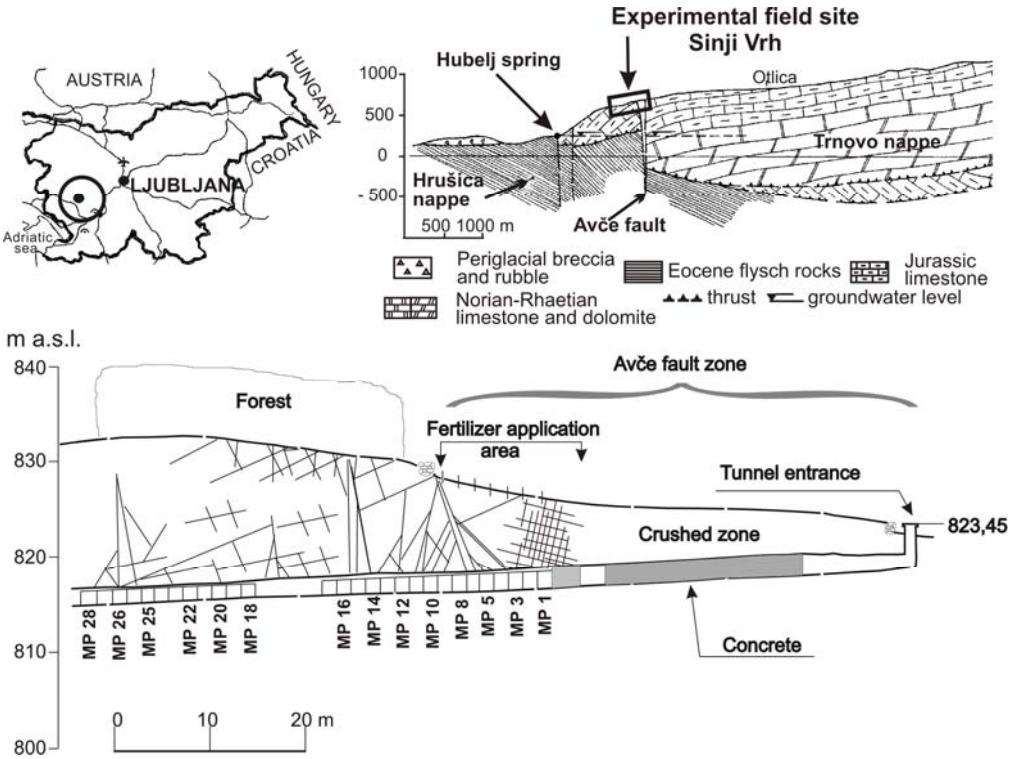

Figure 1: Location of the research field site Sinji Vrh (RFS Sinji Vrh) with a geological cross-section of Trnovo plateau (Janež, 1997, Veselič and Čenčur Curk 2001)

2 MATERIALS AND METHODS

2.1 Research field site Sinji Vrh

RFS Sinji Vrh is located in the western part of Slovenia (Figure 1) at the edge of the Trnovski Gozd plateau (mean altitude of 900 m a.s.l), which is an over thrust (Trnovo nappe) of carbonate rock over impermeable Eocene flysch rocks (turbidites sediments, mainly changing of marble and sandstone). This area is composed of Jurassic limestone, which passes laterally into crystalline dolomite. This territory is crossed by the sub vertical Avče fault with a Dinaric direction (NW-SE), resulting in crushed and fractured rock. The geological profile of the Trnovski Gozd plateau is presented in Figure 1 (Janež, 1997).

The groundwater horizon lies extremely deep and appears on the surface at the lowest point of the impermeable flysh border in karstic spring Hubelj. Average annual precipitation in the catchment is roughly 2450 mm. In this region mainly thin (10-50 cm) carbonate soil types are found. They have low water retention capacities facilitating fast infiltration rates (Matičič, 1997).

The research field site in the unsaturated zone of fractured and karstified rock presents a 340 m long artificial tunnel, 5 to 25 meters below the surface (Figure 1). The surface is covered with grassland and small beech forests which usually cover outcrops. The unsaturated fractured and karstified limestone has a negligible matrix porosity and very high fracture density with some larger conduits (Čenčur Curk and Veselič 1999).

An agrometeorological station (Figure 2) has been installed on the surface, where precipitation,

evaporation, air temperature, air moisture, wind speed and direction (both at two levels) are continuously measured. It is located near the tunnel entrance at a height of 825 m above sea level.

On the grassland, an area of 150 m^2 was used for a fertiliser application experiment (Figure 2). The grassland close to the tunnel entrance is cut twice a year and used for livestock feed; there are no other crop and irrigation practices. Suction cups were installed at two levels (depth of 15 and 45 cm) in the karstic soil above the tunnel. For these experiments a special water collecting structure was made and installed in the tunnel. Each sampling segment consists of metal girders across which a plastic sheet is tightened. Special funnels were made to collect water in narrow sampling containers (Figure 3). The water seeping from the ceiling of the tunnel is gathered in 28 segments (each 1.5 m long); each has a total collecting surface of 2.2 m^2 (Figure 1 and 3).

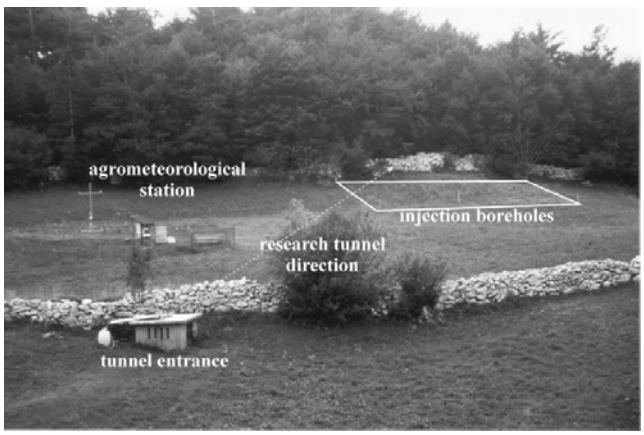

Figure 2: Agrometeorological station and area where fertiliser was applied (rectangle) on the research field site Sinji Vrh

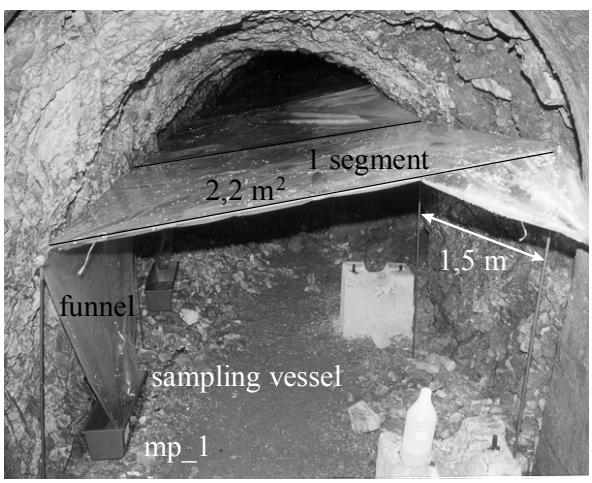

Figure 3: Construction for collecting water samples in the tunnel

2.2 Soil parameters

Standard soil parameters and hydraulic functions were determined at the Biotechnical Faculty, Agronomy Department. The 0.2 – 1 m thick soil is a typical karstic pocket soil (calcaric brown soil), very similar to a rendzina but with characteristic deeper pockets extended along weak zones like fractures in the underlying rock (Figure 4). In a 0.7 m deep soil profile the Ah-horizon has a thickness of 0.15 m, and the B-horizon 0.55 m. In the latter a horizon with more roots and higher organic content (Brz1) can be distinguished (Table 1). The soil has a middle value of cationic

exchange capacity, since the soil particles bound only 25 – 35 meq $100g^{-1}$ of soil. On the sorptive part of the soil particles 15 – 25 meq bases $100g^{-1}$ of soil are bound. The V-ratio is higher than 50 %; therefore the soil is eutric and saturated with bases.

Figure 4: Soil profile at the RFS Sinji Vrh

Table 1: Results of soil profile analysis on the research field site Sinji Vrh

Parameter	Unit	Soil horizon Depth	A horizon 0-15 cm	Brz1 15-33 cm	Brz 33-70 cm
pH in 0.1M KCl	-		5.6	7.1	7.6
P_2O_5	mg $P2O_5$ $100g^{-1}$ soil		4.5	1.8	1.1
K_2O	mg K2O $100g^{-1}$ soil		10.7	10.4	8.4
N – total	%		0.42	0.15	0.08
Humus	%		7.04	2.51	1.19
H+	meq $100g^{-1}$ soil		17.81	7.5	2.81
K+	meq $100g^{-1}$ soil		0.26	0.25	0.20
Ca++	meq $100g^{-1}$ soil		12.68	24.32	22.38
Mg++	meq $100g^{-1}$ soil		3.41	1.46	0.98
S	meq $100g^{-1}$ soil		16.39	26.07	23.59
T	meq $100g^{-1}$ soil		34.2	33.57	26.40
V	%		47.9	77.7	89.4
sand	%		17.1	12	25.2
silt	%		34.3	16.6	14.7
clay	%		48.6	71.4	60.1
texture class	-		SC-C	C	C

meq = miliequivalent; S = total sum of bases, T = sorption capacity, V = S/T, SC = silty clay, G = clay

Before the first fertiliser application experiment soil samples were taken from two different depths and analysed on nitrate and ammonium nitrogen. In depth of 10-20 cm there was 6.5 mg N-NO3 kg^{-1} soil and 9.6 mg N-NH3 kg-1 soil, whereas in depth of 30-40 cm there was 7.8 mg N-NO3 kg^{-1} soil and 5.1 mg N-NH3 kg^{-1} soil. Isotopic composition (δ15N) of soil water was not determined due to small amount of sampled water.

Soil samples were taken in two depths (10 – 20 cm and 30 – 40 cm) for analysis of nitrate and ammonium nitrogen (Table 2).

Table 2: Nitrate and ammonium concentrations in soil samples at two depths

Depth (cm)	mg N-NO_3 in kg soil	mg NO_3 in kg soil	mg NH_4 in kg soil	mg N-NH_4 in kg soil
10-20	6.5	28.6	12.4	9.63
30-40	7.8	21.5	6.5	5.05

The soil samples were taken for determination of the hydraulic functions of the soil in two depths: 30 cm and 60 cm (Figure 5). The pF curves are very similar and differ only for some mass % at low-tension conditions. The data demonstrates that the water retention capacity of the soil at the research field site is relatively low. Soil samples were also taken for determination of hydraulic conductivity. The hydraulic conductivity of these soil samples varies from 9E-4 to 1.25 cm min^{-1}; the average value is 0.4 cm min^{-1}.

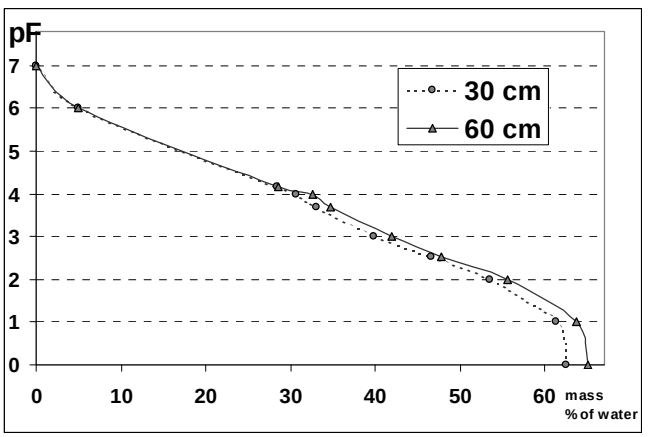

Figure 5: pF curves for the soil depths of 30 and 60 cm

2.3 Fertilizer application experiments

Three fertiliser application experiments were performed at the research field site Sinji Vrh. For all experiments the mineral fertilizer KAN (calcium ammonium nitrate) was used in accordance with appropriate agricultural practice. KAN was selected, because this is widely used mineral fertilizer in Slovenia. For the meadow with mowing twice a year, a norm for application rate is 40 kg N ha^{-1}, therefore 0.6 kg N is needed for 150 m^2 of test area (Figure 2). Regarding 28% N portion in KAN, 2.25 kg of that fertilizer is needed.

In-situ measurements of temperature, pH, Eh and electrical conductivity of seepage water in the research tunnel were performed weekly or after each rain event, whereas samples for chemical and isotopic composition were taken in case of higher electrical conductivity values. Nitrate ion and nitrite ion concentrations were determined with portable WTW photometer MPM 3000 (Institute of Mining, Geotechnology and Environment) with detection limit for nitrate 4,4 mg L^{-1} and 0,16 mg L^{-1} for nitrite. Nitrogen isotope 15N was determined at the Institute Josef Stefan.

3 RESULTS OF FERTILISER APPLICATION EXPERIMENTS

In the first fertiliser application experiment (experiment G1) increased nitrate concentration was detected in water samples of almost all sampling points after approximately 30-35 days (Figure 6), which was five days after the heavy rain (4-July; 51.7 mm), followed by more or less constant rain (Čenčur Curk et al. 2000, Veselič et al. 2001). The first precipitation event (Figure 6) caused dissolution of the fertiliser and transported it into the soil zone. The highest nitrate concentration (15.1 mg L^{-1}) appeared at the measuring point MP5 (Figure 6); δ15N value of

total dissolved N was 0.2 ‰, which refers to mineral fertiliser. It should be noted that for MP5 nitrate concentration before fertilizer application was 16.5 mg L^{-1}, which is higher than the nitrate peak after the fertiliser application. δ15N value after the application was +3.1 ‰, therefore we can assume that there were extensive mineralization processes in the soil because of vegetation. In MP2 the nitrate concentration before the application was even higher: 58.93 mg L^{-1}, but afterwards there was not enough water for analyse. Samples could be taken only after 11th August, when nitrate concentration was very low (5.05 mg L^{-1}). Previous tracer experiments and structural mapping identified this sampling point as a fast channel with the potential to permit large contaminant fluxes. Natural background unsaturated zone concentrations measured in springtime, which are dominated by mineralization processes in the soil, were higher than the observed unsaturated zone nitrate concentrations following fertiliser application.

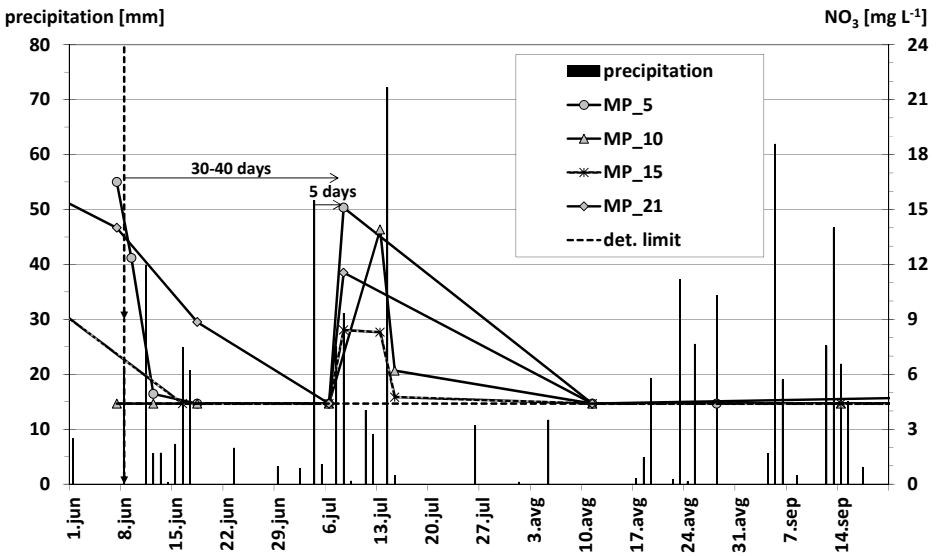

Figure 6: Precipitation events (mm) and nitrate concentrations (mg L^{-1}) (detection limit: 4.4 mg L^{-1}) in sampling points MP5, MP10, MP15 and MP21 for experiment G1 (dashed vertical arrow presents fertiliser application date on 8 June)

The second fertiliser application experiment (G2) was performed in order to obtain further information on nitrate percolation through the soil cover. For this reason, suction cups were installed in the soil and isotope analysis was carried out on water obtained from multiple sampling points (MP2, MP5, MP10 and MP21 in Figure 7). The nitrate appeared one week after one very large precipitation event (68.8 mm; 21-Sept.), about 22 days after fertiliser was applied. Since the isotope composition of the synthetic fertilizer KAN is about 0 ‰, the isotope data δ15N (Figure 7) confirmed that the nitrate source was the fertiliser applied on the meadow in experiment G2 (Čenčur Curk et al. 2000). The nitrate transport along the fast channel at measuring point MP5 showed the first breakthrough of nitrate after 8 days. In some measuring points another flush of the nitrate was detected (MP21 and MP10). After another very large precipitation event in the following spring (1-March; 85.1 mm) the nitrate was flushed again, which released nitrate previously retained in micro fractures of the unsaturated zone (MP2 in Figure 7).

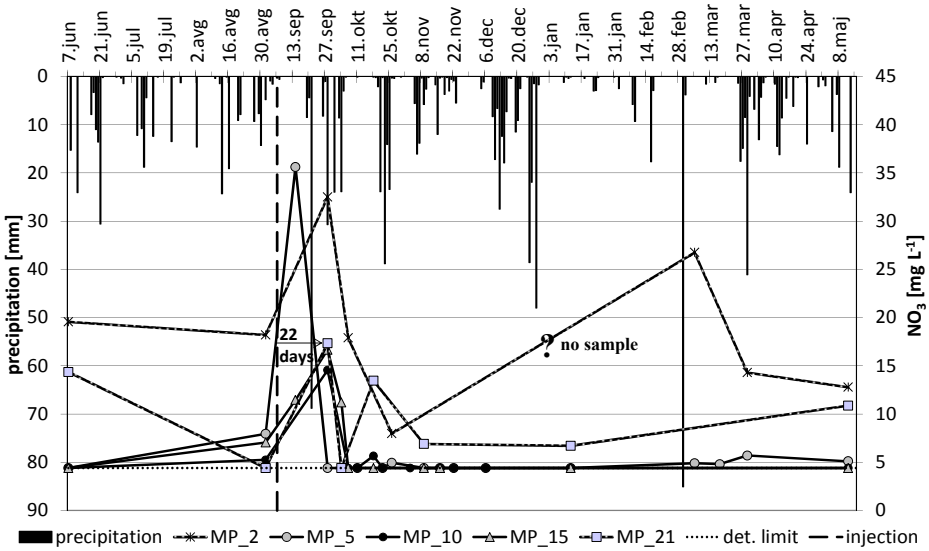

Figure 7: Precipitation events (mm) and nitrate concentrations (mg L^{-1}) (dashed horizontal line: detection limit at 4.4 mg/L) in sampling points MP2, MP5, MP10, MP15 and MP21 for experiment G2

The third fertiliser application experiment (G3) was performed on 8th August. Measuring position MP2 had slightly higher nitrate concentration on 18th September, but this cannot be attributed to fertilization, because there was no precipitation before 15th September and the precipitation event between 15th and 17th September only dissolved the applied fertilizer. On 26th October (57 days after fertiliser application) nitrate concentration in MP2 was extremely high (116.6 mg L^{-1}). δ15N value was 0.5 ‰ (Table 2), which means that this high concentration of nitrate can be result of fertilization. Even δ15N values at MP5 were at the beginning lower and then rose again. The first increase of nitrate concentration on 26th October (7.0 mg L^{-1}) cannot be attributed with certainty to fertilization, because in that time there was not enough water for the isotopic analysis of nitrogen. The next concentration increase on 13th November (9.1 mg L^{-1}) was probably due to fertilization (δ15N = + 0.2 ‰).

Table 2: Nitrate concentrations (mg L^{-1}) and nitrogen isotope composition (‰) in sampling points MP2, MP5, MP10 and MP15 for experiment G3

	MP2		MP5		MP10		MP15	
Date	NO_3^- [mg L^{-1}l]	$\delta^{15}N$ [‰]	NO_3^- [mg L^{-1}l]	$\delta^{15}N$ [‰]	NO_3^- [mg L^{-1}l]	$\delta^{15}N$ [‰]	NO_3^- [mg L^{-1}l]	$\delta^{15}N$ [‰]
31.8.			1.7	11.4	1.7	-4.1	2.1	-0.5
18.9.	23.8	-1.2	1.3	8.8	5.3	3.2	3.0	0.9
6.10.	15.6	0.8	0.6	2.5	4.7	3.0	2.3	-2.1
9.10.			1.3	0.9			7.2	-2.3
11.10.								
26.10.	116.6	-0.5	7.0		86.3		11.8	
2.11.					48.8		19.9	
13.11.			9.1	0.2	48.4		19.3	
15.11.			3.4		47.9		26.5	
17.11.			3.0	-1.5	37.7	0.4	23.3	
20.11.			1.6	2.6	27.1		18.4	
23.11.	0.6	-0.8	0.8	4.0	0.0	-1.1	0.2	4.5
25.11.	2.2	-0.1	0.7	4.4	0.0	1.4	0.2	
27.11.	3.7		0.6	5.5	0.1	2.5	0.3	1.9
29.11.	5.2		0.7	3.6	0.1	-3.8	0.3	-3.9
6.12.			0.8	2.9	0.5	-1.9	0.7	-6.0
13.12.	2.6	-0.9	0.7		0.1		0.5	0.4
4.1.	2.8	0.4	1.1	6.2	0.3		0.4	-0.2

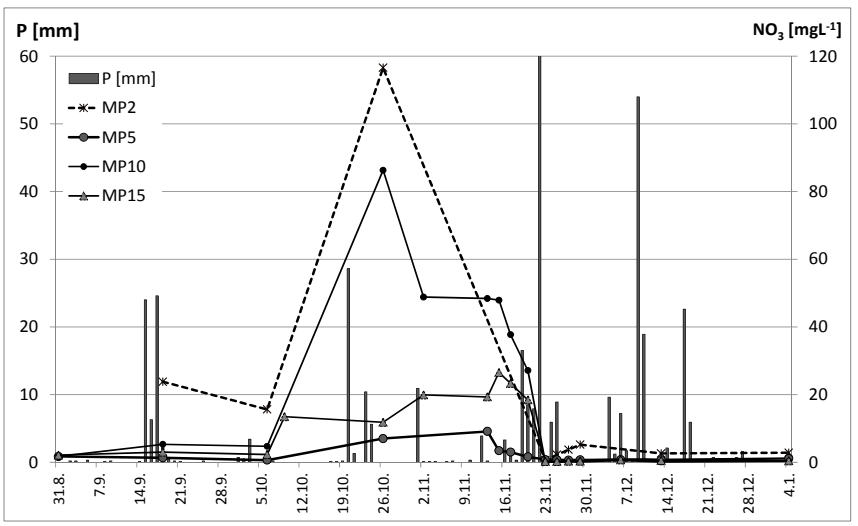

Figure 8: Precipitation events (mm) and nitrate concentrations (mg L^{-1}) (dashed horizontal line: detection limit at 4.4 mg/L) in sampling points MP2, MP5, MP10, MP15 and MP21 for experiment G3

4 CONCLUSIONS

Fractured and karstified rocks are highly heterogeneous and complex, therefore knowledge of the rock structure is of paramount significance for predicting flow and transport. The transport of pollutants depends on i) saturation rate of the soil and unsaturated zone, ii) on precipitation events and iii) on the presence of channels and interconnected fracture networks. Pollution

remains in the soil and in fractures of the upper part of the unsaturated zone and is flushed by subsequent large precipitation events which can occur several months or years afterwards. The degree of flushing is highly dependent on the antecedent moisture conditions within the unsaturated zone.

Results of the fertiliser application experiments have shown that a thin autochthonous soil cover on karstic rock is insufficient to retain nitrate and prevent pollution of groundwater. In most cases the maximum nitrate concentrations were lower than natural background values and the drinking water limit. Therefore the usage of fertilisers should be in accordance with strictly defined standards. Further detailed studies on this aspect are needed within the frame of the research into karstic aquifer vulnerability. Additional experiments with an emphasis on biogeochemical transformations (e.g. measurement of nitrogen gas, N_2O, soil nitrogen compounds, dissolved nitrogen compounds and their isotopic composition, and microbiological processes) is planned in order to better understand the behaviour of nitrate in soil and the unsaturated zone.

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Agris category code: f03

Determination of an optimal priming duration and concentration protocol for pepper seeds (*Capsicum annuum* L.)

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ABSTRACT

Seed priming is a simple pre-germination method to improve seed performance and to attenuate the effects of stress exposure. The objective of this study was to determinate an optimal priming protocol for three pepper cultivars (*Capsicum annuum* L.): 'Beldi', 'Baklouti' and 'Anaheim Chili'. Seeds were primed with three solutions of NaCl, KCl and $CaCl_2$ (0, 10, 20 and 50 mM) for three different durations (12, 24 and 36h). Control seeds were soaked in distilled water for the same durations. After that, all seeds were kept to germinate in laboratory under normal light and controlled temperature. Results indicated that priming depends on concentration, duration and cultivar. The best combinations that we obtained were: KCl priming (10 mM, 36h) for 'Beldi' cultivar, $CaCl_2$ priming (10 mM, 36h) for 'Baklouti' cultivar and finally NaCl priming (50 mM, 24h) for 'Anaheim Chili' cultivar. Generally, priming had an effect on total germination percentage, mean germination time, germination index and the coefficient of velocity compared to control seeds. The beneficial effect of seed priming could be used for improving salt tolerance on germination and early seedling growth for pepper cultivar.

Key words: priming protocol, mean germination time, germination index, coefficient of velocity, 'Beldi', 'Baklouti', 'Anaheim Chili'

IZVLEČEK

DOLOČITEV OPTIMALNEGA ČASA PREDOBDELAVE SEMEN IN KONCENTRACIJSKI PROTOKOL ZA IZBOLJŠANJE KALITVE SEMEN PAPRIKE (*Capsicum annuum* L.)

Predobdelava semen pred kalitvijo je preprosta metoda za izboljšanje kalitve semen in zmanjšanje učinkov izpostavljenosti stresu. Predmet raziskave je bil izdelava optimalnega protokola predobdelave semen za tri sorte paprike (*Capsicum annuum* L.): 'Beldi', 'Baklouti' and 'Anaheim Chili'. Semena so bila pred kalitvijo obdelana z raztopinami NaCl, KCl in $CaCl_2$ (0, 10, 20 in 50 mM) v treh različnih trajanjih (12, 24 in 36h). Kontrolna semena so bila za enak čas namočena v destilirano vodo. Po predobdelavi je bil z vsemi semeni narejen kalitveni test v laboratoriju pri sobni svetlobi in nadzorovani temperaturi. Rezultati so pokazali, da je učinek predobdelave odvisen od koncentracije in vrste raztopin, trajanja predobdelave in sorte. Najboljša kalitev je bila dosežena pri predobdelavi s KCl (10 mM, 36h) za sorto 'Beldi', s $CaCl_2$ (10 mM, 36h) za sorto'Baklouti' in z NaCl (50 mM, 24h) za sorto 'Anaheim Chili'. V splošnem je imela predobdelva v primerjavi s kontrolo učinek na odstotek kalitve, povprečen čas kalitve, kalitveni indeks in koeficient hitrosti kalitve. Blagodejni učinek predobdelave semen bi lahko uporabljali za izboljšanje tolerance kalitve na slanost in hitrejšo zgodnjo rast sadik različnih sort paprike.

Ključne besede: protokol predobdelave, povprečni čas kalitve, kalitveni indeks, koeficient hitrosti kalitve, *Capsicum annuum* 'Beldi', 'Baklouti', 'Anaheim Chili'

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1 INTRODUCTION

Priming involves exposing seeds to an external water potential low enough to restrict germination and yet permit pre-germinative physiological and biochemical activities (Bradford, 1986).

Priming treatment such as NaCl, KCl and $CaCl_2$ can increase and accelerate seed germination and seedling emergence under salt stress for many species. It was effective in alleviating the effect of salt stress on wheat plants through altering the levels of different plant phytohormones (Iqbal *et al*., 2006). Generally, primed seeds germinate faster and more uniformly than unprimed seeds of the same lot (Soughir *et al.*, 2013; Elouaer and Hannachi, 2012). The difference between primed and control seeds are greater in arid and semi-arid conditions when there are more exposure to difficult environmental conditions such as salinity or water deficit. According to Levitt (1980), plants must be exposed to salt stress to develop salt tolerance. This means that seeds should germinate in saline conditions. For example, it has been shown that NaCl priming could be used as an adaptation method to improve salt tolerance of some vegetable seeds (Cayuela *et al.*, 1996; Cano *et al.*, 1991). Also, Khan et *al.* (2009) observed that NaCl priming of seeds improved seedling vigor of pepper under salt stress conditions. Similarly, Soughir *et al.* (2013) demonstrated that seed priming could be used successfully to ameliorate the emergence of fenugreek plants cultivated in pots under salt stress. So, the optimization of priming technique is important to determinate the optimal soaking duration and the optimal concentration of priming agent.

Hot pepper (*Capsicum annuum* L.) is an important vegetable in Tunisia. It is an excellent source of vitamin but the production of this crop is affected by environmental stress such as drought, salinity, chilling and heat (Almansouri *et al.*, 2001). In fact, salinity can affect pepper germination and seedling growth either by creating osmotic pressure that prevent water uptake or by toxic effects of sodium and chloride ions (Hopper *et al*., 1979). So, the need to develop new cultivars with higher salt tolerance has increased strongly. Certainly, priming is one of the cheapest methods which improve seeds performance but seeds priming response depends on many factors such as cultivar, osmotic potential, temperature and duration of the treatment.

This is why the mean objective of this study is to evaluate the effect of soaking pepper seeds in three different solutions (NaCl, KCl and $CaCl_2$) with three different concentrations and durations on germination parameters.

2 MATERIAL AND METHODS

This study was conducted in the research unit "conservation and valorization of vegetal resources by creation of botanical garden", of the High Institute of Agriculture (Chott Mariem, Tunisia).

Plant material was composed of three cultivars ('Beldi', 'Baklouti' and 'Anaheim Chili') of pepper seeds (*Capsicum annuum* L.). These cultivars are the most cultivated in Tunisia. Seeds were initially sterilized with a 1 % solution of sodium hypochlorite for 20 min and then rinsed 3 times with distilled water.

Three solutions (NaCl, KCl and $CaCl_2$) were used as priming agents with 3 concentrations for each solution (10, 20 and 50 mM). Seeds from each cultivar were soaked in every solution for 3 different durations (12, 24 and 36 hours) at 25°C.Control seeds were soaked in distilled water for the same durations. At the end of these durations, all seeds were removed and dried. Following this, every 20 seeds were placed between two filter papers and set to germinate in 90 mm diameter Petri dishes. Seeds were kept to germinate in laboratory under normal light and controlled temperature (25 °C). Each treatment includes 10 Petri dishes in a completely randomized design.

For the next 7 days, filter papers were moistened with 10 ml of distilled water and the number of germinated seeds was counted daily. Based on the result of the higher germination percentage that we have obtained, we fixed the best priming

concentration for each cultivar and we repeated the germination experience for 7 other days (seeds of each cultivar were primed with the concentration which gave the best germination percentage) to choose the best combination between priming duration and concentration for each cultivar. Parameters measured in this stage are given below:

Total germination (GT) was measured on the final days using the formula GT (%) = (total number of germinated seeds/ total seed) x 100.

Mean germination time (MGT) was calculated according to the equation: MGT = Σ Dn / Σn (Moradi *et al.*, 2008), where (n) is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination.

The germination Index (GI) was calculated as described in the Association of Official Seed Analyst (1983) by following formula: GI = ∑ (Gt / Tt) where Gt is the number of seeds germinated on day t and Tt is the number of days. The coefficient of velocity (CV) was calculated using the following formula (Scott *et al.*, 1984): CV= 100 [∑Ni / ∑Ni Ti]. All the data were subjected to an analysis of variance using SPSS 13.0 software and the difference between means were compared by a Duncan multiple range test at 5% level of probability (Table1). For each cultivar, every studied parameter (MGT, GI, and CV) is compared alone (Duration and priming solution are the variables). Means of the same column, followed by the same letter, are not significantly different.

3 RESULTS AND DISCUSSIONS

According to the results, all studied traits were affected by the experimental factors. In fact, there was difference between control seeds (soaked in distilled water) and primed seeds. The highest final germination percentage obtained for 'Beldi' cultivar (95 %) belonged to KCl priming (10 mM) at 36 hours (Figure 1). Concerning 'Baklouti' cultivar, the highest final germination percentage (91 %) belonged to $CaCl_2$ priming (10 mM) at 36 hours (Figure 5) and finally the highest final germination percentage for 'Anaheim Chilli' cultivar (97 %) belonged to NaCl priming (50 mM) at 24 hours (Figure 9). In general, priming with KCl, $CaCl_2$ and NaCl proved superiority over control but it depends on variety, duration and also concentration of priming agent (Figure 1-9). For example, in 'Beldi' cultivar, we obtained 95 % of germination for KCl primed seeds with 10 mM at 36 hours but germination regressed to 65 % when osmotic potential increased to 20 mM (for the same duration). The three cultivars responded differently to priming agent but also to duration and concentration. The same result was obtained by using $CaCl_2$ priming in 'Baklouti' cultivar; the germination percentage of primed seeds decreased from 91 % to 60 % by increasing solution concentration from 10 mM to 20 mM when seeds were soaked for 36 hours.

Priming with KCl, $CaCl_2$ or NaCl in pepper seeds had better effects on seed germination compared with control seeds. These ameliorations in primed seeds might be due to pre-germinative metabolic activities which prepare seeds for radicle protrusion. It can also be the results of metabolic repair processes or osmotic adjustments during priming (Bray *et al.*, 1989).

This good response of seed priming is coherent with the findings of Coolbear and Grierson (1979) who declared that higher germination rate in primed seeds was a result of higher levels of nucleic acid found in tomato cultivars.

According to table 1, the maximum value of coefficient of velocity (CV) was obtained for 'Anaheim Chilli' cultivar (26.8) after soaking seeds for 24 hours in NaCl solution (50 mM). For 'Beldi' cultivar, the maximum value of coefficient of velocity (21, 5) was obtained after soaking seeds for 36 hours of in KCl solution (10 mM). Finally, for 'Baklouti' cultivar, the highest coefficient of velocity (20, 8) was obtained after soaking seeds for 36 hours in $CaCl_2$ solution (10 mM). Priming affects the lag phase in germination and causes early DNA replication (Bray *et al.*, 1989), it increases RNA and protein synthesis (Fu *et al*, 1988), makes greater ATP availability (Mazor *et al.*, 1984) and repairs deteriorated seed parts

(Shaha, 1990). Also, the must ameliorative effect of priming should be the repair of damaged DNA. Primed seeds have more time to complete the process of repair because of water uptake is slower in priming (Varier et al., 2010).

According to table 1, germination index (GI) was the highest for 'Anaheim Chilli' cultivar (4,93) after soaking seeds for 24 hours in NaCl solution (50 mM). Then, germination index was the highest (4, 4) for 'Baklouti' cultivar after soaking seeds for 36 hours in $CaCl_2$ solution (10 mM).

Concerning 'Beldi' cultivar, the best germination index (4,1) was obtained after soaking seeds for 36 hours of in KCl solution (10 mM). According to Soughir *et al.* (2012), higher value of germination index in fenugreek primed seeds indicate a better vigor of seeds than control. Ruan *et al*. (2002) demonstrated that KCl and $CaCl_2$ seed priming improved germination index of rice. Also, Sadeghi *et al*. (2011) showed that osmopriming increased total germination percentage, germination index while decreased mean germination time in soybean seeds.

The shortest mean germination time (MGT) was 3,72 days and it was obtained for 'Anaheim Chilli' cultivar (Table 1) after soaking seeds for 24 hours in NaCl solution (50 mM). The best MGT obtained for 'Beldi' cultivar was 4,65 days and that was obtained after soaking seeds for 36 hours of in KCl solution (10 mM). For 'Baklouti' cultivar, the least MGT (4,81) was obtained after soaking seeds for 36 hours in $CaCl_2$ solution (10 mM). Yamauchi and Winn (1996) found that seeds priming broke down dormancy which resulted in an earlier emergence. This earlier emergence may be related to a rapid water uptake comparing to the control treatment. Similarly, Kaya *et al*. (2006) signaled that primed seeds had more rapid water uptake than control seeds in sunflower. Priming improves mean germination time by accelerating imbibition, which facilitate the emergence phase and the rapid multiplication of radicle cells. Rapid germination in primed seeds can be due to the increasing activity of the degrading enzymes, such as α-amylase, synthesis of RNA DNA, ATP and the number of mitochondria.

It becomes evident that priming increase free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase in seeds (Afzal *et al.*, 2006). The values of total germination mean germination time, coefficient of velocity and germination index were better than in control seeds. These results are consistent with the work of several researchers (Soughir *et al*., 2012; Elouaer and Hannachi, 2012).

We conclude that the use of priming technique can enhance the germination of pepper seed under normal or under saline conditions. Nevertheless, beneficial effects of priming for later growth and development stages of plants remain unclear (Farhoudi and Sharifzadeh, 2006). In fact, Passam and Kakouriotis (1994) reported that benefits of NaCl priming did not persist beyond the seedling stage in cucumber. So, additional work is needed to evaluate the effect of seeds priming on early seedling growth of these plants under field conditions.

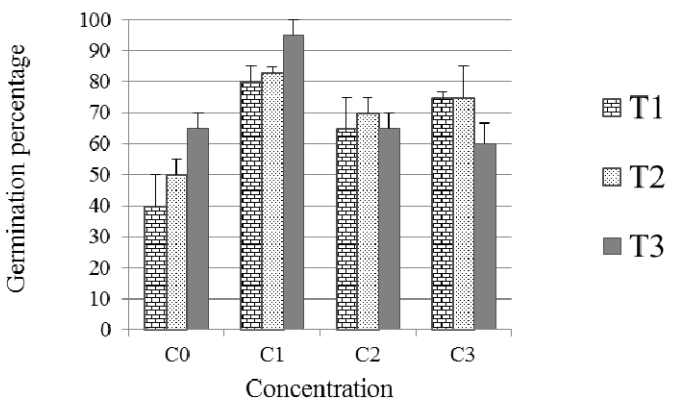

Figure1: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of KCl priming agent on the germination percentage (%) of 'Beldi' cultivar.

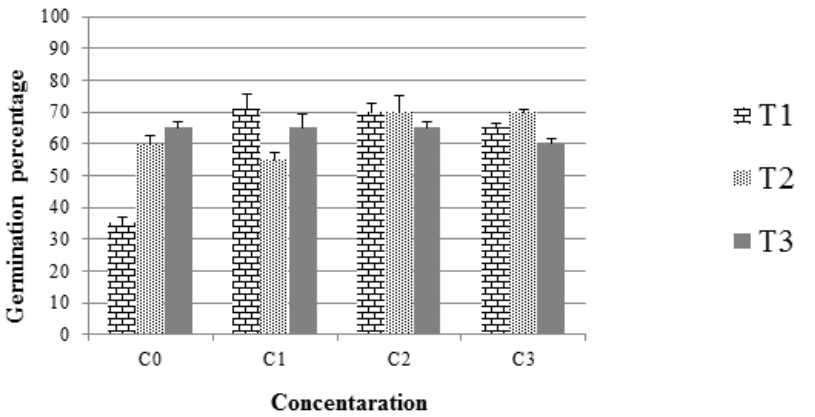

Figure 2: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of KCl priming agent on the germination percentage (%) of 'Baklouti' cultivar.

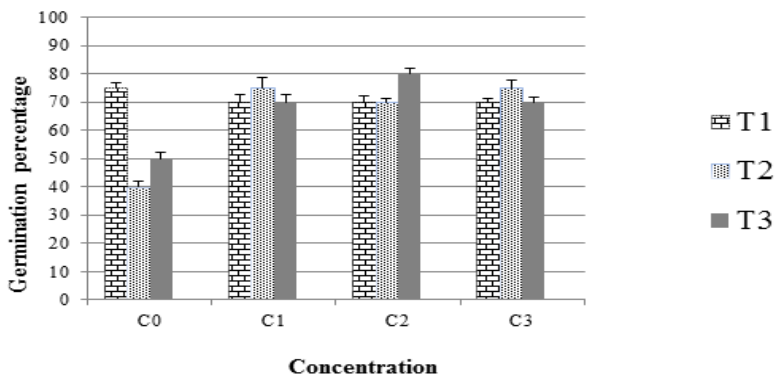

Figure 3: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of KCl priming agent on the germination percentage (%) of 'Anaheim Chilli' cultivar.

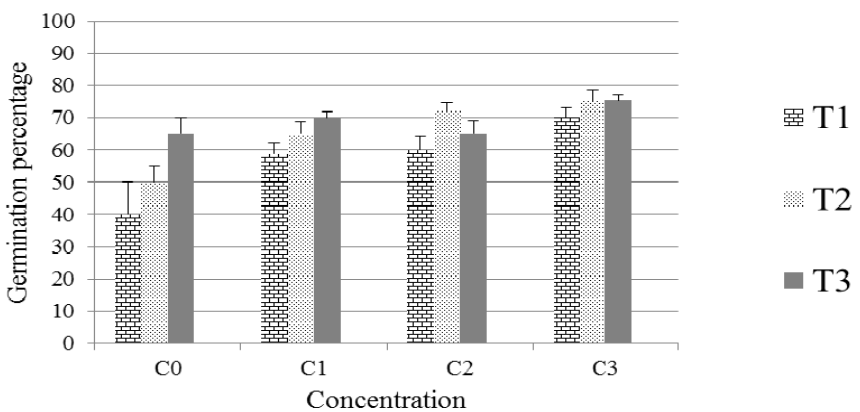

Figure 4: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of $CaCl_2$ priming agent on the germination percentage (%) of 'Beldi' cultivar.

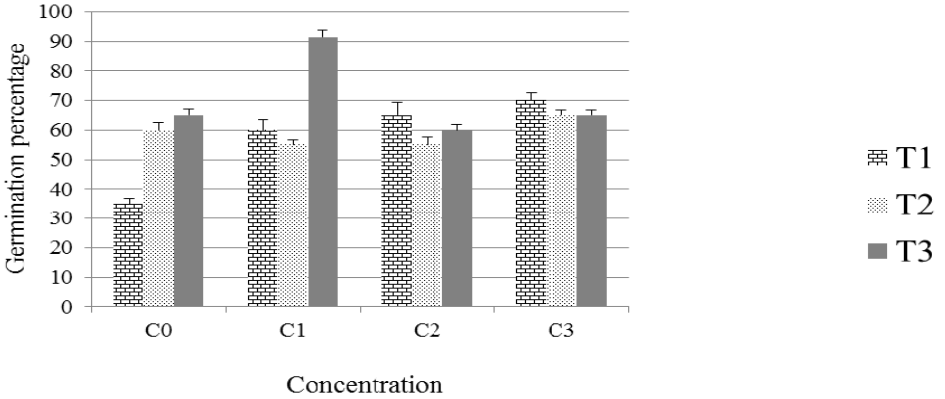

Figure 5: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of $CaCl_2$ priming agent on the germination percentage (%) of 'Baklouti' cultivar.

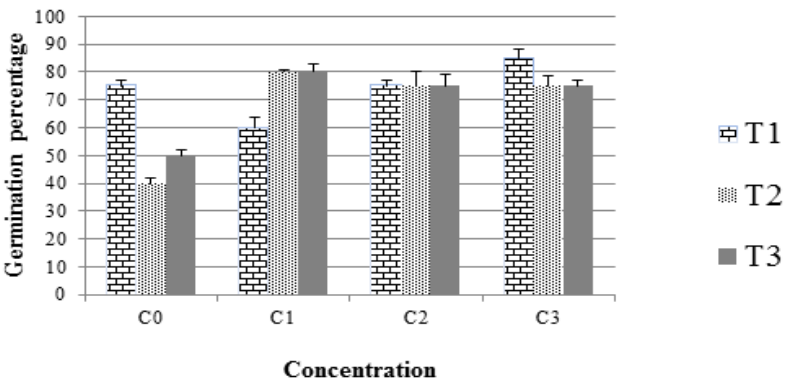

Figure 6: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of $CaCl_2$ priming agent on the germination percentage (%) of 'Anaheim Chilli' cultivar.

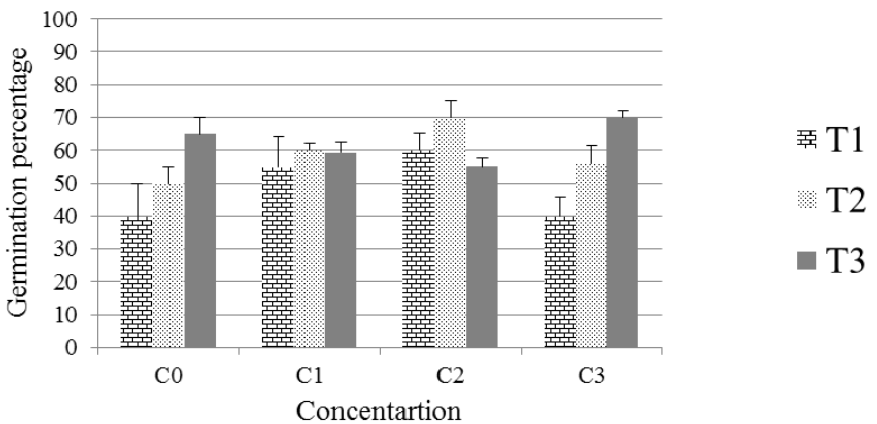

Figure 7: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of NaCl priming agent on the germination percentage (%) of 'Beldi' cultivar

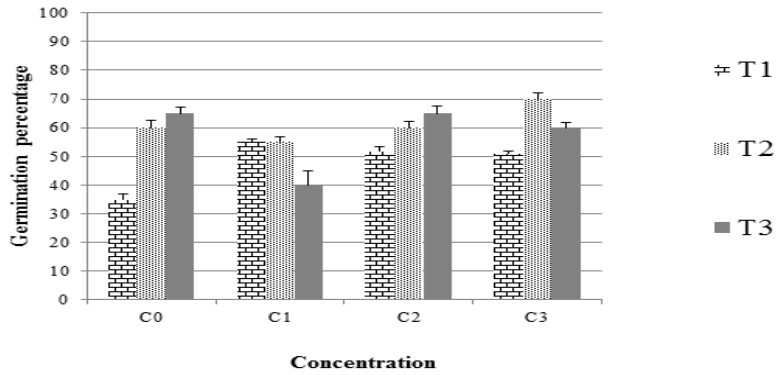

Figure 8: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of NaCl priming agent on the germination percentage (%) of 'Baklouti' cultivar

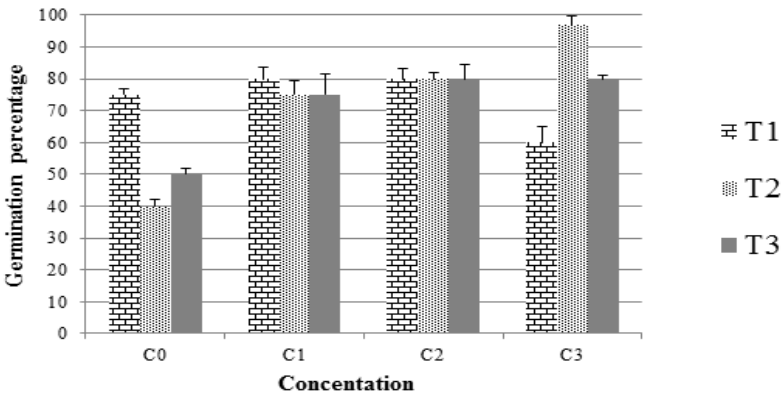

Figure 9: Role of duration (T1= 12h; T2 =24h; T3 = 36h) and concentration (C0= control; C1=10 mM; C2=20 mM; C3= 50 mM) of NaCl priming agent on the germination percentage (%) of 'Anaheim Chilli' cultivar

Table1: Effect of priming treatment with KCl, $CaCl_2$ and NaCl on the germination index (GI), Coefficient of velocity (CV) and Mean germination time (MGT) of three pepper cultivars 'Beldi' , 'Baklouti' and 'Anaheim Chilli'

		KCl (10 mM)			$CaCl_2$(10 mM)			NaCl (50 mM)		
	T(hours)	GI	CV	MGT	GI	CV	MGT	GI	CV	MGT
Beldi	12h	2.5^{b}	13.9^{c}	7.19^{a}	2.6^{b}	14.7^{b}	6.80^{a}	2.1^{b}	12.5^{b}	8^{a}
	24h	2.9^{b}	15.6^{b}	6.41^{a}	2.4^{b}	13.8^{b}	7.24^{a}	2.2^{b}	12.8^{b}	7.81^{a}
	36h	4.1^{a}	21.5^{a}	4.65^{c}	2.25^{b}	13.1^{b}	7.63^{a}	2.3^{b}	13.3^{b}	7.51^{a}
	Control	2.45^{b}	14.58^{c}	6.93^{a}	2.45^{b}	14.58^{b}	6.93^{b}	2.45^{b}	14.58^{b}	6.93^{a}
Baklouti	12h	2.8^{b}	15.3^{b}	6.53^{b}	3.1^{b}	16.1^{b}	6.21^{b}	1.9^{c}	11.9^{c}	8.40^{a}
	24h	2.58^{b}	13.8^{c}	7.24^{a}	2.9^{b}	15.3^{b}	6.53^{b}	2.6^{b}	13.1^{b}	7.63^{a}
	36h	2.45^{b}	12.8^{c}	7.81^{a}	4.4^{a}	20.8^{a}	4.81^{c}	2.3^{b}	12.8^{b}	7.81^{a}
	Control	2.85^{b}	14.03^{b}	7.13^{a}	2.85^{b}	14.03^{b}	7.13^{a}	2.85^{b}	14.03^{b}	7.13
Anaheim Chilli	12h	3.2^{b}	14.2^{b}	7.04^{a}	3.3^{b}	18.6b	5.37^{b}	3.2^{b}	17.4^{b}	5.74^{b}
	24h	2.85^{c}	14.5^{b}	6.89^{a}	3.2^{b}	17.4^{b}	5.74^{b}	4.93^{a}	26.8^{a}	3.72^{c}
	36h	2.9^{c}	15.6^{b}	6.41^{a}	3.11^{b}	16.3^{b}	6.13^{a}	3.15^{b}	18.3^{b}	5.46^{b}
	Control	3.16^{b}	18.2^{b}	5.50^{b}	3.16^{b}	18.2^{b}	5.50^{b}	3.16^{b}	18.2^{b}	5.50^{b}

4 CONCLUSIONS

Priming gave better results than control for the three cultivars. This shows that the profit of this method is a general phenomenon. But the best results were obtained for the seeds of "Anaheim Chilli" cultivar which were soaked in a solution of NaCl (50 mM, 24 h). The best combinations that we chose were: KCl priming (10 mM, 36h) for 'Beldi' cultivar, $CaCl_2$ priming (10 mM, 36 h) for 'Baklouti' cultivar and finally NaCl priming (50 mM, 24 h) for 'Anaheim Chili' cultivar. Additional molecular research is needed to explore priming advantages and to confirm these results.

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Different species of basil need different ammonium to nitrate ratio in hydroponics' system

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ABSTRACT

Basil is a very important medicinal plant and culinary spice, and is marketed fresh, dried or frozen. In crop nutrition, nitrogen is essential for plant growth and as a macro-element, is part of the proteins' structure and participates in the metabolic processes involved in the synthesis and energy transfer. It has been shown that a balance between ammonium and nitrate favors plant growth and that the degree of benefit varies among crops. This study was conducted to evaluate the growth of two varieties of basil in function of four nutrient solutions containing different NH_4^+/NO_3^- ratios. Results showed that different variety response differently to nutrient solution. Although the highest yield in both varieties (sweet and purple) was obtained when fed by nutrient solution without ammonium but their response on quality indices were different due to nitrate ammonium ratio in nutrient solutions. The highest total phenol content of sweet and purple basil was 92 and 100 mg gallic acid equivalent per gram of dry weight respectively, while the highest antioxidant capacity was obtained in purple variety grown in nutrient solution 2 (NH_4^+:1/NO_3:4) and the lowest value were related to sweet variety with the same nutrient solution. Moderate content of total nitrogen can be suitable for sweet variety while for purple variety nutrient solution with low amount of ammonium can be more suitable.

Key words: NFT, antioxidant, phenol, vitamin C, chlorophyll, yield

IZVLEČEK

RAZLIČNI BIOTIPI BAZILIKE POTREBUJEJO V RAZMERAH HIDROPONSKEGA GOJENJA RAZLIČNA RAZMERJA NITRATA IN AMONIJA

Bazilika je zelo pomembna zdravilna rastlina in začimba, ki se prodaja sveža, suha ali zmrznjena. V prehrani rastlin je dušik esencialni makroelement, potreben za rast, sintezo beljakovin, druge presnovne procese in pretvorbo energije. Znano je, da ravnovesje med amonijsko in nitratno obliko dušika izboljšuje rast in, da se njuno ugodno razmerje spreminja glede na posamezne rastline. V raziskavi smo ovrednotili rast dveh biotipov širokolistne bazilike (zeleno- in vijoličnolistno) v štirih hranilnih raztopinah, ki so vsebovale različna razmerja NH_4^+/NO_3^-. Rezultati so pokazali različen odziv obeh biotipov na razmerja hranil v raztopinah. Čeprav je bil pridelek obeh (zelene in vijolične) največji v hranilni raztopini z amonijsko obliko dušika, so kvalitetni kazalci variirali glede na razmerja nitrata in amonija. Največja vsebnost celokupnih fenolov v zeleni in vijolični baziliki je bila 92 in 100 mg ekvivalenta galne kisline na gram suhe mase, vendar je bila največja antioksidativna sposobnost dosežena pri vijolični baziliki, ki je rastla v hranilni raztopini 2 (NH_4^+:1/NO_3:4) in najmanjša vrednost pri zeleni baziliki, ki je uspevala v isti hranilni raztopini. Zmerna vsebnost celokupnega dušika je primerna za gojenje zelene širokolistne bazilike, medtem ko je za gojenje vijolične primernejša hranilna raztopina z manjšo vsebnostjo amonija.

Ključne besede: NFT hranilna raztopina, antioksidanti, fenoli, vitamin C, klorofil, pridelek

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1 INTRODUCTION

Among the various medicinal and culinary herbs, some herbs such as basil (*Ocimum basilicum* L.) are of particular interest because they may be used for the production of raw materials or preparations containing phyto-chemicals with significant antioxidant capacities and health benefits (Exarchou *et al*. 2002). Basil is a very important medicinal plant and culinary spice, and is marketed fresh, dried or frozen. Traditionally, basil has been used as a medicinal plant in the treatment of headache, cough, diarrhea and kidney malfunctions (Simon *et al*. 1984), against insect bites, acne (Waltz, 1996), and it has long been used to flavor foods, as well as dental and oral products (Simon *et al*., 1984).

In crop nutrition, nitrogen is essential for plant growth, and is the fourth most abundant element found in plant tissues after carbon, oxygen and hydrogen. As a macro-element, it is part of the proteins' structure and participates in the metabolic processes involved in the synthesis and energy transfer. It is absorbed by the plant roots in the form of ammonium ($NH4^+$) or nitrate (NO_3^-) ions. The form in which N is supplied influences directly the absorption and the rhizosphere pH (Trejo *et al*. 2008). In hydroponics, both nitrate and ammonium forms are used in nutrient solutions. It can be argued that either of the two is beneficial or that either may equally cause growth problems or imbalances in the nutrient solution. It has been shown that a balance between ammonium and nitrate favors plant growth and that the degree of benefit varies among crops (Mengel and Kirkby, 1987).

For most plant species, NO_3^- supply combined with low quantities of NH_4^+ favors growth, but the response depends on the species and the age of the plant. Mengel and Kirkby (1987) reported plant species that grow better when nitrogen is administered as NO_3^- instead of NH_4^+. Reports have indicated that the incorporation of nitrogen in N-NH_4^+ form is toxic for many species, even in low concentrations (Salsac *et al*. 1987).

In other hands the yield depends to a large degree on the content of photosynthetically active pigments. Authors of numerous papers showed a close correlation between the level of these pigments and nitrogen content in leaves determined by the dose and time of fertilization (Baghour *et al*. 2000; Biczak *et al*. 1998; Smith 1999; Swiader and Moore 2002).

Hydroponic systems have consented to improve the cropping management and to reach higher produce quality standards in leafy vegetables (Santamaria *et al*. 2002). However, the identification of optimal soilless practices such as plant density and nutrient solution composition are critical factors in order to increase yield, reduce pathogen incidence, enhance dry matter production, improve antioxidant activity, and reduce nitrate content (Chen et al. 2004; Fravel and Larkin 2002).

This study was conducted to evaluate the growth of two variety of basil in function of four nutrient solutions containing different NH_4^+/NO_3^- ratios to test basil species response to nutrient solution prepared with NO_3^- combined with NH_4^+.

2 MATERIALS AND METHODS

In order to determine, the effect of different nutrient solution on the yield and quality indices of sweet and purple basil in a NFT culture, an investigation was conducted in a PVC greenhouse in 2010 in a completely randomized experimental design with three replications.

Basil seeds were sown in May 2010 and transferred to NFT system after two weeks. Harvesting took place during July to September 2010 (four times). Nutrient solutions (Tables 1-2) were prepared with municipal tap water and delivered to plants by a mist irrigation system.

Yield, dry mater and ash percent, total soluble solid, total acidity, total carotenoid, total phenol, vitamin C and antioxidant capacity of basil leaves were determined.

2.1 Determination of dry matter, total soluble solids, and ash:

the dry matter of samples was determined by drying at 75±5 °C until they reached constant weight (AOAC 1984) and then ashes in electrical oven. Titrable acidity (TA) was measured by the titrimetric method (AOAC 1984).

2.2 Determination of ascorbic acid:

Ascorbic acid was quantitatively determined according to 2, 6- dichlorophenolindophenol dye method (Ranganna 1997). The ascorbic acid of fresh samples (10 g) was extracted by grinding in a suitable medium with a small amount of sand and using 3% metaphosphoric acid (v/v) as a protective agent. The extract was made up to a volume of 100 ml mixed and centrifuged at 3000 g for 15 min at room temperature. Ten ml was titrated against standard 2, 6-dichlorophenolindophenol dye, which was already standardized against standard ascorbic acid. Results were expressed as mg/100 g on fresh weight (FW) basis.

2.3 Determination of total chlorophyll:

Total chlorophyll mg/100g was determined by a modified method of Ranganna (1997) using acetone and petroleum ether as extracting solvents and measuring the absorbance by spectrophotometer.

2.4 Determination of total phenolic compound:

the methanol extracts of basil were used for the determination of total phenolics. Total phenolic content was evaluated by colorimetric analyses using Folin-Ciocaltaue's phenol reagent (Singleton and Rossi, 1965). The content of total phenolics was expressed as mg galic acid equivalent per 100 g of leaves.

2.5 2-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity:

the free radical-scavenging activity against DPPH radical was evaluated according to the method of Leong and Shui (2002) and Miliauskas et al. (2004) with minor modification. According to principle of this method, in the presence of an antioxidant, the purple color intensity of DPPH solution decays and the change of absorbance are followed spectrophotometrically at 517 nm. The scavenging activity was expressed as IC_{50} (mg/ml).

2.6 Nutrient content and nitrate analysis:

Phosphorus, Calcium and magnesium in leaves were measured by spectrometry (JENWAY 6105 U.V/V). Nitrate was measured in following to Humphries (1956).

The resultant data were subjected to analysis of variance using SAS statistical program. Means were separated by Tukey's Multiple Range Test.

3 RESULTS AND DISCUSSION

Due to significant interaction between nutrient solution and variety we are not able to propose a significantly better nutrient solution for both varieties so we discuss only about interaction.

Interaction between variety and nutrient solution on total phenol showed that the highest amount of phenol was obtained in purple variety with nutrient solution 2 (NH_4^+:1/NO_3:4) and the lowest value were related to sweet variety with the same nutrient solution (NH_4^+:1/NO_3:4). This variety show the highest phenol content when fed with nutrient solution 3 (NH_4^+:0.5/NO_3:4). These values were significantly different from other values (figure 1).

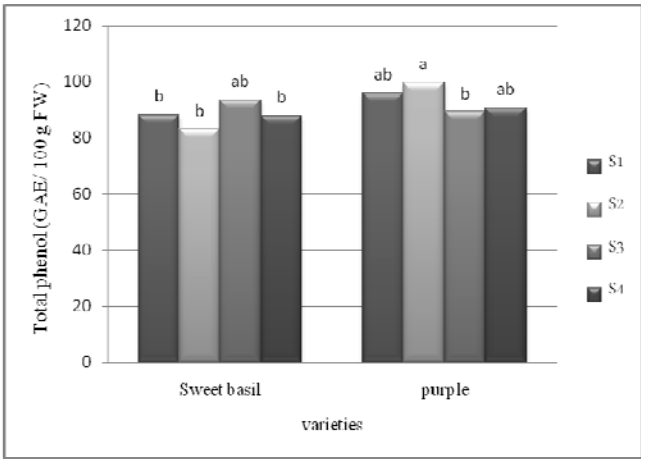

Figure 1: Nutrient solution and variety interaction on basil total phenol (GAE: Gallic acid equivalent)

The highest total phenol content of sweet and purple basil was 92 and 100 mg gallic acid equivalent per gram of dry weight of basil leaves, respectively. The highest value in this recent study was higher than the value (51.1 mg gallic acid equivalent per gram of dry weight) reported by Juliani and Simon (2002). These results were not doubtful because phenolic compounds in plant foods are largely influenced by genetic factors and environmental conditions including nutrition condition (Bravo, 1998). The difference in phenolic content could affect the antioxidant capacity of plants, because many phenolic compounds in plants are good sources of natural antioxidants (Amiot *et al*. 1997; Ho 1992).

The highest number of antioxidant capacity was obtained in purple variety with nutrient solution 2 (NH_4^+:1/NO_3:4) and the lowest value were related to sweet variety with the same nutrient solution. This variety showed the highest antioxidant capacity when fed with nutrient solution 3 (NH_4^+:0.5/NO_3:4) and 4 (NH_4^+:0/NO_3:5) (figure 2).

Antioxidants, including vitamin C, carotenoid, phenols and chlorophyll, are responsible for the level of antioxidant capacity of a leaf extract. Thus, enhanced amounts of the biologically active reduced form of antioxidants can be indicative of an increased antioxidant capacity, i.e. the healthy properties of basil leaves extracts (Sgherri *et al*., 2010).

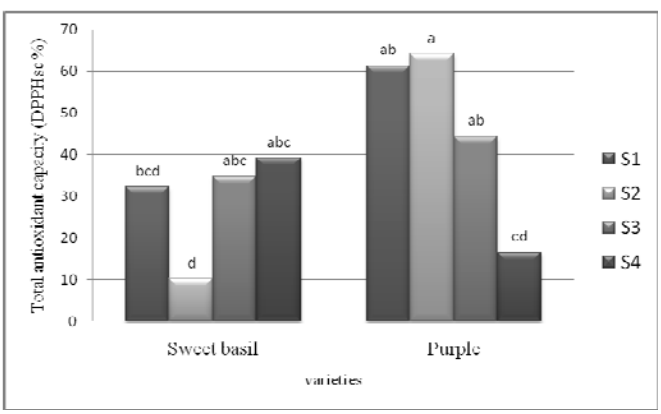

Figure 2: Nutrient solution and variety interaction on basil antioxidant capacity

Interaction between variety and nutrient solution on total chlorophyll showed that the highest chlorophyll was obtained in purple variety with nutrient solution 3 (NH_4^+:0.5/NO_3:4) but nutrient solution did not affect sweet basil total chlorophyll (figure 3). Nutrient solution has influenced plant

biochemical characteristics such as concentrations of photosynthetic pigments. Surprisingly sweet and purple varieties total chlorophyll was similar in spite of their different color.

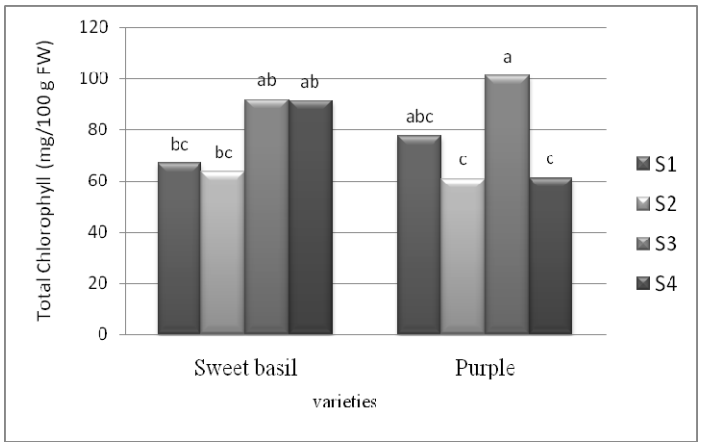

Figure 3: Nutrient solution and variety interaction on total chlorophyll content in fresh basil

Interaction between variety and nutrient solution on Mg, P and Ca content showed that the highest level of these elements in purple variety was obtained in plants that were feed on with nutrient solution 3 (NH_4^+:0.5/NO_3:4), while the highest level of Mg and Ca in sweet basil was obtained in plants that were feed on with nutrient solution 4 (NH_4^+:0/NO_3:5). On the other hand the highest level of P was obtained when the sweet basil was feed on with nutrient solution 2 (NH_4^+:1/NO_3:4).

Interaction between variety and nutrient solution on yield showed that the highest yield was obtained in purple and sweet variety when they were grown in nutrient solution 4 (NH_4^+:0/NO_3:5) and the lowest value were related to sweet variety with nutrient solution 2 (NH_4^+:1/NO_3:4) (figure 7). For most plant species, an $NO3^-$ supply combined with low quantities of $NH4^+$ favors growth, but the response depends on the species and the age of the plant. Mengel and Kirkby (1987) reported plant species that grow better when nitrogen is administered as $NO3^-$ instead as $NH4^+$. Reports have indicated that the incorporation of nitrogen in N-$NH4^+$ form is toxic for many species, even in low concentrations (Salsac *et al* 1987). A positive response was also observed when basil plants were fed with both nitrogen sources (NH_4^+ and NO_3^-). This coincides with Zornoza et al. (1988), who found that sweet peppers increased their yield for 10% when they were fertigated with a nutrient solution containing 80/20 (NO_3^-/NH_4^+) compared to the fertigation with NO_3^- as the only source of nitrogen in the nutrient solution. Goyal et al. (1982) and Xu et al. (1992) also stated that plant growth is notably favored when treated with both forms of nitrogen, but it is needed to find the optimal nitrate/ammonium ratios for each crop.

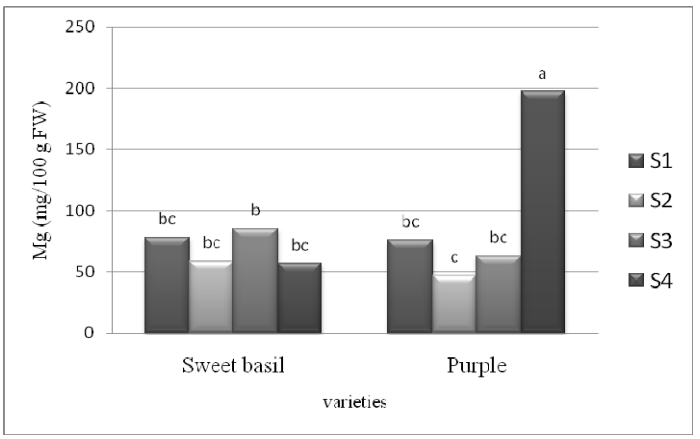

Figure 4: Nutrient solution and variety interaction on Mg content in fresh basil

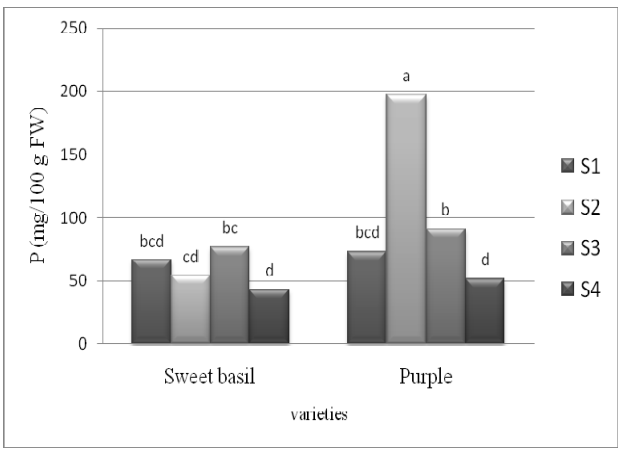

Figure 5: Nutrient solution and variety interaction on P content in fresh basil

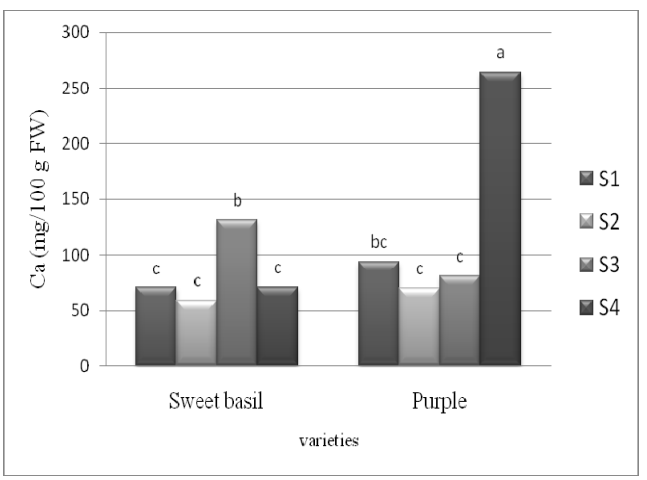

Figure 6: Nutrient solution and variety interaction on Ca content in fresh basil

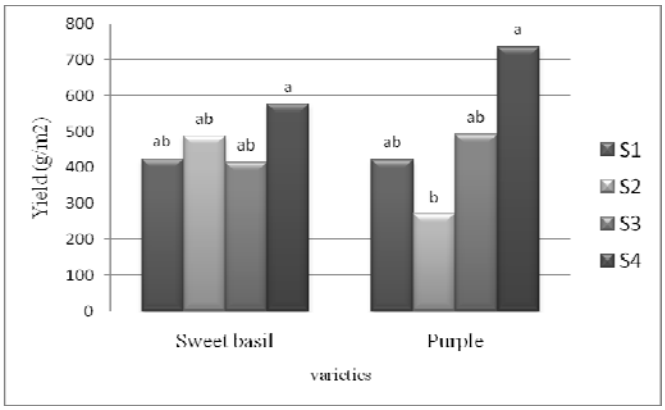

Figure 7: Nutrient solution and variety interaction on basil yield in g/m^2

Interaction between variety and nutrient solution on ash showed that the highest ash was obtained in purple variety with nutrient solution 4 (NH_4^+:0/NO_3:5) and the lowest value were related to sweet variety with nutrient solution 2 (NH_4^+:1/NO_3:4). This variety showed the highest ash content when fed with nutrient solution 3 (NH_4^+:0.5/NO_3:4). These values were significantly different from other values (figure 8).

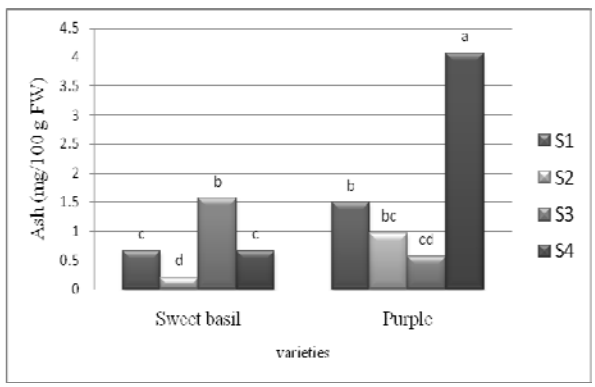

Figure 8: Nutrient solution and variety interaction on basil ash

Interaction between variety and nutrient solution on vitamin C content showed that the highest vitamin C was obtained in purple and sweet variety with nutrient solution 3 (NH_4^+:0.5/NO_3:4) and 1 (NH_4^+:1.5/NO_3:3.5) respectively (figure 9).

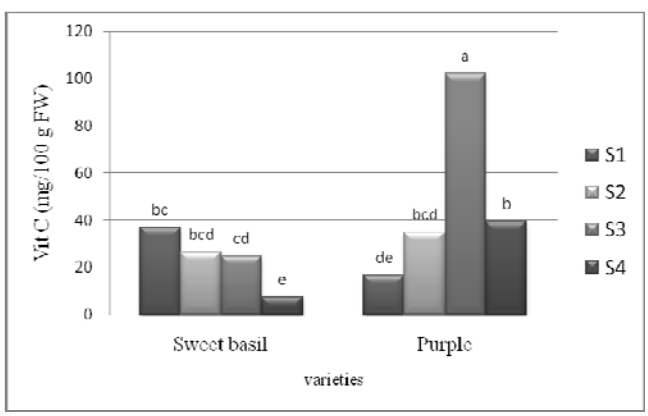

Figure 9: Nutrient solution and variety interaction on basil vit. C

Interaction between variety and nutrient solution on nitrate accumulation showed that the highest and the lowest nitrate accumulation was obtained in both varieties with nutrient solution 4 (NH_4^+:0/NO_3:5) and 1 (NH_4^+:1.5, NO_3:3.5) respectively (figure 10).

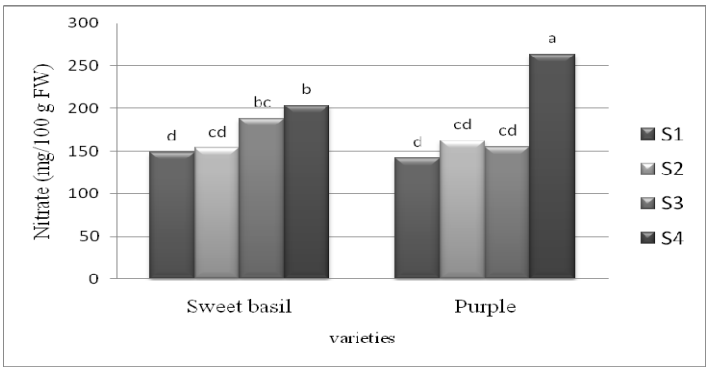

Figure 10: Nutrient solution and variety interaction on basil Nitrate

4 CONCLUSION

Results indicated that sweet basil that have green color and high chlorophyll content responded not significantly to ammonium content in nutrient solution on yield and some other indices while nitrate accumulation is a big problem when this variety irrigated with high nitrogen content nutrient solution so a moderate content of total nitrogen such as nutrient solution 2 or 3 can be suitable for this variety cultivation. In purple variety with lower amount of chlorophyll NO3:NH4 ratio is important. Nutrient solution with low amount of ammonium such as nutrient solution 3 or 4 can be more suitable for this variety in soilless culture.

Table 1: Macronutrients used in nutrient solutions.

	Meq/l										
Nutrient solution	KNO_3	KH_2PO_4	K_2SO_4	NaCl	$CaNO_3$	$MgSO_4$	$MgHPO_4$	NH_4NO_3	$CaSO_4$	SO_4	NO_3
1	1	1.5	2	0.1	1	0.5	1.5	1.5	1.5	4	3.5
2	2	1.5	1	0.1	1	0.5	1.5	1	1.5	3	4
3	2	1.5	1	0.1	2	0.5	1.5	0.5	0.5	3	4
4	3	1.5	0	0.1	2	0.5	1.5	0	0.5	1	5

Table 2: Micronutrient used for nutrient solution preparation.

Compound	Mg/l Irrigation solution
(NH4) 6Mo 7O2/4H2O	0.1
H3BO3	1.5
MnSO4/4H2O	2
CuSO4/5H2O	0.25
ZnSO4/7H2O	1
Sequesteren Fe 136	10

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Agris category code: p01,f40,e90

Dependence of the conservation status of acid grasslands at the Pohorje and Kozjak on socioeconomic parameters

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ABSTRACT

Grassland habitats were studied on twenty farms on the area of the Radlje ob Dravi administration unit, in the transect from Kozjak to Pohorje at different altitudes. The aim of the study was to investigate how environmental and socio-economic parameters influence the diversity of plant species and, consequently, the conservation of grassland on acid soils, which are rare in Slovenia and are therefore more protected. The socioeconomic structure of farms was studied on the basis of an inquiry carried out on farms. Part-time farms prevail; the average age of farmers is 56.5 years, and 30% of farmers has no education or just elementary school. The relationship among the environmental, socio-economic parameters and floristic structures of grasslands was studied using canonic-correspondence analysis. The impact of 16 parameters was analysed, of which six were determined not to be statistically significant. The occurrence of chosen plant species was analysed in relation to environmental and socioeconomic parameters. The efficiency of agro-environmental subsidies in relation to plant species diversity was evaluated. It was determined that the education and age of farmers influence the intensity of farming and consequently have an impact on the diversity of plants species and the conservation status of grasslands.

Key words: grasslands, habitats, biotic diversity, plant species, socio-economic structure of farms, Kozjak, Pohorje, Slovenia

IZVLEČEK

ODVISNOST OHRANJENOSTI KISLIH TRAVIŠČ NA POHORJU IN KOZJAKU OD SOCIOEKONOMSKIH PARAMETROV

V nalogi proučujemo traviščne habitate na območju Upravne enote Radlje ob Dravi, na dvajsetih kmetijah, na območju Pohorja in Kozjaka in na različnih nadmorskih višinah. Namen naloge je bil proučiti ali socioekonomski dejavniki vplivajo na diverziteto rastlinskih vrst in posledično s tem na ohranjenost travišč, predvsem travišč na kisli podlagi, ki so redkejša in zato še posebej varovana. Na podlagi izvedbe anket na teh kmetijah smo proučili socioekonomsko strukturo kmetij. Prevladujejo mešane kmetije, povprečna starost gospodarjev je 56,5 let in kar 30 odstotkov gospodarjev je brez izobrazbe ali imajo končano osnovno šolo. Z metodo kanonične korespondenčne analize (CCA) smo poskušali ugotoviti, kateri ekološko- socioekonomski dejavniki so najbolj povezani z vrstno sestavo travišč. Določili smo šestnajst spremenljivk, pri čemer je bilo osem statistično neznačilnih. Proučili smo povezanost ekološko-socioekonomskih dejavnikov s pojavljanjem rastlinskih vrst. V raziskavi smo ovrednotili učinkovitost kmetijsko okoljskih plačil na raznolikost rastlinskih vrst. Ugotovili smo, da socioekonomska dejavnika izobrazba in starost gospodarjev kmetijskih gospodarstev vplivata na intenzivnost kmetovanja in posledično s tem na raznolikost rastlinskih vrst in ohranjenost travišč.

Ključne besede: travišča, habitati, biotska raznovrstnost, rastlinske vrste, socio-ekonomska struktura kmetij, Kozjak, Pohorje

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Prispevek je del magistrskega dela interdisciplinarnega podiplomskega študija Varstva okolja Univerze v Ljubljani: KETIŠ, Karmen. *Vpliv gospodarjenja s travišči na naravovarstveno vrednost teh habitatov v upravni enoti Radlje ob Dravi : magistrsko delo = Impact of the grasslands management on their natural protection value in the Radlje ob Dravi administrative unit : M. Sc. thesis*. ; mentor: prof. dr. Franc Batič, somentor: prof. dr. Andrej Udovč.

1 INTRODUCTION

Animal husbandry is the most significant agricultural discipline in Slovenia due to environmental circumstances, and grasslands represent the major part of agricultural land. The orientation toward animal husbandry is even narrower in comparison with crop production. One key factor in this is the size structure of farms, which is more suitable for labour- and income-intensive animal husbandry farms than for specialized crop production or mixed crop-animal husbandry production. The consequence of the inappropriate size structure of farms in Slovenia is also reflected in their socio-economic structure. Part-time farms prevail; farms are too small to enable income solely from agricultural activity. Due to these unfavourable conditions, small farms seek income in labour-intensive production activities, such as cattle rearing and milk production (Hrustel Majcen, 2005).

Problems in the area of agriculture and environment are connected with the abandonment of agriculture in marginal areas and with the intensification of activity in lowlands, soil degradation and erosion, eutrophication due to fertilisation and the use of pesticides in agro-ecosystems, regulation of water regime, irrigation and loss of buildings due to abandonment and decay (Pregled …, 2001).

Vrišer (2005) stresses that size structure of farms represents the most important factor of socio-economic basis in the agricultural economy. The type of agricultural activity depends largely on this parameter, which also determines the social role of agriculture. The inappropriate size structure of farms does not allow proper income, what is then often a source of discontent of poorer farmers as also a cause for their social activity.

The essential problem of Slovenian farms is their small size, which is additionally enhanced by the high level of fragmentation of areas in agricultural land use. The consequences of the unfavourable size structure of Slovenian farms are also reflected in their socio-economic structure (Cunder, 2002). As already stated, part-time farms prevail, because farm enterprises are too small to provide income solely from agricultural activity.

With an average size of 6.5 ha, Slovenia has some of the smallest average farm sizes in the world (Poročilo …, 2009).

Numerous areas, critical for habitat conservation and biotic diversity, are linked to agricultural land use areas. In the past agricultural land use caused the development of habitat types not existing in pristine nature (Zechmeister et al., 2003).

The fragmented structure of agricultural and forestry land use contributes to high biotic diversity, conservation of habitats and to high natural and landscape diversity. The fragmentation of units of land use types conserves a mosaic structure of the landscape in which semi-natural areas are interwoven with croplands. In natural areas of high value, it is necessary to conserve the fragmentation of units of land use by subsidies and thereby help farmers to equalise economic lost, caused by these structural circumstances (Program razvoja …, 2007).

Batič et al. (2002) emphasised that agricultural activity changes natural ecosystems and decreases biotic diversity but also creates new habitats and increases biotic diversity. The increase or decrease of biotic diversity is dependent on the type of agricultural activity, which depends on natural pedoclimatic circumstances and on socio-economic relations. In general, high intensity agriculture decreases biotic diversity while extensive agriculture increases biotic diversity. Intensive agricultural activity, linked to the chemicalisation of the environment and aspiration for higher yields turned out in several occasions to be unacceptable, but it simultaneously enabled the growth and spread of humanity on the Earth. Due to natural conditions, partly also with them linked agricultural praxes and socio-economic circumstances, the intensity of agriculture is generally low in Slovenia, with the exception of some lowlands and areas under intensive use, e.g. orchards and vineyards, in comparison to other developed countries of the European Union. This is reflected in several fields and in Slovenia's biotic diversity, which is quite high with regards to the small size of the country.

The abandonment of grassland use and encroachment with shrubs and pioneer forest, in progress for more than fifty years, is a consequence of soil impoverishment in nutrients and therefore lower productivity potential, expressed as lower productivity of grasslands and lower fodder quality. Connected to the impoverishment of soil fertility are changed socio-economic circumstances for living in such areas. In the structure of land use, cultivated agricultural lands comprise only 25% of all agricultural lands in Slovenia. The proportion of permanent grasslands in use comprises 60%; this has remained unchanged for last 50 years. In this time, many fields were transformed to meadows and pastures, and former pastures were transformed to shrublands and pioneer forest, which according to the land management law already belonged to the forest sector (Vidrih, 2007).

Zechmeister et al. (2003) and Strijker (2005) warned about inefficient, overly general agricultural-environmental measures that have been implemented in order to conserve grassland habitats from the state policies. Greater flexibility according to local natural and socio-economic circumstances should be necessary in taking and formulating these measures, in addition to other recommendations by Critchley et al. (2003) and Kleijn et al. (2001).

The role of agriculture in environment management has been drastically changed with technological development and socio-economic changes in that this discipline is evaluated as the major threat to global biodiversity and stability of ecosystems (Sala et al., 2001).

Areas significant for species and habitat conservation are linked to agricultural land use. Agriculture can positively or negatively influence conservation and the sustainable use of components of biotic diversity. Many investigations in this field, carried out throughout the world, show how direct activities in agriculture, such as fertilisation, mowing technologies, pasture regime, etc., influence grassland habitats. Several studies dealing with the evaluation of efficiency of agricultural-environmental measures give different conclusions. Critchley et al. (2003) in Great Britain, Paschlod et al. (2005) in Sweden, and Taylor & Morecroft (2008) in south England concluded that agro-environmental schemes contributed to the conservation of grassland species diversity. In contrast, Bakker & Heerdt (2005) concluded that agro-environmental measures in the Netherlands had no positive effect on the conservation of grasslands. There are fewer investigations dealing with the connection between nature conservation and socio-economic parameters. Some investigations of this kind were carried out by Zechmeister et al. (2003), Paschlod & WallisdeVries (2002) and Wytrzens & Mayer (1998). There are few similar studies for Slovenia; exceptions are that of Plajnšek (2005) for the district of Goričko, showing some correlation between the socio-economic characteristics of farms and conservation status of critical habitats for nature conservation and Žgavec et al. (2013) for the Radensko polje, which also pointed to certain unefficiency of implemented socio-economic measures on conservation status of grassland habitats.

Studies carried out in Germany revealed how changes in land-use type drastically decreased grassland habitats in limestone, sandstone and wet grasslands (Poschlod et al., 2005). The abolition of fertilizers in ecological farming in Netherlands decreased the productivity and fodder quality of pastures but increased their species diversity (Bakker & Heerdt, 2005). The effects of increased use frequency of grassland habitats and problems after the new decrease of their use in the 1963–2003 period are shown in the study of Hodgson et al. (2005).

There are many fewer holistic studies, which would include all factors influencing the condition of grassland habitats. According to Poschlod et al. (2005), the socio-economic factors are the most important. The linkages between nature conservation and socio-economic factors are highly complex, in the majority indirect, and therefore difficult to evaluate, but necessary and urgent for understanding the changes in the field, as emphasised by Poschlod et al. (2005). In this aspect, investigations of Zechmeister et al. (2003), Poschlod & Wallis deVries (2002) and Wytrens & Mayer (1998) are rare exceptions.

Grasslands on acid soils are very rare in Slovenia due to the prevailing limestone bedrock and

therefore are under special protection. Among such grasslands are those on the Pohorje and Kozjak mountain range. The nature conservation value of those habitats depends on type of land use, and their protection is not possible merely by administration acts but only with prescribed land use, in which the socio-economic parameters connected to farmers' populations plays a significant role.

In this study, we wanted to evaluate the connections among the size and socio-economic structure of farms, the educational and age structure of farm holders and the intensity of farming. We were also interested to what extent agro-environmental payments contribute to plant species diversity and conservation of habitat types and what the relationship between the floristic composition of grasslands and socio-economic parameters is.

2 MATERIAL AND METHODS

The study was carried out on farms chosen in the transect over the Drava valley, from Kozjak to Pohorje, within the Radlje ob Dravi administration unit. Productional orientation of farms is in the majority of farms in animal husbundry, fodder production and supplemented farming. Natural circumstances supporting agriculture enable cattle rearing for milk and meat and sheep and goat rearing. Position and exposition of grasslands supports both types of use, e.g. grazing and mowing. Whole area of the Radlje ob Dravi administration unit belongs to the areas in which natural circumstances limit agricultural activity (LFA areas), in which about 80% of farms belongs to mountainous and steep areas. Use of agricultural mechanisation is limited in these farms due to the high altitude, steepness of the terrain, remoteness of grasslands from main farm buildings.

According to agricultural census from 2010 (Popis, 2002; www.stat.si) were in the area 823 farms in total, from which 511 or 62% were specialized in animal husbandry. From this we selected twenty farms of different types, e.g. with intensive production, to extensive production to farms having already abandoned agricultural land. In the choice of farms, other parameters were also considered in order to include the entire variation of demographic and socio-economic circumstances to the greatest extent possible. With this choice, we obtained gradients of the factors that impact on state of grasslands.

The study was limited to grasslands. The first issue in the choice of farms was altitudinal position of farms on both banks of the River Drava. On the left bank, on the slopes of Kozjak, there were farms from the valley (ca. 300 m above sea level), on the first terrace above the river to 700 m of altitude. On the right bank, on the slopes of Pohorje, the transect of farms extended to 1000 m a.s.l. Data about farms were obtained by interviews on twenty farms chosen in the transect; characteristics of grasslands' floristic composition were obtained by mapping plants on grasslands (meadows, pastures) belonging to the analysed farms, before the first mowing in spring.

With inquiries among farm holders, in addition to environmental parameters (altitude, steepness, exposition and soil characteristics, also we analysed socio-economic and production characteristics of farms (ownership and size of farms, kind and manner of land use, history of land use, technology of grassland management (grazing, mowing, fertilisation, use of mechanisation, application of agro-environmental measures).

The inquiry was of a combined type with 49 questions, some with sub-questions.

The inquiry was carried out at the end of August 2009. Each interview lasted from one half hour to two hours; so one to four interviews were done per day. Data were analysed by methods of descriptive statistics and use of MS Excel; they are presented in graphs and tables.

Data on grassland mapping were processed by CCA statistical analysis. For elaboration of the figures, the programme CANOCO 4.5 for Windows was used (Braak and Šmilauer, 2002). The obtained results are presented in ordination graphs.

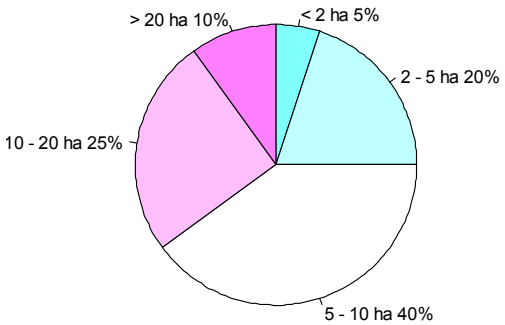
3 RESULTS AND DISCUSSION

3.1 Size structure of farms

Farmers cultivate their own land and hired land, some farms also rent their land out. In the area analysed, more than half of agricultural land is farmers' property (65%) but only 3% of land is hired. Land rented to other farmers was not detected.

Analysis of the size structure of agricultural lands showed that majority (40%) of the farms researched belonged to the medium size class, between 5 and 10 ha. The proportion of small farms (to 2 ha) is 5%. There is 10% of big farms (over 20 ha) in the analysed area (Fig 1). Sizes of the researched farms are comparable to average farm sizes in Slovenia, according to the state inquiry carried out in 2000 (5.3 ha), in which 75% of farms are slightly bigger than 5 ha (Popis.., 2002).

A total of 7% of farms are classified in montane belt, 5% to hilly and 5% to steep areas; only 15% of farms are not classified to areas considered less favourable for agriculture (LFA).



Slika 1: Delež anketiranih kmetij v letu 2009 po velikostnih razredih
Figure 1: The proportion of farms surveyed in 2009 by size classes

The biggest part of the researched farm lands is forest: 68% (450.7 ha). The majority cultivate their own land, and only a smaller portion of the land is hired. The proportion of meadows is 16% (106.4 ha); the proportion of fields, gardens and pastures is slightly above 14% (103.0 ha). Orchards comprise about 1% of the entire area (8.5 ha) (Table 1). Each farm still possesses a high stem orchard meadow, meaning that traditional land use still exists and that farmers are aware of it. Ninety per cent of these orchards are well tended, the remainder belonged to abandoned farms and were not tended. The majority of these orchards are used for supplying fruits to farmers; they are usually smaller than 10 acres and are classified as meadows. In the analysed area, there were no agricultural land rented to other people.

Preglednica 1: Zemljišča anketiranih kmetij in njihova raba v letu 2009
Table 1: Agricultural areas of the surveyed farms and their use in 2009

Land categories	Property (ha)	Hired (ha)	Rented (ha)	Together (ha)
Fields and gardens	22.4	39.3	0.0	61.7
Orchards	8.5	0.0	0.0	8.5
Meadows	94.9	11.5	0.0	106.4
Intensive pastures	0.0	0.0	0.0	0.0
Pastures	29.5	3.3	0.0	32.8
Forests	450.7	0.0	0.0	450.7
Together (ha)	**606.0**	**54.1**	**0.0**	**660.1**

Uncultivated land comprises 4.6 ha (slightly more than 2%). The reason for the lack of cultivation is inappropriate terrain for machinery or excessive distance from the farm.

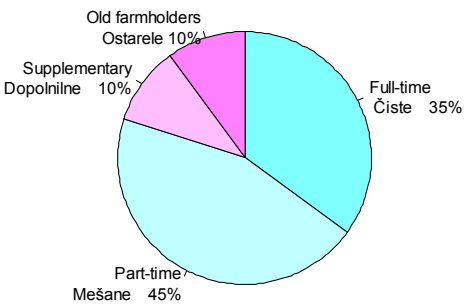
The farmers researched possess 7.8 ha of agricultural land on average. All cultivated their own land, on average 10.5 ha per farm. On average, the agricultural land of their farm consisted of seven pieces, (ranging from one to 19 pieces), mostly due to the configuration of the terrain.

3.2 Socio-economic characteristics if inquired farms

The socio-economic type of farm shows from which sources farms earn income, and also how much of the income is from agricultural activity (Kovačič, 1996).

Criteria for socioeconomic types of farms were taken from Udovč et al. (2006).

Part-time farms prevail among the researched farms (Fig. 2) and there are nine such farms, accounting for 45% of the total; seven farms are full-time, earning all income by farming, (35%), 20% of farms (two farms) are supplementary farms and two farms are with old farm holders.

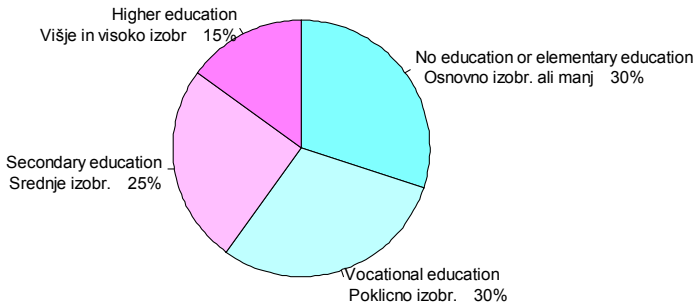


Slika 2: Socio-ekonomska struktura anketiranih kmetij v letu 2009
Figure 2: Socioeconomic structure of the surveyed farms in 2009

3.3 Educational and age structure of farm holders

A proper educational structure of farm holders has considerable impact on farm management due to a better adjustment to contemporary economic and environmental issues. Thirty per cent of farm holders have no education or just elementary school. The same percentage has vocational agricultural school or another vocational school. Twenty-five per cent of farm holders have degrees from agricultural college or another college. Higher agricultural school or other higher school accounts for 15% of farm holders (Fig. 3). The reason for the lower education level is the high proportion of older farm holders; their children have higher levels of education.

The age structure allows determination of human potential and possibilities for further development of farms.



Slika 3: Nivo dosežene izobrazbe gospodarjev na anketiranih kmetijah v letu 2009
Figure 3: Level of educational attainment of managers on the farms surveyed in 2009

Preglednica 2: Starost gospodarjev na anketiranih kmetijah v letu 2009
Table 2: Age structure of managers on the surveyed farms in 2009

	Farm holders age categories s (years)					
	under 35	3 –44	45–54	55–64	over 64	**Together**
N	1	3	7	2	7	20
%	5	15	35	10	35	100

3.4 Influence of eco-socio-economic factors on floristic composition of grasslands

With the method of canonic-correspondence analysis (CCA), we attempted to determine which of the environmental, or socio-economic factors has the best linkage with the floristic composition of grasslands. We analysed 16 impacting parameters, which were assumed to have greater impact on grasslands:

-Altitude (m) (Alt)
-Average inclination of grassland (%) (Incl)
-Exposition of grassland (%) (Eksp)
-Soil type (PKE) (Soil)
-Fertilisation (kg N/ha/year) (N)
-Grassland use (Use)
-Frequency of grassland use (Freq)
-Type of farm (FType)
-Number of LSU on farm (LSU)
-Age of farmholder (Age)
-Education of farmholder (EDU)
-Size of farm (Size)
-Remoteness (km) (Remot)
-Burden by LSU/ha (LSU)
-Grassland origin (Origin)
-Mineral fertilisers (kg/ha) (Min)

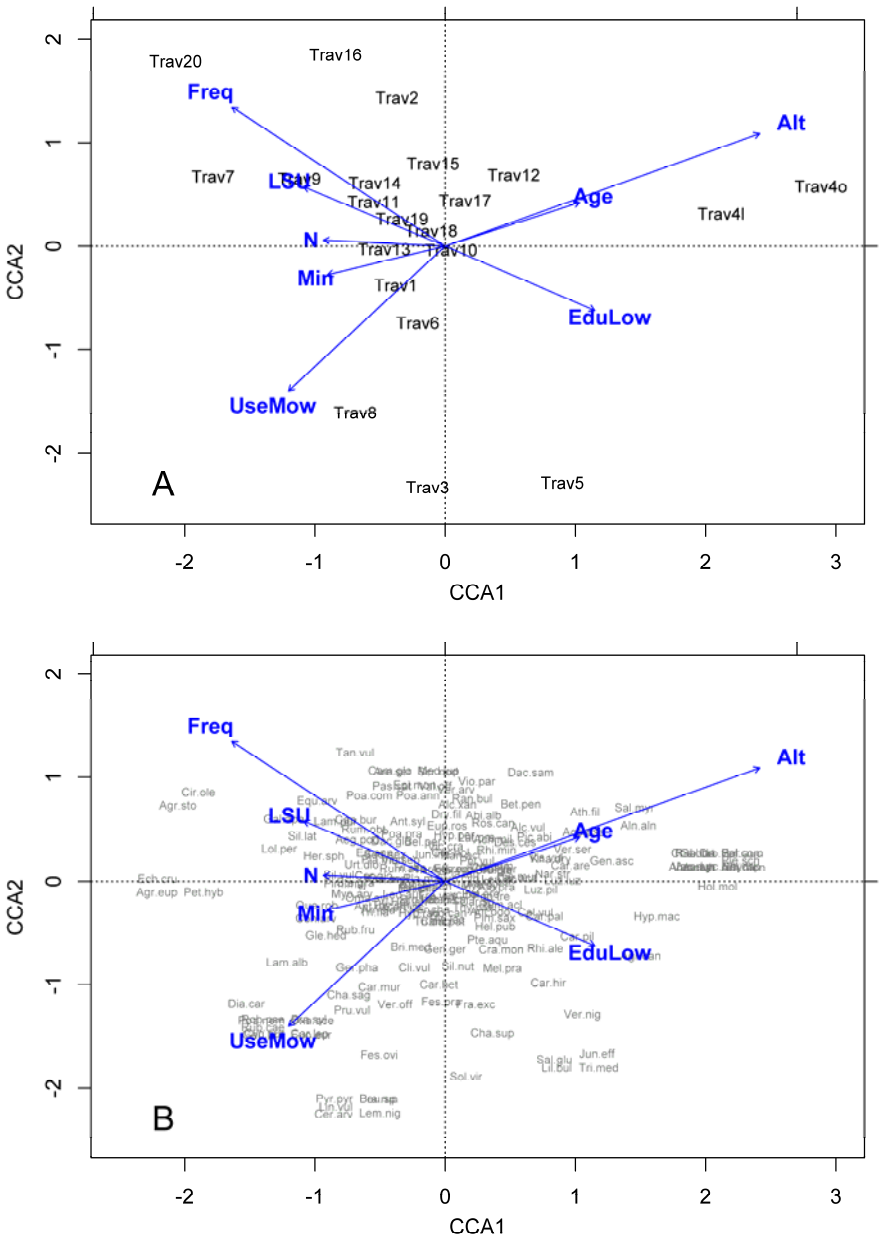
From all analysed parameters, the following eight had statistically insignificant impact:

-Average inclination of grassland (%) (Incl)
-Exposition of grassland (%) (Eksp)
-Soil type (PKE) (Soil)
-Type of farm (FType)
-Number of LSU on farm (LSU)
-Size of farm (Size)
-Remoteness (km) (Remot)
-Origin of grassland (Origin)

The results of CCA are presented in an ordination diagram in which the linkage between individual parameters with the floristic composition of grassland is presented with the length of the vector.

The characteristics of grasslands marked with T7, T9, T20 (Table 3) are: greater frequency of cutting (more than 3 times per year), bigger burden by LSU/ha, more fertilisation with N (kg N/ha /year), greater consumption of mineral fertilizers (kg/ha/year), higher education of farm holders, lower ages of farm holders and lower altitude. These grasslands are more intensively used, in contrast to grasslands marked T3, T5, T8, which are used more extensively. On the grasslands marked T40 and T4l, we conclude that following factors are responsible for the occurrence of endangered plants: higher altitude, lower education and higher age of farm holder, lower burden by LSU/ha, small input of organic fertilizers, (kg

N/ha/years), absence of mineral fertilizers, and combined grassland use (grazing and mowing).



Slika4: Ordinacijski diagram za prvi dve osi pri kanonični korespondenčni analizi. A – povezanost vegetacijske sestave travišč in okoljsko-socioekonomskih dejavnikov. B – povezanost pojavljanja rastlinskih vrst na obravnavanih travišči in okoljsko-socioekonomskih dejavnikov.

Figure 4: Ordination diagram for the first two axes of the canonical correspondence analysis. A – relationship between species composition of investigated grasslands and the environmental-socioeconomic variables . B – relationship between the plant species occurence in the investigated grasslands and environmental-socioeconomic variables. The abbreviations for the variables used: UseMow – type of grassland use (mowing yes/no), Freq – frequency of grassland use, Min – mineral fertilizers added or not, N – quantity of nitrogen fertilization, LSU – stocking rate, Alt – altitude, Age – farmholder age, EduLow – Education of farmholder (lower than secondary school yes/no).

Fig. 4 shows the ordination in linkage between occurrence of certain species groups and impacting factors (environmental, socioeconomic). On grasslands that are used predominantly for mowing, marked by Use, the following species occur: *Veronica officinalis* (Ver. off), *Carex muricata* (Car. mur), *Geranium phaeum* (Ger. pha), *Rubus fruticosus* (Rub. fru), *Dianthus carthusianorum* (Dia. car) and *Carpinus betulus* (Car. bet).

On grasslands with more intensive use, i.e. more than three cuttings per year, marked by Freq1, the following species prevail: *Poa pratensis* (Poa. pra), *Vicia sepium* (Vic. sep), *Aegopodium podagraria* (Aeg. pod), *Rumex obtusifolius* (Rum. obt), *Heracleum sphondylium* (Her. sph) and *Equisetum arvense* (Equ. arv).

Species that appear on grasslands fertilised by mineral fertiliser (marked with Min) are: *Leontodon hispidus* (Leo. his), *Centaurea jacea* (Cen. jac), *Lamium album* (Lam. alb) and *Convolvulus arvensis* (Con. arv).

Fertilising with nitrogen (kg N/ha/year), marked by N, causes the appearance of *Rumex obtusifolius* (Rum. obt) and *Stellaria graminea* (Ste. gra).

A greater burden by LSU/ha, marked by LSU, influences the appearance of *Lolium perenne* (Lol. per), *Silene vulgaris* (Sil. vulg), *Rumex acetosa* (Rum. ace) and *Pimpinella major* (Pim. maj).

Higher education of farm holders, marked by Edu, influences the appearance of *Silene latifolia* (Sil. lat), *Lamium purpureum* (Lam. pur), *Capsella bursa-pastoris* (Cap. bur), *Knautia arvensis* (Kna. arv) and *Rumex acetosa* (Rum. ace).

With higher altitude, marked by Alt, the following species appear: *Luzula campestris* (Luz. cam), *Viscaria vulgaris* (Vis. vul), *Nardus stricta* (Nar. str), *Veronica serpyllifolia* (Ver. ser), *Acer pseudoplatanus* (Ace. pse) and *Salix myrsinifolia* (Sal. myr).

Higher age of farm holders, marked by Age, linked with higher altitude, marked Alt caused appearance of following species: *Euphrasia rostkoviana* (Eup. ras), *Rhinanthus minor* (Rhi. min), *Achillea millefolium* (Ach. mil), *Galium verum* (Gal. ver), *Ajuga reptans* (Aju. rep) and *Picea abies* (Pic. abi).

3.5 Number of plant species on analysed grasslands in relation to agro-environmental measures

Table 3 presents the number of agro-environmental measures claimed by the farmers on the analysed grasslands and demarcation of grasslands. Half of the farmers claim direct payment under the KOP scheme (Kmetijsko okoljski program – Agro-environmental program); 10% of farmers were also included in the SKOP scheme (Slovenski kmetijsko okoljski program – Slovenian agro-environmental program); 40% of farmers applied just for the basic agricultural measures. The total analysed grasslands had an area of 58.4 ha. For this area, all farmers claimed basic payments from the first pillar of subsidies and payments for the areas with limited conditions for agricultural production (LFA). Twenty per cent of farmers claimed the measure for ecological farming (EK), comprising 12.6 ha of agricultural lands The measure called "co-natural rearing of domestic animals" (REJ) was claimed by 40% of farmers (23.3 ha of agricultural land): 60% of farmers claimed just the measure of mowing of steep meadows (inclinations of 35–50%= (S35) on 21.0 ha of agricultural land and 45% of farmers used the measure for mowing steep meadows with inclination over 50% (S50) on 11.0 ha of agricultural land. Analysis of plant species on the studied grasslands showed that on a quarter of analysed grasslands, fewer than 50 plant species were found (27 to 49). For these grasslands, farmers claim just the basic measures. Within these basic measures, were also grasslands (1%) on which we found from 60 to 70 plant species. On the rest of grasslands, where farmers also claim measures from KOP and SKOP, we found from 51 to 78 plant species.

Preglednica 3: Število vrst na proučevanih travniščih ter vrste ukrepov za proučevana travišča
Table 3: Number of species in the surveyed grasslands and the types of measures for surveying the grasslands

Demarcation of grasslands	Number of plant species	Kinds of measures on analysed grasslands
Trav1	62	KRM, OMD, UP, EK, S35 (271 ar), S50 (128 ar)
Trav2	57	KRM, OMD, UP, EK, S35 (88 ar) , S50 (54 ar)
Trav3	70	KRM, OMD, UP
Trav4o	58	KRM, OMD, UP, REJ
Trav4l	37	KRM, OMD, UP, REJ, S35 (100 ar)
Trav5	55	KRM, OMD, UP, REJ, S35 (150 ar), S50 (90 ar)
Trav6	64	KRM, OMD, UP, EK, S35 (241 ar), S50 (368 ar)
Trav7	47	KRM, OMD, UP
Trav8	72	KRM, OMD, UP
Trav9	38	KRM, OMD, UP
Trav10	78	KRM, OMD, UP, REJ, S35 (544), S50 (142 ar)
Trav11	64	KRM, OMD, UP, REJ, S35 (116 ar)
Trav12	70	KRM, OMD, UP, EK, S35 (54 ar), S50 (32 ar)
Trav13	59	KRM, OMD, UP, REJ, S35 (150 ar), S50 (180 ar)
Trav14	68	KRM, OMD, UP, REJ, S35 (20 ar), S50 (23 ar)
Trav15	64	KRM, OMD, UP, REJ, S35 (107 ar)
Trav16	30	KRM, OMD, UP
Trav17	51	KRM, OMD, UP, REJ, S35 (260 ar), S50 (99 ar)
Trav18	60	KRM, OMD, UP
Trav19	49	KRM, OMD, UP
Trav20	27	KRM, OMD, UP

4 CONCLUSIONS

Part-time farms prevail, comprising 45% of the analysed areas, which is more than average for Slovenia (35.1%) for the year 2000 (Udovč et al., 2006). Part-time farms earn their income from agricultural activities and activities outside the farm.

Farmers cultivate their own and hired land; 65% of agricultural land is owned by the farmers, on average 7.8 ha per farm. Farms between 5 and 10 ha represent the biggest share (40%).

The educational structure shows that 38% of farm holders have elementary or lower education. The reason for lower education could be the markedly high age of farmers.

The age structure of farm holders is unfavourable; 38% of them are older than 55 years. The average age of farmers in the analysed area is 56.5 years.

CCA analysis showed which eco-socio-economic factors are best linked to the composition of grasslands. Most notable were three grasslands that were intensively used, at lower altitudes, having higher frequency use (more than three cuttings per season), having greater impact of LSU/ha, they are more fertilized with N (kg N/ha/year), used only by mowing and having higher usage of mineral fertilizer (kg/ha/year). With regard to socioeconomic factors, these grasslands were linked with lower ages of farmers and their higher education. In contrast, linked with eco-socioeconomic factors, was group of three grasslands with extensive use, on two of which the occurrence of endangered plants on acidic soils was linked with higher altitude, lower education levels of farm holders, higher age of farm holders, smaller impact by LSU/ha, small input of organic fertilisers (kg/ha/year), absence of mineral fertiliser use and combined grassland use (grazing and mowing).

Socio-economic factors, such as the age and education of farm holders, significantly influence the intensity of farming. Our results showed that

farmers on intensively used grasslands are younger and have higher education levels than those on grasslands, which are more extensively used by farm holders that are older and less educated. From this, we can conclude that the younger generation of farmers is inclined to more intensive farming than older ones, and that younger farmers are not yet ecologically educated.

In the evaluation of the efficiency of agro-environmental measures in relation to plant species diversity and the conservation of habitats in the analysed area, we have concluded that the majority of farmers take part only in basic measures. Such grasslands are poor with regard to the number of plant species, averaging less than 50 species. In contrast, the grasslands for which additional measures have been taken (KOP and SKOP measures), the number of plant species is higher, between 51 to 78 species, among them some sensitive ones.

For better evaluation of the efficiency of these agro-environmental measures, longer-term monitoring would be necessary.

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The impact of wheat production on the occurrence of mycotoxins DON (deoxynivalenol) and ZEA (zearalenone) on wheat grains (*Triticum aestivum* L.)

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ABSTRACT

The current study has been conceived to draw attention to the factors that should be avoided in the production of cereal grains (such as high doses of nitrogen) by analysing differences in cereal grain contamination with mycotoxins DON (deoxynivalenol) and ZEA (zearalenone), depending on methods of wheat production. We studied the occurrence of DON and ZEA at very different production intensities in the 'Reska' and 'Savinja' wheat cultivars which were grown in a long-term field experiment (designed in 1992) at Jable near Ljubljana in the years 2006 and 2008. The cultivars 'Reska' and 'Savinja' have been considered in crop rotation of wheat-maize-oats in 5 methods of production with variants ranging organic up to intensely conventional. The results obtained have shown that adequately chosen agri-technical measures significantly reduced the risk of contamination of wheat flour by the mycotoxins DON and ZEA. Too abundant N (nitrogen) fertilization of either inorganic or organic origin is to be avoided. The extensive wheat growing reduces considerably the risk of DON and ZEA occurrence in comparison to the intensive one. Cultivar plays an important role in this process. The comparison of both cultivars has shown that the cultivar 'Savinja' was more resistant to the contamination with DON and ZEA than the cultivar 'Reska'.

Key words: mycotoxins, deoxynivalenol, zearalenone, wheat, nitrogen fertilization, food safety, production method

IZVLEČEK

VPLIV OKOLJSKIH DEJAVNIKOV NA POJAVNOST MIKOTOKSINOV DON (DEOKSINIVALENOL) IN ZEA (ZEARALENON) NA ZRNJU PŠENICE *(Triticum aestivum* L.*)*

V raziskavi smo analizirali razlike v kontaminiranosti žitnih zrn z mikotoksinoma DON (deoksinivalenol) in ZEA (zearalenon) v odvisnosti od načina pridelovanja, da bi opozorili na okoljske dejavnike, kot na primer visoke odmerke dušika, ki bi se jim morali v pridelavi pšenice izogibati. Pojavljanje mikotoksinov DON in ZEA smo proučevali v letih 2006 in 2008 pri različnih postopkih pridelovanja pšeničnih kultivarjev Reska in Savinja v okviru trajnega poljskega poskusa, zasnovanega leta 1992 v Jablah pri Ljubljani. Kultivarja Reska in Savinja sta bila posejana v kolobarju pšenica-koruza-oves v 5-ih postopkih pridelovanja, ki vključujejo variante, od ekološko prijaznih do intenzivnih konvencionalnih. Rezultati so pokazali, da lahko z ustreznimi agrotehničnimi ukrepi pomembno zmanjšamo tveganje za pojav kontaminacije pšenične moke z mikotoksinoma DON in ZEA. Pri ekstenzivni pridelavi pšenice je tveganje za pojav DON in ZEA občutno manjše kot pri intenzivni. Pomembno vlogo ima tudi kultivar: primerjava kultivarjev je pokazala, da je Savinja bolj odporna na kontaminacijo z DON in ZEA kot Reska.

Ključne besede: mikotoksini, deoksinivalenol, zearalenon, pšenica, varnost hrane, gnojenje z dušikom, metode pridelovanja

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1 INTRODUCTION

When eating food, humans and animals are exposed to various mycotoxins which are formed as degradation products of the metabolism of the *Fusarium* spp. present in cereals. Long-term intake of cereal products (bread, pasta, biscuits, etc.) that are contaminated with these mycotoxins may be the cause of serious developmental and hormonal disorders, chronic poisoning, malignant tumours and other diseases, as well as deformities (Smith et al., 1994; Gregorčič et al., 2009; Casteel and Rottinghaus, 2000). Williams and Hammitt (2001) considered the consumers to be insufficiently aware of the threats posed by the presence of mycotoxins in food. They stated that the consumers were certain that it were primarily the pesticides and not the presence of mycotoxins that put their health at risk, however, the authors' opinion was just the opposite: human health was exposed to increased risk due to the potential effects of mycotoxins rather than to the residues of fungicides in food.

Mycotoxins which are the result of the secondary metabolism of mycotoxicogenic mold (inter alia fungi *Fusarium* spp.) occur in cereals (often in wheat, maize, barley and triticale) as fungal infections. Visible signs of disease (FHB - *Fusarium* head blight - is a devastating disease of wheat with spikelets exhibit symptoms of premature bleaching shortly after infection by the fungal plant pathogen of genus *Fusarium* spp.*)* that may be present in all parts of the plant, especially in the grains and inflorescences (spikes, cobs and wiper), reduce the quantity and quality of crop yields. Among the most problematic mycotoxins belong mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) which are formed by the metabolism of *Fusarium* spp. (*F. graminearum* Swabe, *F. culmorum* (W.G. Smith) *Sacc.*, etc). DON poses a significant threat to the health of domestic animals and humans because of its deleterious effects on the digestive system and disturbances in normal cell function by inhibiting protein synthesis. DON has high cytotoxic and immunosuppressive properties. Humans consuming flour made from wheat contaminated with DON will often demonstrate symptoms of nausea, fever, headaches, and vomiting. At high doses, DON induces acute gastrointestinal toxicity; chronic, at low doses immunotoxicity has been reported (Nogueira da Costa et al., 2011); at the molecular level DON disrupts normal cell function by inhibiting protein synthesis via binding to the ribosome and by activating critical cellular kinases involved in signal transduction related to proliferation, differentiation and apoptosis (Waśkiewicz et al., 2014). Because of concerns related to DON, the United States FDA has instituted advisory levels of 5 µg/g for grain products for most animal feeds and 10 µg/g for grain products for cattle feed (Waśkiewicz et al., 2014). ZEA is a phytohormone, which displays, apart from its anabolic properties, mainly estrogenic effects. Because of its estrogenic properties, ZEA may induce fertility disorders in animals with clinical signs of hyperestrogenism – an aspect of a disease which although reported mainly in hogs, is described in other species such as cow, horse and sheep, too. The potential health risk for man induced by this mycotoxin, which is taken up with foods of vegetable or animal origin, is extensively discussed. As an immunotoxic compound similar to estrogen and some endocrine disruptors ZEA has the toxic effects on the immune function, promote reduction in body weight gain (which is not fully explained by diminished food consumption), cause thymic atrophy with histological and thymocyte phenotype changes and decrease in the B cell percentage in the spleen and also weaken the antibody production and peroxide release by macrophages (Hueza et al., 2014). These two mycotoxins have therefore become the subject of worldwide intensive research in recent decades (Atroshi et al., 2002; European Commission, 1999; Smith et al., 1994; Srey at al., 2014).

A tolerable daily intake (TDI) for mycotoxins DON and ZEA has been evaluated by the Scientific Committee for Food (SCF); a TDI for DON of 1 µg/kg body weight and a provisional TDI of 0.2 µg/kg body weight for ZEA (Commission regulation, 2004).

Analysis of the levels of ZEA and DON in wheat and other cereals has become a legal obligation in many countries, including Slovenia. In 2006, the European Union decreed the establishment of the monitoring of contamination of cereals and cereal products as well as the maximum content of these

mycotoxins in food products. The maximum permissible content in wheat for adults is 750 µg DON kg^{-1} of flour and 75 µg ZEA kg^{-1} of flour. The criteria are stricter for children, with less than 200 µg DON kg^{-1} of flour and below 20 µg ZEA kg^{-1} of flour being allowable (Commission Regulation, 2006a).

Although scientists have conducted intensive research into ways in which to avoid the contamination of crops and mould-resistant plant species and new fungicides have been developed, no highly effective protective methods of avoiding the occurrence of mycotoxins in crops have been ascertained as yet (Jakovac-Strajn et al., 2004). Nevertheless suspensions or solutions with 1 % of Chinese galls (*Galla chinensis)* or 1 % of tannic acid had an antifungal toxicity and inhibited growth of *Fusarium graminearum* by 98–100 % or by 75–80 % in wheat that was artificially inoculated with *Fusarium graminearum* and *F. crookwellense* and then treated with 5 % suspensions of tannic acid and Chinese galls, whereas dried bark from buckthorn (*Rhamnus catharticus* L.) showed no effect at this concentration. In field experiments with two wheat varieties and artificial or semi-natural inoculations, mean DON reductions of 66 % (with tannic acid) and 58 % (with *Rhamnus catharticus* L.) were obtained (Forrer et al., 2014).

Previous studies have paid too little attention to the impact of production methods on the occurrence of mycotoxins in wheat grain; such occurrence has been discussed primarily in relation to the precipitation, to the crop rotation and to the cultivars and has rarely assessed the differentiated and simultaneously precise circumstances and methods of production. Rather than examining individual modes of production, surveys have been directed at the study of the impact of other factors, such as warm and humid weather, crop rotation of cereal grain crops or cultivars with differences in the resistance to mycotoxins (Schachermayr and Fried, 2000). Some scientists in Slovenia have investigated the influence of the environment on the occurrence of mycotoxins in wheat crops. Zemljič et al. (2008) found that location played an important role in the occurrence of DON while other mycotoxins, including ZEA, have not been identified in crop yields. Gregorčič et al. (2009) examined mycotoxin contamination in wheat and detected DON in the majority of the tested samples, ZEA in half of the samples and the threshold values of both DON and ZEA were exceeded in one fifth of all samples. Jakovac-Strajn et al. (2010) studied grain contamination with mold and the incidence of mycotoxins in cereals cultivated by farmers and used for animal feed. It was found that 73 % of the samples were contaminated, the majority with the mold *Fusarium* spp. causing the mycotoxin DON, and to a lesser extent ZEA and other toxins. Kalcher-Tavčar et al. (2007) examined the contamination of animal feed and, inter alia, found an average content of 178 µg ZEA kg^{-1} grains in 42 % of all tested samples.

The above findings confirm that the control of mycotoxins in grain production is necessary and justified in Slovenia. By following the principles of good agricultural practice and the optimization of production methods, the occurrence of *Fusarium* fungi can be reduced and the risk factors for the occurrence of mycotoxins can be limited to the lowest level (Commission Regulation, 2007).

This investigation forms part of a wider research into the relationship between the occurrence of DON and ZEA and the methods of production. Its purpose is the identification of environments and production systems in which mycotoxins occur with greater or lesser intensity. The aim of the study was to find one or more appropriate agricultural practices of wheat production in order to avoid the contamination with mycotoxins DON and ZEA promoting fungi.

2 MATERIALS AND METHODS

In order to conduct the agricultural labour research, we used the static long-term field experiment, which is a part of the network of international field trials known as ′Internationaler organischer Stickstoff-Düngungsversuch/International organic nitrogen fertilization long-term experiment′ (IOSDV), which have been conducted at Jable near Ljubljana (SI) since 1992. The design of this long-

term field experiment was described in detail in the previous descriptions together with their environmental parameters (Tajnšek, A., 2003; Tajnšek L. and A. Tajnšek, 2004; Tajnšek L., 2004).

The location of IOSDV at Jable is an experiment in the alpine climate area where long periods of drought rarely occur. Depending on the texture of the soil, the average annual rainfall is often too high (1343 mm a^{-1}, with an upward trend; Tajnšek et al., 2013) to ensure crops of wheat that are stable and of good quality, and the temperature is suitable (9.5 ºC a^{-1}). Given the long-term average rainfall and temperature, the climate conditions for the production of high-quality wheat at Jable, the fluctuations of precipitation between different years are higher than in those regions of the world where the most favourable conditions for the production of wheat exist.

The experiment has been conducted in the rotation of three crops (maize-wheat-oats) in three repetitions of basic parcels, each measuring 30 m^2. Wheat, maize and oats are sown each year in one of the three plots (fields, each measuring 1800 m^2) in crop rotation, so that various crops returns every third year to the same parcel. Each of these three plots is divided into three blocks and each of the blocks is divided into 10 basic parcels (of 30 m^2) with specific production methods. The process of production of each of these 10 basic parcels of all three field crops took place in a specific manner throughout the period of the experiment. From all these 10 methods of production 5 methods were chosen for the purposes of this study (Table 1). The specificity of permanent experiments conceived in this way is such that the methods of production lead to systematic differences between the level and the quality of crops in each of the years studied.

Table 1: Methods of production at IOSDV, Jable (chosen methods), 1993 – 2008

Preglednica 1: Metode pridelovanja v poskusu IOSDV Jable (izbrane metode), 1993 - 2008

The method of production	Code of production method	Annual rate of mineral nitrogen (N-min) for wheat	Level of production method with mineral nitrogen (N-min)
System A:			
Without organic fertilization	AN0	0	Without fertilization with N-min
System B:			
Fertilization with FM**			
(300 dt ha^{-1} FM every third year; before sowing maize)	*BN0**	0	Without fertilization with N-min
	BN2	130	Moderate rate of N-min
System C:			
Ploughed in maize stalk, straw of wheat, barley and oats; 60 kg ha^{-1} N-min before sowing oilseed radish	*CN0**	0	Without fertilization with N-min
	CN2	130	Moderate rate of N-min

In addition to all parcels in the fertilization experiment 100 kg P2O5 ha-1 a-1 and 180 kg K2O ha-1 a-1 were given
* Methods written in italics, BN0 and CN0, are the approximation of the principles of sustainable farming
**FM = farmyard manure

In Table 1, management without nitrogen and organic fertilization is indicated by code A, management with FM is indicated by code B and management with ploughing in the by-products (of wheat, barley or oats straw and of maize stalk) and oilseed radish as a green manure after harvesting barley or oats before planting maize, is indicated by code C. Wheat yield obtained in the years 2006 and 2008 was used to analyse the impact of production procedures on the occurrence of mycotoxins DON and ZEA. During these years, two cultivars of wheat, 'Reska' and 'Savinja', were sown in the long term experiment IOSDV; so both cultivars were included in our study which enables additional comparisons between the two cultivars.

Slovenian cultivar 'Reska' was acknowledged in 1996 (Grižon et al., 2011); characteristics of the cultivar are thick straw and thick grains, the absolute mass more than 44 g, a high protein content, up to 17.3 % and sedimentation value 25-32 ml (Tajnšek et al., 2010). Comparison with the standard at that time (cultivar 'Marija') showed that 'Reska' is of a very good quality for baking bread (Pavlic Nikolić, 2005). Because of long awns on the ear the cultivar 'Reska' is suitable especially for the areas where there is a greater risk of yield lowering caused by birds and wildlife. The cultivar is very resistant to high temperature stress even at very high temperature (Ristic et al., 2008).

Cultivar 'Savinja' is also a Slovenian cultivar, acknowledged in 2010 (Grižon et al., 2011). The cultivar is fertile in particular at the subalpine climate conditions with moderate rates of nitrogen (Čergan and Tajnšek, 2010). Depending on baking quality cultivar 'Savinja' is a typical improved cultivar and a good bread making cultivar (Tajnšek A. and Tajnšek L., 2011).

The years 2006 and 2007 at the location at IOSDV Jable were more dry during the period of flowering-ripening than year 2008 (average of April-July 2006: precipitation 98.5 mm, temperature 15.9°C; average of April-July 2007: precipitation 81.5 mm, temperature 16.7 °C; average of April-July 2008: precipitation 168.7 mm, temperature 15.9 °C) (SURS (Statistical Office of the Republic of Slovenia), 2011), thus for the analysis of wheat grains contamination with mycotoxins DON and ZEA the year 2007 (with the driest flowering-ripening period) was set aside, as several researchers found that wet weather had a significant impact on the contamination of wheat with mycotoxins DON and ZEA (Whitlow and Hagler 2009, Prandini et al. 2009). In both years studied, samples of wheat grains (1.5 - 2 kg) were taken from the harvested wheat from all plots after weighing, and stored in freezers at T = -20 °C prior to the analysis.

For the mycotoxicologic analysis of the presence of DON and ZEA a total of 60 grains samples (of 1.5 - 2 kg) were collected from basic parcels in both years. One half of the samples belonged to the cultivar 'Savinja' and the other half to the cultivar 'Reska'. Appropriate flour samples were prepared prior to laboratory analysis. Wheat grains were ground in the Brabender wheat mill suitable for grinding smaller size samples (MPI R. O. »Tehnićke usluge« Tip: S – 150 M, Atest: TU 78/1). The flour was separated by German typisation (DIN 10355) into white flour (type 405), dark flour (type 1050) and bran which was not sent for further analysis. From every wheat sample 110 – 120 g of white and dark flour were ground and weighed and a total of 120 flour samples were sent for the analysis of DON and ZEA mycotoxin content to the reference laboratory LUFA Speyer in Germany in which 240 chemical analysis were performed; i.e. 120 for DON and 120 for ZEA.

The standardized method used for detection of the mycotoxin DON in wheat flour developed in the reference laboratory LUFA Speyer is called ELISA LUFA SP 22005 (limit of detection and quantification is 200 ppm / 200 µg DON kg^{-1} flour) and that used for detection of the mycotoxin ZEA is called ELISA LUFA SP 22006 (limit of detection and quantification is 5 ppm / 5 µg ZEA kg^{-1} flour). The quantification of the mycotoxins content by those methods is based on competitive ELISA (the enzyme-linked immunosorbent assay), where a solid-phase enzyme immunoassay (EIA) is used to detect a presence of mycotoxins in a wet sample, based on the colour change reaction of the sample (Usleber et al., 1991; see also In Vitro Test, R-Biopharm AG, Darmstadt, and http://www.r-biopharm.com).

A competitive enzyme immunoassay used for the quantitative analysis of DON in wheat is RIDASCREEN®FAST DON (Art. No.: R5901, 96

wells / R5902, 48 wells) and the one used for the quantitative analysis of ZEA in wheat is RIDASCREEN® Zearalenon (Art. No.: R1401).

The RIDASCREEN®FAST DON can't differentiate between DON and 3-acetyl-DON (cross reactivity 213 %) and has a negligibly low or no cross reactivity to other related substances such as Nivalenol, 15-acetyl-DON or Triacetyl-DON.

The specificity of the RIDASCREEN® Zearalenon test was established by analysing the cross reactivity to corresponding mycotoxins (100 % for ZEA, 41.6 % for α-zearalenol, 27.7 % for zearalenol and 13,8 % for β-zearalenol).

All reagents for both enzyme immunoassay – including standards – are contained in the test kit in sufficient quantity for 96 determinations, including standards. A microtiter plate spectrophotometer is required for quantification.

Sample preparation include extraction and filtration (and dilution at sample preparation for determining ZEA), time requirement for sample preparation (for 10 samples) is 10 minutes for DON and 20 minutes for ZEA.

Test principle is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-DON antibodies and with specific antibodies to ZEA. DON/ZEA standards or sample solutions, DON/ZEA enzyme conjugate and anti-don antibodies are added. Free DON and DON-enzyme conjugate compete for the DON-antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-DON antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen is added to the wells, bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a colour change from blue to yellow. The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the DON concentration in the sample.

Materials required for determining DON and ZEA are reagent distilled or deionized water for determining DON and methanol (for determining ZEA) and equipment followed: microtitre plate spectrophotometer (450 nm), graduated cylinder-for DON (plastic or glass, 100 ml, 1 l), glassware for preparing sample extract: filter funnel and 50 ml flask for DON and 100 ml flask for ZEA, grinder (mill), Ultra-Turrax or equivalent shaker-optional for DON (shaker required for ZEA) filter paper (Whatman No. 1 filter) and variable 20-200 µl and 200-1000 µl micropipettes. Equipment required additionally for determining ZEA are rotary evaporator or another equipment for evaporation of solvents, pasteur pipettes and graduated pipettes are required.

Table 2: Reagent required for the determination of mycotoxins DON and ZEA in wheat competitive enzyme immunoassay RIDASCREEN®FAST DON and RIDASCREEN® Zearalenon used for the quantitative analysis of DON and ZEA in wheat

Preglednica 2: Potrebni reagent pri določanju mikotoksinov DON in ZEA v pšenici z encimsko imunskim testoma RIDASCREEN®FAST DON in RIDASCREEN® Zearalenon

Reagent*	For determining DON	For determining ZEA
Microtitre plate	96 or 48 wells (12, R5901 or 6 strips, R5902 resp., with 8 removable wells each)	96 wells coated with antibodies against ZEA (12 strips with 8 wells each)
Standard solutions	5 x DON standard solutions (1.3 ml each): 0 ppm -zero standard, 0.222 ppm, 0.666 ppm, 2 ppm, 6 ppm DON in water, ready to use; the dilution factor 20 for the sample has already been considered, therefore, the DON concentrations of samples can be read directly from the standard curve.	6 x standard solutions (1,3 m each): 0 ppt-zero standard, 50 ppt, 150 ppt, 450 ppt, 1350 ppt, 4050 ppt ZEA in aequeous solution
Conjugate	6 ml, R5901 and 3 ml, R5902; (red cap) peroxidase conjugated DON; ready to use	0.7 ml; (red cap); peroxidase conjugated ZEA concentrate
Anti-mycotoxin antibody	1 x anti-DON antibody (6 ml, R5901 and 3ml R5902; ready to use)	/
Substrate / Chromogen	Substrate / chromogen (10 ml; stained red, brown cap)	Substrate (7 ml; contains urea peroxide, green cap) Chromogen (7 ml) contains tetramethylbenzidine (blue cap)
Stop solution	14 ml; contains 1 N sulphuric acid; yellow cap	14 ml; contains 1 N sulphuric acid; yellow cap
Buffer	Buffer salt (washing buffer) for preparation of a 10 mM phosphate buffer (pH 7.4); Contains 0.05 % Tween 20.	Buffer 1 (50 ml) sample and conjugate dilution buffer (white cap)

*All the reagents from the Table 2 are contained in each kit (sufficient materials for as many as 91 or 43 analysis, plus 5 standard analysis; storage of kit at 2-8 ºC).

The preparation of samples is carried out according to the following steps in the Table 3 (the samples should be stored in a cool place, protected of light; a representative sample should be ground and thoroughly mixed prior to proceeding with the extraction procedure):

Table 3: Steps of determination of mycotoxins DON and ZEA in wheat competitive enzyme immunoassay RIDASCREEN®FAST DON and RIDASCREEN® Zearalenon used for the quantitative analysis of DON and ZEA in wheat

Preglednica 3: Postopki priprave vzorca za določanje mikotoksinov DON in ZEA v pšenici z encimsko imunskim testoma RIDASCREEN®FAST DON in RIDASCREEN® Zearalenon

Step	For determining DON	For determining ZEA
Weighing 5 g of ground sample into a suitable container	And addition of 100 ml of distilled water (sample size may be increased if required, but the volume of water must be adapted accordingly: e.g. 25 g in 500 ml of distilled water or 50 gin 1000 ml of distilled water)	And addition of 25 ml of methanol/water (70/30); sample size may be increased if required, but the volume of water must be adapted accordingly: e.g. 10 g in 50 ml of methanol/water (70/30)
Shaking	Blending the sample by ultra-turrax (or equivalent for 2 minutes or shaking vigorously for three minutes (manually or with shaker)	Shaking vigorously for three minutes (manually or with shaker)
Filtering / centrifuging	Filtering the extract through Whatman No. 1 filter (or equivalent)	Centrifuging the extract: 10 min / 3500 g / room temperature (20 – 25 ºC) or filtering the extract through filter (Whatman No.1 filter)
Diluting	Diluting the filter sample extract 1:4 (1+3) with distilled water (e.g. 1 ml of the extract + 3 ml of distilled water)	Diluting the supernatant or filtrate 1:7 (1+6) with sample dilution buffer (buffer 1)
Filtrate / supernatant	Using 50 µl of the filtrate per well in the test	Using 50 µl of diluted supernatant or filtrate per well in the test

Test implementation includes procedures followed:

1. Insertion of a sufficient number of wells into the microwell holder for all standards and sample to be run. Recording standard and sample positions.

2. Pipetting of 50 µl of standard (solutions) or prepared sample into separate wells; using a new pipette tip for each standard or sample.

3. For determining DON: addition of 50 µl of enzyme conjugate (red cap) to each well.

4. Addition of 50 µl of anti-DON antibody solution (black cap) to each well (50 µl of the diluted enzyme to each well for ZEA). Mixing gently by shaking the plate manually and incubating for 5 min (+/- 1) at room temperature (20–25 ºC); the specific reaction starts with the addition of the specific antibody.

5. Pouring the liquid out of the wells into a sink. Taping the microwell holder upside down vigorously against absorbent paper/onto a clean filter towel (three times in a row) to ensure complete removal of liquid from the wells. Using a wash bottle or multichannel pipette, fill the wells (250 µl per well) with washing buffer-buffer salt from test kit (with distilled water for ZEA). Empty the wells again and remove all remaining liquid. The washing procedure must be repeated two more times.

6. Addition of 100 µl of substrate/chromogen (brown cap) to each well. Mixing gently by shaking the plate manually and incubate for 3 minutes (+/- 0.5) for DON (for 30 minutes for ZEA) at room temperature (20 – 25 ºC) in the dark.

Time requirement for test implementation (incubation time) for 10 samples is 8 minutes for determining DON and 150 minutes for ZEA.

A specific software, the RIDA®SOFT Win (Art. No. Z9999), is available for evaluation the RIDASCREEN® enzyme immunoassays (for single determinations logit/log evaluation and for double or multiple determinations cubic spline should be used). The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit.

For the calculation without software the percentage of absorbance is calculated according to the equation:

Percent (%) absorbance = (absorbance standard (or sample) / absorbance zero standard) x 100

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semi-logarithmic graph paper against the DON concentration ($mgkg^{-1}$) ZEA concentration (μgkg^{-1}).

The DON concentration in $mgkg^{-1}$ corresponding to the extinction of each sample can be read from the calibration curve.

The ZEA concentration in μgkg^{-1} actually contained in a sample, the concentration read from the calibration curve must be further multiplied by the corresponding dilution factor. When working in accordance with the regulation stated, the dilution factors is 35.

Since in the first year (2006) observation (2006) no contamination of grains either with DON (limit of detection = 200 µg DON kg^{-1} flour) or with ZEA (limit of detection = 5 µg ZEA kg^{-1} flour) was detected, the statistical evaluation of results was performed only for the second year (2008). The data on the contamination of white and dark flour in dependence of production method were then analysed by the method of statistic variance. First the transformation value (*tv*) was made for all data using the formula: $tv = sqr(x+½)$ so that we were able to evaluate the data statistically using the analysis of variance for the normal distribution of data (Weber, 1961).

3 RESULTS

3.1 Mycotoxin DON found on the wheat grain samples of the cultivars 'Reska' and 'Savinja'

On the location of IOSDV Jable in 2006 the mycotoxin DON was not detected in any of both cultivars (in frame of detection). White and dark bread baked from the flour of both cultivars would be completely safe according to the EU criterion concerning the safety of flour from contamination with DON (European Commission, 2006) irrespective of any production method included in the experiment.

Contamination of flour with the mycotoxin DON was detected in both cultivars in the intensive methods of production under weather conditions in 2008 when an above the average precipitation quantity occurred on the IOSDV Jable location (1593 mm in 2008, 1064 mm in 2006; SURS (Statistical Office of the Republic of Slovenia), 2011). In the cultivar 'Reska' grown in the method with stable manure and N-min (BN2) the white flour was contaminated in two out of three repetitions. In the same flour type the CN2 production method was contaminated in all three repetitions (see Table 2) while the dark flour of the same cultivar was contaminated with DON in all three repetitions of intensive production (BN2, CN2). Within the limits of possible determination using the method chosen in the cultivar 'Savinja' the white flour was contaminated with DON in all three repetitions of the method BN2 and two repetitions in dark flour (Table 2). The methods of production without fertilization with N-min (AN0, BN0, CN0) were not contaminated with DON in any of flour types belonging to both wheat cultivars.

Table 4: White and dark flour* contamination with the mycotoxin DON above the detection level (200 µg DON kg-1 flour) in the cultivars 'Reska' and 'Savinja' on IOSDV Jable in 2008 (µg DON kg-1 flour)

Preglednica 4: Kontaminacija bele in črne moke* z miktoksinom DON nad mejo detekcije (200 µg DON kg^{-1} moke) pri kultivarjih 'Reska' in 'Savinja' na IOSDV Jable v 2008 (µg DON kg^{-1} flour)

µg DON kg^{-1} flour	'Reska'								'Savinja'							
	White flour*				Dark flour*				White flour				Dark flour			
Block	I	II	II	Mean	I	II	III	Mean	I	II	I	Mean	I	II	III	Mea
AN0	-**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BN0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CN0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BN2	250	-	630	293	630	260	1000	630	330	270	530	477	220	-	500	240
CN2	570	300	670	513	670	280	250	400	-	-	-		-	-	-	-

*White flour of the type 405, dark flour of the type 1050
** <200 µg DON kg^{-1} flour

For practical and theoretical reasons it is important to know whether the occurrence of mycotoxin DON in wheat flour is influenced statistically significantly by the flour type, the method of production and the cultivar. The relevant analysis is presented in the Table 5. From the presentation it is evident that the contamination with the mycotoxin DON (at $p \leq 0.05$) of both the white and the dark flour of the cultivar 'Reska' is significantly influenced by the method of production.

Table 5: Significance of contamination of wheat grain (white and dark flour*) of two cultivars with the mycotoxin DON in dependence on production methods, cultivar ('Reska' and 'Savinja') and flour type; IOSDV Jable, 2008; transformed values of contamination $\sqrt{(1/2 + x)}$

Preglednica 5: Značilnost kontaminacije pšeničnega zrnja z mikotoksinom DON v odvisnosti od metode pridelovanja, kultivarja ('Reska' in 'Savinja') in tipa moke, IOSDV Jable, 2008; transformirane vrednosti $\sqrt{(1/2 + x)}$

Variability source	'Reska'				'Savinja'			
	White flour		Dark flour		White flour		Dark flour	
	F-test	Significance	F-test	Significance	F-test	Significance	F-test	Significance
Blocks	1.03	$p \geq 0.05$	1.00	$p \geq 0.05$	1.00	$p \geq 0.05$	0.28	$p \geq 0.05$
Methods	6.88	$p \leq 0.05$	22.26	$p \leq 0.00$	88.22	$p \leq 0.05$	3.52	$p \geq 0.05$

*White flour of type 405, dark flour of type 1050

In cultivar 'Savinja' the contamination of white flour with mycotoxin DON depends significantly on the production methods while in dark flour the differences are not statistically significant (Table 5).

From the results presented in Tables 4 and 6 it is evident that in extensive production methods with no N-min fertilization neither white nor dark flour of any of the two cultivars included in the experiment were contaminated with the mycotoxin DON. Since in the dark flour of the cultivar 'Savinja' even in intensive production methods (BN2, CN2) the content of DON did not increase statistically significantly in comparison to the extensive production methods (AN0, BN0, CN0) and in the white flour of this cultivar only that in the production method BN2 was contaminated

with DON statistically significantly, we have concluded that the cultivar 'Savinja' was more resistant to the contamination of grains with DON than the cultivar 'Reska'. In the latter, both intensive production methods (BN2, CN2) were contaminated with DON more significantly than the extensive methods (AN0, BN0, CN0) (Table 6).

Table 6: Statistical difference between the contamination with DON in various production methods in white and dark flour* of the cultivars 'Reska' and 'Savinja', IOSDV Jable, 2008; transformed values of contamination *√(1/2 + x)*

Preglednica 6: Statistična razlika med kontaminacijo z DON pri različnih metodah pridelovanja v beli in črni moki kultivarjev 'Reska' in 'Savinja', IOSDV Jable, 2008; transformirane vrednosti kontaminacije *√(1/2 + x)*

Transformed values of mycotoxin DON (√1/2+x)				
Production methods	'Reska'		'Savinja'	
	White flour	Dark flour	White flour	Dark flour
BN2	14.14 a**	24.29 *a*	18.87 a	13.10 a
CN2	19.32 a	19.49 *a*	0.71 b	0.71 a
CN0	0.71 b	0.71 *b*	0.71 b	0.71 a
BN0	0.71 b	0.71 *b*	0.71 b	0.71 a
AN0	0.71 b	0.71 *b*	0.71 b	0.71 a

*White flour of type 405, dark flour of type 1050
* Values designated with the same letter do not differ statistically significantly at $p = 0.05$ (Duncan's test);

Since the white flour of the cultivar 'Savinja' was evidentiary contaminated with DON in all three repetitions of the method BN2 and the dark flour only in two of them (Table 4) it is logical to speculate whether the difference between the contamination of dark and white flour was statistically significant. Testing of the characteristics of differences between the contamination of dark and white flour in the method BN2 was performed using the 'method of pairwise comparison' (Weber, 1961). The test showed that the contamination of white and dark flour with DON in this production method did not differ statistically at $p \leq 0.05$ (Table 7).

Table 7: Testing differences in the contamination with the mycotoxin DON between three repetitions of samples of white (w) and three repetitions of samples of dark (d) flour of the cultivar 'Savinja' in IOSDV Jable in 2008; transformed value of infection *√ (½ + x)*

Preglednica 7: Razlike v kontaminaciji z mikotoksinom DON med vzorci treh ponovitev bele (w) in treh ponovitev črne (d) moke kultivarjev 'Savinja' v IOSDV Jable 2008; transformirane vrednosti kontaminacije *√ (½ + x)*

Method	Arithmetic mean	Variance	(x_1-x_2)	$T_{izr(0,05)}$	$T_{tab(0,05)}$	Significance (p=0.05)
wBN2*	19,22	11,65	6,58	1,93	2,78	No
dBN2**	12,64	120,99				

*White flour, method BN2
**Dark flour, method BN2

3.2 Mycotoxin ZEA found on the wheat grain samples of the cultivars 'Reska' and 'Savinja'

The contamination of wheat flour with mycotoxin ZEA was not detected in any of the production methods on the location of IODSV Jable in 2006. However, in the year 2008 mycotoxin ZEA appeared in the dark flour samples of the cultivar 'Reska' (within the limit of detection <5 µg ZEA kg^{-1} flour) in all repetitions of intensive production methods (BN2, CN2) and in one repetition of extensive method in which the stable manure without min-N fertilization had been applied (BN0). From the Table 10 it is evident that in the white flour of the cultivar 'Reska' the mycotoxin ZEA did not occur in any of the production methods (in frame of the limit of detection using the method chosen). The dark flour of the cultivar 'Reska' was contaminated with ZEA in all three repetitions of both intensive production methods (BN2, CN2) and only one repetition of the method BN0 among extensive production methods. Based on the above findings it may be concluded that in both intensive production methods of the cultivar 'Reska' contamination with the mycotoxin ZEA was significantly more expressed in the dark flour than in the white one in which within the limit of detection using the chosen method the mycotoxin ZEA was not detected.

In the cultivar 'Savinja' the contamination with ZEA was proven in lesser number of samples than in the cultivar 'Reska' and it was less dependent on the production methods (Table 8). The differences between the occurrence of mycotoxin ZEA in dependence on production method and flour type in the cultivar 'Savinja' were not as pronounced as in the cultivar 'Reska' and the occurrence of the mycotoxin ZEA was less frequent, too. The statistical significance of the influence of production methods on the contamination of flour with the mycotoxin ZEA is presented in Table 9.

Table 8: Contamination of white and dark flour* (<5 µg ZEA kg^{-1} flour) of the cultivars 'Reska' and 'Savinja' with the mycotoxin ZEA in dependence on the production methods in IOSDV Jable, year 2008 (µg ZEA kg^{-1} flour)

Preglednica 8: Kontaminacija bele in črna moke* (<5 µg ZEA kg^{-1} moke) kultivarjev 'Reska' in 'Savinja' z mikotoksinom ZEA v odvisnosti od načina kultivacije, IOSDV Jable, 2008 (µg ZEA kg^{-1} moke)

µg ZEA kg^{-1} flour	'Reska'								'Savinja'							
	White flour				Dark flour				White flour				Dark flour			
Block	I	II	III.	Mean	I	II	III	Mean	I	II	III.	Mean	I	II	III	Mean
AN0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BN0	-	-	-	-	14	-	-	4.7	-	-	-	-	-	-	-	-
CN0	-	-	-	-	-	-	-	-	-	-	-	-	22	-	-	7.3
BN2	-	-	-	-	16	9	79	38	-	7	-	2.3	18	44	-	20.7
CN2	-	-	-	-	22	3	12	15.7	-	-	-	-	6	-	-	2.0

<5 µg ZEA kg^{-1} flour
*White flour of the type 405, dark flour of the type 1050

Table 9 displays a statistically significant influence of production methods in the dark flour of the cultivar 'Reska' on the occurrence of the mycotoxin ZEA. In the cultivar 'Savinja', too, ZEA occurred more often in the dark flour than in the white one. Using F-test did not help prove a significant influence of production methods on the occurrence of mycotoxin ZEA either in dark flour or in the white one.

Table 9: Significance of contamination of white and dark flour of two cultivars with the mycotoxin ZEA in dependence on production methods; IOSDV Jable, 2008; transformed values of contamination $\sqrt{(1/2 + x)}$

Preglednica 9: Značilnost kontaminacije bele in črne moke dveh kultivarjev (‘Reska’ in ‘Savinja’) z mikotoksinom ZEA v odvisnosti od metode pridelovanja; IOSDV Jable, 2008; transformirane vrednosti $\sqrt{(1/2 + x)}$

Variability source	‘Reska’ Dark flour		‘Savinja’ White flour		‘Savinja’ Dark flour	
	F-test	Significance	F-test	Significance	F-test	Significance
Blocks	0.46	$p \geq 0.05$	0.94	$p \geq 0.05$	1,67	$p \geq 0.05$
Methods	6.12	$p \leq 0.00$	1.75	$p \geq 0.05$	1,92	$p \geq 0.05$

Table 10: Significance of statistical differences between production methods as to the contamination of white and dark flour* with the mycotoxin ZEA in two wheat cultivars; IOSDV Jable, 2008; transformed values of contamination $\sqrt{(1/2 + x)}$

Preglednica 10: Značilnost statističnih razlik med postopki pridelovanja glede na kontaminacijo bele in črne moke z mikotoksinom ZEA pri dveh kultivarjih pšenice; IOSDV Jable, 2008; transformirane vrednosti $\sqrt{(1/2 + x)}$

Production methods	‘Reska’		‘Savinja’	
	White flour	Dark flour	White flour	Dark flour
BN2	0.71	5.80 *a**	1.38 a	3.89 a
CN2	0.71	3.98 *a*	0.71 a	1.31 a
CN0	0.71	0.71 *b*	0.71 a	2.05 a
BN0	0.71	1.74 *b*	0.71 a	0.71 a
AN0	0.71	0.71 *b*	0.71 a	0.71 a

Values designated with the same letter do not differ statistically significantly at $p = 0.05$ (Duncan's test);
*White flour of type 405, dark flour of type 1050

Results presented in Table 10 show a significantly higher degree of contamination of the dark flour of the cultivar ‘Reska’ with the mycotoxin ZEA in the two intensive production methods (BN2, CN2) than in the extensive methods (AN0, BN0 and CN0) while the white flour was not contaminated with ZEA in any of the methods.

The cultivar ‘Savinja’ also shows higher degree of contamination found in the two intensive methods than in the extensive ones (with the exception of ZEA found in the dark flour of the method CN0); however, it was not possible to prove the significance of the differences using the F-test. The use of statistical method of pairwise comparison (t-test) has shown that in the method BN2 in which the wheat flour had the highest degree of contamination with mycotoxin ZEA, this contamination was significantly higher in the dark flour than in the white one (Table 11).

Table 11: The difference in the contamination with mycotoxin ZEA between white (w) and dark (d) flour variants BN2 of the cultivar 'Savinja' on the site IOSDV Jable in 2008 (t-test); transformed values of contamination $\sqrt{(1/2 + x)}$

Preglednica 11: Razlika v kontaminiranosti z mikotoksinom ZEA med belo moko (w) postopka BN2 in moko (d) postopka BN2 pri kultivarju 'Savinja' na lokaciji IOSDV Jable leta 2008 (t-test); transformirane vrednosti $\sqrt{(1/2 + x)}$

Method	1st block	2nd block	3rd block	Arith. mean	Variance	$(x_1 - x_2)$	Tcomp	Ttab	Sign.
wBN2	0.71	2.74	0.71	1.39	3.10	3.89	2.56	2.45	Yes*
dBN2	4.30	6.67	0.71	3.89	32.16				

*At $p \leq 0.05$

4 DISCUSSION

The results obtained in the investigation have shown the contamination of wheat flour to be dependent on all the factors studied: from the influence of production methods, cultivar and year of production to the weather. The year of production had a great influence on the occurrence of the mycotoxins DON and ZEA. Since the experiment was conducted on the same location in both years with the same two cultivars and equal production methods (with regard to the degree of intensity), the fact that DON and ZEA occurred only in 2008 and not in 2006 proves that the weather had a significant impact on the occurrence of both mycotoxins. This finding is in accordance with the investigations in which the impact of weather on the contamination of wheat grain with the mycotoxins DON and ZEA was established (Whitlow and Hagler, 2009, Prandini et al., 2009). Some other researchers talk about the impact of location (Zemljič et al., 2008), but also their findings can be related to the influence of weather or climate as other location may also imply other weather conditions.

As it is almost impossible to influence the yearly weather conditions, from the producer's standpoint the result saying that the production method has also a great influence on the occurrence of DON and ZEA is more useful. We have proved that in this frame an important role is played by the intensity of N-min (mineral nitrogen) fertilization. In both cultivars, the methods with medium rate of N-min (130 kg N ha^{-1}) were as a rule significantly more contaminated than the production methods with no N-min fertilization. In an investigation conducted in similar natural conditions (Zemljič et al., 2008) it was impossible to establish whether fertilization with N-min had an impact on the occurrence of mycotoxin DON, the content of which was studied in wheat grains. However, opposite to our investigation conducted under the circumstances of a permanent field experiment, their field experiment was conducted only one year and only in variants of more or less intensive methods and not in the variants of extensive ones. Since without nitrogen fertilization the mineralization of organic matter in the soil decreases to the point in which the fertilization with nitrogen becomes more pronounced, static permanent field experiments with diversified nitrogen rates which also include variant with no nitrogen fertilization allow better options (Smith et al., 2001).

For a safe production and use of food the results obtained with the grinding of wheat grain in white (type 400) and dark flour (type 1000) are important as well as the establishment of difference between them as far as the contamination with DON and ZEA are concerned. With the extensive production methods no statistically significant differences were found between the white and dark flour in the sense of contamination with DON, however, in this respect there was a significant difference in the

intensive production methods. With the cultivar 'Reska' the white flour was not proven to be ZEA contaminated in all production methods but with the cultivar 'Savinja' the dark flour in the method BN2 (the only method in which the mycotoxin ZEA occurred in the white flour) was significantly more contaminated with the mycotoxin ZEA than the white flour. As for babies the safety threshold lies at 20 µg ZEA kg^{-1} flour, so the dark flour grown in the method of wheat fertilization with 130 kg N ha^{-1} was not safe enough for baby food in view of the fact that the average content of mycotoxin ZEA in dark flour of the method BN2 in both cultivars exceeded the allowable 20 µg ZEA kg^{-1} flour. If we want to consume healthy bread, considering the results of the present study we should avoid eating dark bread which the public considers to be healthier than the white one.

The content of mycotoxin DON in white wheat flour of the cultivar 'Reska' did not exceed in any sample the allowable content of 750 µg DON kg^{-1} flour which is the limit value valid for adult persons. However, the content of 200 DON kg^{-1} of flour, which is the limit value for safe food for children, was exceeded in white flour in all three repetitions of the method CN2 and in two repetitions of the method BN2. In the dark flour of the cultivar 'Reska' in one repetition of the method BN2 the allowable limit value for food safety for adults, which is 750 DON kg^{-1} flour, was exceeded by 250 DON kg^{-1} flour. In the remaining repetitions of intensive production methods (BN2, CN2) the DON content exceeded the limit value for food safety for children while in extensive production methods both the mycotoxin DON content was not detected either in white or in dark flour samples.

In the flour of the cultivar 'Savinja' the content of DON mycotoxin were detected only in the BN2 method, i.e. in three repetitions they were higher in white flour than the limit value of safe food for children. In dark flour only two repetitions of the method BN2 were contaminated with the mycotoxin DON, their content being above the limit of food safety for babies. All extensive production methods did not present any detectable contamination with the mycotoxin DON. The presumption that there was less mycotoxin DON in white flour than in the dark one was not confirmed, however, an increasing tendency towards Savinja as being less susceptible to the contamination with this mycotoxin than Reska has been observed.

Beside fertilization with N-min the contamination with DON and ZEA was also significantly influenced by the fertilization with organic fertilizers. The comparison of the methods BN2 and CN2 has shown that the contamination with DON was as a rule higher in BN2 than in CN2. One of the main reasons was the exceeded total N (nitrogen) in the method BN2 in comparison with CN2 as the ploughed straw contains less N (nitrogen) than the ploughed stable manure.

The comparison of the resistance of both cultivars to contamination with DON and ZEA has shown that the cultivar 'Savinja' was more resistant to the contamination with the two mycotoxins studied than the cultivar 'Reska'. The cultivar 'Reska' witnessed thus in the method including fertilization with FM and N-min (BN2) on the average of the repetitions an almost three times (2.6 times) higher contamination with DON than the cultivar 'Savinja' while in the method with straw (CN2) a contamination with DON in this cultivar was not observed at all. Similar to that, the contamination with ZEA in the cultivar 'Reska' was almost twice (1.84 times) as high in the method BN2 and almost eight times higher in the method CN2 than in the cultivar 'Savinja'.

5 CONCLUSIONS

Based on the findings of the current investigation it may be concluded that adequately chosen agro-technical measures significantly reduce the risk of excessive contamination of foodstuffs of cereal origin with the mycotoxins DON and ZEA. In this frame a too abundant fertilization with N (nitrogen) of either inorganic or organic origin is to be avoided. The extensive wheat production implies an appreciably lesser risk of DON and ZEA occurrence than the intensive one. With regard to the results obtained it may be assumed that the occurrence of the mycotoxin ZEA is less dependent on the intensity of N (nitrogen)

fertilization than the occurrence of the mycotoxin DON.

The influence of various production methods on the contamination of wheat grain with the mycotoxins DON and ZEA may be reliably confirmed only in frame of a long-term field experiment which includes both intensive and extensive methods of N (nitrogen) fertilization.

In spite of the fact that two cultivars were included in the current investigation we were able to prove that the cultivars differ with regard to resistance to the contamination with the mycotoxins DON and ZEA. It would therefore require establishing the susceptibility of individual cultivars to the contamination with mycotoxins in order to be able to avoid those more susceptible to it.

The conclusion saying that dark flour which is usually recommended as healthier was more contaminated with the mycotoxins DON and ZEA than the white one would also require further examination. As far as the presence of bran in various foods it would be worth while studying the content of both mycotoxins in bran.

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Adverse effects and intoxications related to medicinal/harmful plants

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ABSTRACT

Many wild plants around us have beneficial effects on our body and can be used as food. People are more and more interested in the medicinal plants. Many of them began gathering and preparing plants for the relief of symptoms of diseases or as a food dietary. Due to the lack of knowledge of plants, mistaking plants that contain toxins for medical plants may happen and cause adverse effects or even poisoning. The Poison Control Centre in Ljubljana keeps records of patients who have been admitted to the department because of adverse effects from the ingestion of certain plants. We analysed 64 cases, which were registered by the Poison Control Centre between January 2000 and December 2013. The aim of the present study was to determine which plants cause the most intoxications in Slovenia.

Key words: medicinal plants, intoxication, misidentification, abuse, suicidal attempt

IZVLEČEK

ŠKODLJIVI UČINKI IN ZASTRUPITVE Z ZDRAVILNIMI/STRUPENIMI RASTLINAMI

Mnogo rastlin, ki nas obdaja, ima blagodejen vpliv na naše zdravje in so lahko dodatek k prehrani. Ljudje se vse bolj zanimajo za zdravilne rastline. Ljudje so začeli sami nabirati divje rastoče rastline in jih pripravljati za lajšanje bolezenskih simptomov ali kot dodatke k hrani. Zaradi nepoznavanja rastlin pa se lahko dogajajo zamenjave zdravilnih rastlin z rastlinami, ki vsebujejo strupene snovi in lahko povzročajo neželene učinke ali celo zastrupitve. Center za zastrupitve v Ljubljani beleži paciente, ki so bili sprejeti na njihov oddelek zaradi neželenih učinkov, ki so jih utrpeli po zaužitju nekaterih rastlin. Preverili smo 64 primerov, ki so bili zapisani na Centru za zastrupitve v časovnem obdobju od januarja 2000 do decembra 2013. Cilj raziskave je bil prikazati, katere rastline predstavljajo največji problem v Sloveniji.

Ključne besede: zdravilne rastline, zastrupitev, napačna identifikacija, zloraba, samomorilnost

1 INTRODUCTION

Several wild plants have beneficial effects on human health. In developing countries herbal drugs play an important role in health care (Matthews et al., 1999; Philomena, 2011). In the past two decades there has been an increase in the use of medicinal herbs. People are increasingly seeking for herbal remedies in self medication which includes complement to conventional therapies, and maintaining their overall health condition and well-being (Spiteri Staines, 2011). There are additional advantages of medicinal herbs, some of which are that they are cheap, easily produced, in proper combination they can meet nutritional needs and they are found in all climates and terrains. There is a general belief amongst consumers around the world that medical herbs are inherently safe, because they are "natural". However, just because the product is natural, that does not assure their safety (Colombo et al., 2010; Spiteri Staines, 2011; Philomena, 2011; Matthews et al., 1999).

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All medical agents have potentially unexpected effects including toxicity and interactions, and herbs are not different. However, they are less likely adverse effects than in using conventional drugs and if such effects occur, they are mild and only affecting a small number of people (Philomena, 2011; Spiteri Staines, 2011). Contact with plants was rarely responsible for serious poisoning (Fuchs et al., 2011). Flowering plants produce thousands of secondary metabolites (alkaloids, flavonoids, saponins, tanins, essential oils, etc.), which can be either useful or very noxious for human health. It is believed they are involved in the production of poison that they use for protection from browsing animals and plant-eating insects (Colombo et al., 2010). When overdosed, incorrectly used or used regularly over a long period of time, some plants that possess "harmful effects", have the potential to induce adverse effects (Matthews et al., 1999). Consumers are largely uninformed about the possibility of adverse effects. Although most botanical products are probably safe under most conditions, some are known to be toxic at high doses and others may have potentially adverse effects under some conditions. Just as with many foods and pharmaceuticals, there is a possibility of allergic reactions (Philomena, 2011). Any pharmaceutically active agent has the potential to result in synergistic or antagonistic interaction when consumed with other pharmaceutically active compounds. Increased self-medication also increases the chance of adverse reactions to these products as well as adverse drug/herb or herb/herb interactions (Matthews et al., 1999). Patients may also substitute more conventional therapies for herbal remedies without informing the doctor. Negative effects can result from bad communication between the patient and a healthcare professional, which include adverse effects or drug-herb interactions (Kosalec et al., 2009; Spiteri Staines, 2011; Ghosh and Ghosh, 2009). The solution for that problem would be proper education and effective communication. Occasionally, plants can grow in contaminated environments. There are biological and chemical contaminants in the air and in the soil, which accumulate into the herbs (Kosalec et al., 2009). Plants can therefore contain unwanted constituents, such as heavy metals, or can be even deliberately adulterated with pharmaceutical ingredients (Philomena, 2011).

Accidental poisoning with plants should also be mentioned. Using plants as food can lead to accidental poisoning. In order to eat fresh or cooked herbs and plants, mainly during spring and summer, some people prefer to spend their free time outdoors picking up young shoots, young leaves and buds of wild plants without having sure knowledge of plant identification (Colombo et al., 2010). They use them to make fresh salads or cook them in soups. In Europe, severe plant poisoning is a rare event for which a small number of specific plants appear to be mainly responsible. In Slovenia serious poisoning in adults is most commonly caused by autumn crocus (*Colchicum autumnale* L.). Wrong identification between autumn crocus and wild garlic (*Allium ursinum* L.) leads to poisoning. The use of wild garlic has increased dramatically in the last few years. This plant, often used as a spice, grows wild in groundlayer vegetation of several broadleaved forestsin Central Europe. Leaves of wild plants collected during spring for food purpose may be responsible for intoxications. Its raw leaves are mainly used to flavour spreads based on cottage cheese, soups and sauces (Gilotta and Brvar, 2010; Fuchs et al., 2011). Dried leaves usually have a very faint odour (Colombo et al., 2010). Because of their great similarity the patients mistakenly ingested autumn crocus instead of wild garlic. Accidental colchicine poisoning is not uncommon. All parts of autumn crocus are toxic and contain an alkaloid called colchicine, which blocks the cell division by inhibition of mitosis (Gilotta and Brvar, 2010; Sundov et al., 2005). Concentration of colchicine reaches a maximum content in the seeds (Gaillard and Pepin, 1999). Crude seeds were used for medical purposes (Sundov et al., 2005). Two to five hours after ingestion the patient develops nausea and diarrhoea, along with gastrointestinal symptoms (Gilotta and Brvar, 2010; Brvar et al., 2004; Nagesh et al., 2011; Brnčić et al., 2001; Sundov et al., 2005). In cases when patients consumed higher doses, they died of multi-organ dysfunction (Klintschar et al., 1999). However, there are other poisonous plants with similar elliptical leaves as wild garlic which grow in similar environments and their vegetation period falls into the same time as wild garlic: white hellebore (*Veratrum album* L.) and other species from the genus *Veratrum* and Lily-of-the-Valley (*Convallaria majalis* L.). Both of them contain a mixture of different alkaloids.

Accidental poisoning with white hellebore or Lily-of-the-Valley mistaken for wild garlic is possible. Both plants do not show even traces of garlic odour, but their leaves are particularly similar (Gilotta and Brvar, 2010; Columbo et al., 2010; Klintschar et al., 1999; Meyer et al., 2009). Poisoning with Lily-of-the-Valley rarely occurs, but it is possible because both of them have elongated-elliptic leaves (Meyer et al., 2009). It contains digitalis toxins and is considered a mildly poisonous plant (Klintshar et al., 1999; Gaillard and Pepin, 1999). Interestingly, in accidentally poisoned patients white hellebore was nearly always mistaken for yellow gentian (*Gentiana lutea* L.) (Gilotta and Brvar, 2010; Rauber-Lüthy et al., 2010). Roots of yellow gentian are used to aromatize home-made distilled products. These roots have to be harvested in the autumn, when the yellow flowers disappear and its leaves go brown. Unfortunately, the stem and the leaves of gentians are very similar to those of *Veratrum* species, plants containing a mixure of toxic alkaloids called veratrin. The alcohol in brandy is able to extract the toxic alkaloids from the roots and the "grapa" becomes toxic (Columbo et al., 2010; Grobosch et al., 2008). White hellebore that can be mistaken for yellow gentian contains alkaloids that are soluble in alcohol, so the use of it in an alcoholic drink leads to faster absorbtion of toxic alkaloids and more rapid onset of symptoms (Gilotta and Brvar, 2010). Symptoms that occur after ingestion are mainly nausea, vomiting, vertigo and in some cases headache (Rauber-Lüthy et al., 2010).

There are plants containing psychoactive chemicals and other abusable substances around us. When taken for nonmedical reasons, usually for their mind-altering effects, they are called drugs and their use abuse. The most affected are adolescents and young adults (Ghosh and Ghosh, 2009). Jimson weed (*Datura stramonium* L.), especially seeds, is used because of its psychoactive hallucinatory effects. Concentrations of hallucinogen alkaloids vary from plant to plant, the anatomical part of the plant, the preparation method, and various environmental factors. Positive or negative effects depend on the dose (Krenzelok and Mrvos, 2011). There are many signs of poisoning with jimson weed, some of which are abnormal behavior (delirium), agitation, blurred vision, dry mouth and mucous membranes, thirst, tachycardia, nausea and vomiting, difficulty in swallowing and speech, disturbed bowel function, hyperthermia, hypertension, loss of consciousness and coma (Gaire and Subedi, 2013). The number of patients poisoned by deadly nightshade (*Atropa belladonna* L.), a perennial busy herb with black, shiny and sweet berries in autumn, is also not negligible. Some patients consumed the deadly nightshade by mistake instead of blueberries (*Vaccinium corymbosum* L.). Deadly nightshade is frequently taken by adults for the purpose of suicide or to experience its hallucinogenic effect. Accidental consumption in adults is rare (Cikla et al., 2011). Unfortunately the berries of the deadly nightshade contain some tropane alkaloides (hyoscyamine, atropine and scopolamin) (Columbo et al., 2010). The part of the deadly nightshade that contains the most alkaloids is the stem (Gaillard and Pepin, 1999). Children are most vulnerable because they do not realize the danger, and are attracted by the smell or color of plants. The number of death cases is slightly higher in adults, because most of the deaths are the result of deliberate poisoning by certain toxic plants (Krenzelok and Mrvos, 2011). At greater risk to adverse health effects associated with medicinal herbs may be young children, pregnant women and the elderly, who are more sensitive and thus more vulnerable to adverse effects. Intensity of adverse effects also depends on users age, gender, genetics, nutrition status, concurrent disease states and treatments (Matthews et al., 1999; Staines, 2011). Another important limitation is the difficulty determining the quality of the toxin the patient had been exposed to, because leaves or seeds of the same plant species may contain variable amounts of toxins, depending on the vegetation period, soil, exposure to light, and age of the plant (Fuchs et al., 2011).

The aim of this study was to define the clinical relevance of plant toxicity for humans in Slovenia and to identify which plants may actually lead to severe poisoning. The study obtains an overview of the more common and more relevant plant exposures reported to the University Medical Centre in Ljubljana, Poison Control Centre.

2 MATERIALS AND METHODS

We checked the records of the patients admitted to the Poison Control Centre. In the Poison Control Centre only adults are treated. Among these we further investigated only patients who suffered from the side effects of plant ingestion. In the study we investigated cases that occured during the 13-year period between January 2000 and December 2013. We analysed 64 cases of human plant exposure, which were reported to the Slovenian Poison Control Centre. Data were edited and graphically staged using Microsoft Excel.

3 RESULTS

During the 13-year period, the Poison Control Centre in Ljubljana recorded a total of 64 cases of human exposure to toxic plants. Anual the number of poisonings with ingested plants varied. Peak intoxications with plants occurred in 2011 (12 cases) and in 2003 (7 cases) (Fig 1). The year with the fewest human intoxications with plants was 2008, with just 2 cases. In 2000 there were no reports of human poisoning with plants.

In 46 cases, the exposure was accidental. From these 46 accidental cases, 39 were because of mistakenly ingested plants (Table 1) and 8 because of incorrectly usage (Table 2). In 15 cases it was the result of the abuse and in 3 cases the result of a suicidal attempt. In the case of accidental exposure the most frequent plant was autumn crocus. In the case of abuse, jimson weed was the most common plant. *Aconitum, Atropa belladona* and *Lonicera* species were the plants used in suicide attempts. 13 different plants were responsible for the poisonings and side effects in this study. Autumn crocus was at the top of the list with 20 cases, followed by jimson weed with 15 cases, and white hellebore with 13 cases.

Table 1: Number of poisoned patients that mistakenly ingested wild plants reported in the register book between 2000 and 2013

Wild plant used as food	Ingested part of plant	Use	Misidentified plant	Adverse effects	Cases
Allium ursinum L. Wild garlic	Leaves	Cardiovascular benefits	*Colchicum autumnale* L. Autumn crocus	Abdominal pain, diarrhoea, vomiting, nausea and dizziness, at high doses multi-organ dysfunction	20 (2 fatal)
Allium ursinum L. Wild garlic	Leaves	Cardiovascular benefits	*Veratrum album* L. White hellebore	Vomiting, nausea, increased sweating	6
Gentiana lutea L. Yellow gentian	Roots soaked in brandy	For stomach problems	*Veratrum album* L. White hellebore	Vomiting, nausea, increased sweating,	7
Asarum europaeum L. European wild ginger	Roots	Expectorant	*Ranunculus ficaria* L. Lesser celandine	Nausea, vomiting and diarrhoea	1
Pinus pinea L. Stone pine	Seeds	Food additive	*Ricinus communis* L. Castor bean	Nausea, headache, heavy breathing, vomiting and diarrhoea	1
Robinia pseudoacacia L. Black locust	Flowers	For inflammation	*Laburnum anagyroides* Medic. Golden chain or *Laburnum alpinum* J. Presl Alpine golden chain	Nausea, dizziness, visual disturbances and diarrhoea	3
Ruta graveolens L. Rue or herb-of-grace	Leaves soaked in brandy	For stomach problems	*Arnica montana* L. Arnica	Nausea, dizziness and vomiting	1

Of the 64 cases included in this study 39 were due to accidental exposure, because of mistakenly ingested plants, and all of them were adults (Table 1). The most frequently accidentally ingested plant was autumn crocus (20 case reports of accidentalal poisoning with (*Colchicum autumnale*), and white helleborere (*Veratrum album* L.) (13 cases). Of the 64 cases included in this study, mistletoe (*Viscum album* L.) caused adverse effect because of overdose. One patient suffered adverse effects from regularly consuming club moss (*Lycopodium clavatum* L.) over a long period of time. The plant used as a drug was jimson weed. Shoots of Norway spruce (*Picea abies* (L.) H. Karst.) triggered allergic reactions in one case. Of the 64 poisonings included in this study, 2 adult patients attempted suicide by poisoning using *Aconitum napellus* L.Em. Skalicky, deadly nightshade and *Lonicera* species. In this study 6 different plants were responsible for poisoning, because they were mistakenly ingested. 20 patients ingested autumn crocus, and 13 white hellebore. 26 of the patients ingested the said poisonous plants instead of wild garlic. 7 patients ingested white hellebore instead of yellow gentian. The Poison Control Centre also lists a case, when one patient ingested lesser celandine (*Ranunculus ficaria* L.) instead of European ginger (*Asarum europaeum* L.), and when one patient ingested castor bean (*Ricinus communis* L.) instead of stone pine (*Pinus pine*). 3 patients ingested golden chain species (*Laburnum* spp.) instead of black locust (*Robinia pseudoacacia* L.). One case reported that woman drunk flowers of arnica (*Arnica montana* L.) soaked in brandy instead of rue (*Ruta graveolens* L.).

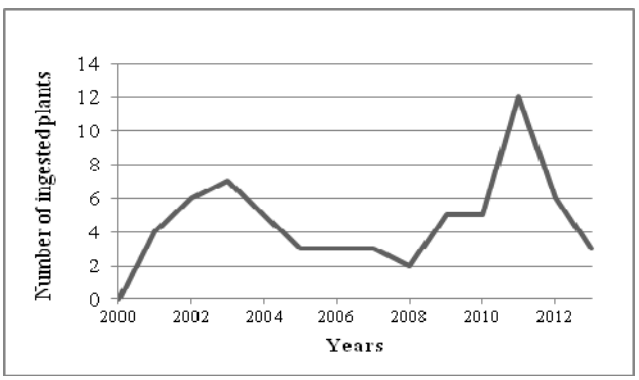

Figure 1: Plant ingestions by year: 2000-2013

Table 2: Number of poisoned patients that incorrectly used wild plants reported in the register book between 2000 and 2013

Plant	Use	Harmless effects	Cases
Viscum album L. Mistletoe	Antispazmolitic performance, for regulation of neurosis and high blood presure	Chest tightness, abdominal pain and diarrhoea	1
Lycopodium clavatum L. Club Moss	For liver and biliary disorders	Fatigue and vomiting	2
Picea abies (L.) Karsten Norway spruce	For respiratory diseases	Allergic reaction	1
Atropa belladona L. Deadly nightshade	Abuse in some cases	Disorientation, hallucinations	4
Datura stramonium L. Jimson Weed	Abuse	Hallucinations	15
Aconitum spp. Aconite	For suicide attempt	Nausea, vomiting	1
Lonicera spp. Honeysuckles	For suicide attempt	Nausea, vomiting	1

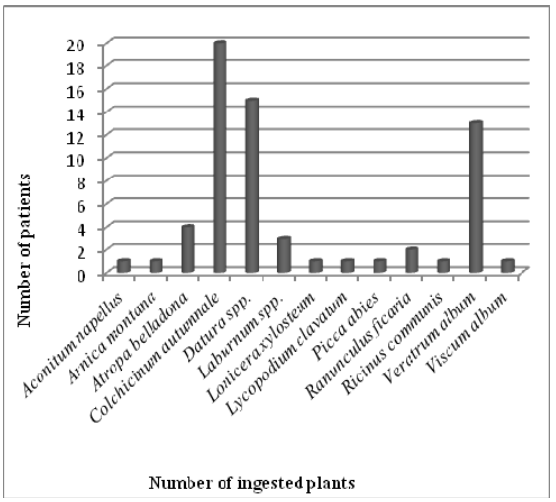

Figure 2: Plants which were ingested by patients in the period between 2000 and 2013

Over 13-year period patients aged 15 to 85 came in contact with poisonous plants (Fig. 3). There were no significant differences between the fequency in males and females. Most adverse effects occurred to those younger than 18 (10 patients) and those adults aged 45 to 60 years (23 patients).

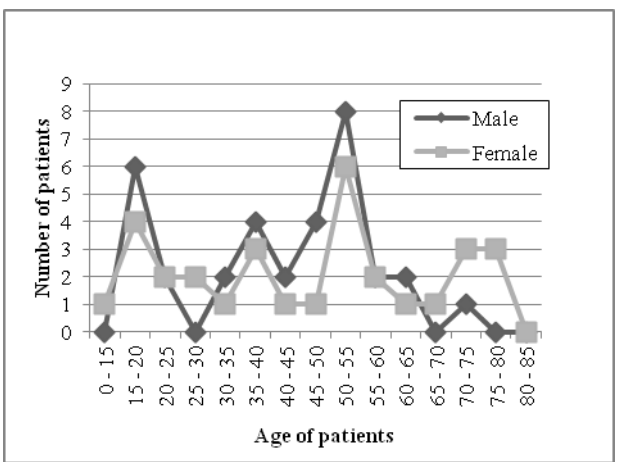

Figure 3: Number of intoxications with plants by sex and age of the patients

4 DISCUSSION

Side effects that occur after the ingestion of plants products are rare. However, the number of such cases is not negligible, and they occur every year. The problem is that people believe just in the benefits of plant products, but there were many more adverse effects about which we do not know about. People would need to be aware that plants contain a number of bioactive compounds which cause adverse effects and toxicity to humans. Small doses of highly toxic plants are sufficient to induce severe symptoms. Severe and fatal poisoning could also be observed in the accidental settings. At the Poison Control Centre in Ljubljana the most frequent cause of poisoning with toxic plants is accidental poisoning. It was followed by deliberate abuse and suicidal intent. Plant intoxications vary according to the season. Thus the most cases of poisoning were reported in spring

and at the end of summer. Many people prepared salads and soups from wild plants. Ingestion of plants for suicidal intent was performed exclusively by adult patients. Since wild garlic became widely known, many people have tried to pick the plant in the nature. Since there are other toxic plants with similar leaves, several cases of poisoning have been report in recent years. Particularly similar are autumn crocus (20 cases, 2 of which were fatal), and white hellebore (6 cases). Especially in spring, the leaves of these two plant species look very much alike and are often confused. In these reports, the most common plant species to have caused poisoning was autumn crocus in 20 cases. Two patients, an elderly couple, died after ingestion. The elderly are more sensitive and they died of multiorgan-failure. Intoxications with autumn crocus are followed by jimson weed, which caused serious poisoning in adolescents. The third most commonly ingested toxic plant, which caused seven intoxications, was white hellebore. People mistakenly soaked roots of white hellebore instead of yellow gentian roots in brandy. Four patients accidentally ingested white hellebore instead of wild garlic because of their great similarity, particularly their elliptic leaves and the fact they grow in the same places during spring even though they have different in smell and taste. Two adults ingested species of golden chain flowers (*Laburnum* spp.). Both of them mistakenly used flowers of said plant instead of black locust. Both of the plants have hanging flowers. One reported case of poisoning was with the castor-oil plant. A man that mistakenly ingested the seeds of that plant thought the seeds belonged to stone pine (*Pinus pinea* L.). There were four cases of poisoning with the deadly nightshade. An elderly woman mistakenly ingested the berries of the deadly nightshade and suffered minor poisoning. That was probably because of her years. In two cases, the mode of poisoning remained unknown. In one case the man ingested the berries of mentioned plan to make a suicide attempt. Mistletoe (*Viscum album* L.) caused adverse effects because of overdose. One patient suffered adverse effects because he had been taking the plant club moss (*Lycopodium clavatum* L.) over a long period of time. The plant taken as a drug was jimson weed: 15 cases were reported, and all of them were adolescents. Shoots of Norway spruce (*Picea abies* (L.) H. Kerst.) triggered allergic reactions in one case. Of the 64 poisonings included in this study, 3 adult patients poisoned themselves with *Aconitum napellus* L.Em. Skalicky and with *Lonicera* in an attempted suicide respectively. Plant-associated poisoning affected all age groups, most frequently adults. In 15 cases, the affected were adolescents and an elderly person, who were poisoned by autumn crocus and the deadly nightshade. There is no significant difference between males and females. The most commonly exposed age groups were those younger than 18 years and those between the ages of 45 and 60 years.

5 CONCLUSION

There are many poisonous plants containing alkaloids that cause adverse effects when ingested. Active molecules in certain plants can cause adverse effects in our body. Serious poisoning with plants seems to be a very rare event in Slovenia. Nevertheless, even accidental ingestions can be responsible for fatal poisonings. When people picking edible wild plants, it is crucial that they are correctly identified, because many are similar to poisonous plants in appearance. Every year in spring such misidentification mistakes regularly result in poisoning accidents. Autumn crocus and white hellebore are easily mistaken for wild garlic. In addition, many edible plants have poisonous parts. It is important to learn how to identify poisonous plants that grow in the house, yard and neighbourhood. We should only eat plants that we can positively identify and know that they are safe to eat. It is suggested to use caution when picking outdoor wild plants. Warnings in the media about such potentially very dangerous mistakes are far too rare.

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The content of minerals in Slovenian organic and conventional produced fruits, herbs and vegetables

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ABSTRACT

The present study aims to compare mineral composition of fruits, herbs and vegetables grown conventionally and according to organic practice. Fruits, herbs and vegetables have been identified as leading dietary source of antioxidants, vitamins and minerals. These compounds are very variable in the composition and in the concentration among cultivars and species. Determination of calcium (Ca), potassium (K), phosphorus (P) sulphur (S) and chlorine (Cl) was performed with Energy dispersive X-ray fluorescence spectrometry (EDXRF). We found that among organic crops basil, parsley, pears, peppers, rockets and celery had higher calcium contents in comparison to conventional ones. Organically produced broccoli, eggplant, parsley, rocket and celery had higher potassium contents as well as pepper, rockets, celery, beetroots and tomatoes had higher phosphorus contents. Likewise, higher sulphur content was found in organically produced parsley, rocket, celery and raspberries. Results of this study demonstrated that in general there are no rules in the content of minerals between different farming systems.

Key words: mineral content, EDXRF, organic farming, conventional farming, fruits, herbs, vegetables

IZVLEČEK

RAZLIKE V VSEBNOSTI MINERALOV MED SLOVENSKIM EKOLOŠKO IN KONVENCIONALNO PRIDELANIM SADJEM, ZELIŠČI IN ZELENJAVO

Namen opravljene študije je narediti primerjavo vsebnosti mineralov ekoloških in konvencionalnih pridelkov glede na način kmetovanja. Sadje, zelišča in zelenjava so poznani kot glavni vir prehranskih antioksidantov, vitaminov in mineralov. Te spojine se razlikujejo glede na sestavo in koncentracijo znotraj posameznih vrst. Energijsko disperzijska rentgenska fluorescentna spektrometrija (EDXRF) je bila uporabljena za določevanje vsebnosti kalcija (Ca), kalija (K), fosforja (P), žvepla (S) in klora (Cl). Ugotovili smo, da so imeli med ekološkimi pridelki večje vsebnosti kalcija bazilika, peteršilj, hruška, paprika, rukola in zelena. Večje vsebnosti kalija so bile v ekološkem brokoliju, melancanu, peteršilju, rukoli in zeleni, v primerjavi s konvencionalnimi pridelki. Večje vsebnosti fosforja so bile v ekološki papriki, rukoli, zeleni, rdeči pesi in paradižniku. Glede na konvencionalne pridelke je bila večja vsebnost žvepla izmerjena v peteršilju, rukoli, zeleni in malinah. Rezultati so pokazali, da se vsebnost mineralov v pridelkih ne razlikuje glede na različen načinov kmetovanja.

Ključne besede: vsebnost mineralov, EDXRF, ekološko kmetijstvo, konvencionalno kmetijstvo, sadje, zelišča, zelenjava

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1 INTRODUCTION

Minerals in the crops are beside vitamins, antioxidants, flavonoids and phytochemicals one of the most important nutrients, which have been reported to contribute to the human health (Rembialkowska, 2007; Crinnion, 2010). It has been suggested that fruits and vegetables may be important for bone health because of the alkaline salts they provide (Macdonald 2007; Žnidarčič et. al., 2011). Conventional farming utilise fertilizers which contain soluble inorganic nitrogen and other elements, which are directly available to plants (Rapisarda in sod., 2005). In addition, organic crops have a longer ripening period compared to conventional ones due to slower release of the supplied nutrients (Oplanić et al., 2009a). Consequently, there could be expected a higher content of these nutrients in the products (Brandt in Molgaard, 2001).

In the organic farming the use of chemical - synthetic pesticides and readily soluble mineral fertilisers are not allowed (Oplanić et al., 2009b). Organic farming practise is diverse range of crops rotation and sustainable soil tillage (Woese in sod., 1997). Although conventional practises results in the reliable high-yield crops, there are worries regarding the negative biological and environmental consequence (Worthington, 2001; Lairon, 2010). Regarding mineral content a number of comparative studies showed lower nitrate content and less pesticide residues, but usually higher levels of vitamin C and phenolic compounds as well as more minerals. Worthington, 2001 found more iron (21 %), magnesium (29 %) and phosphorus in organic crops, compare to conventional. More potassium, magnesium, phosphorus, sulphur and cooper but less iron and manganese were found by Wszelaki et al., 2005. High concentration of iron, potassium, calcium and phosphoruh and low levels of sodium was found in organic vegetables (Schuphan, 1975). In analysis which included only statisfactory quality studies organic products had a significantly higher content of phosphours and organic crops had significantly higher content of nitrogen (Dangour in sod., 2009).

During the last decades many studies compared organic and conventional crops. Most of the crops were bought in the markets (Ismail in sod., 2004; de Souza Araújo in sod., 2014) what is the easiest way for acquiring samples. However, it is hard to get information about environmental influences (Žnidarčič D., 2012). The main problem in the comparative studies between organic and conventional crops is how to select appropriate experimental fields that they truly represent expected environment. To minimize the effect of climatic conditions, variety, irrigation, ripening time and storage it is necessary to have a controlled field, where most of the factors could be either controlled or at least recorded (Perez-Lopez in sod., 2007; Aldrich in sod., 2010; Bavec in sod., 2010).

The objective of the present study was is to assess the influence of agricultural management system including organic and conventional on mineral composition of fruits, herbs and vegetables.

2 MATERIAL AND METHOD

2.1 Plant material

Herbs (basil, parsley, celery) and vegetables (broccoli, beetroot, cucumber eggplant, rocket, tomato, and cherry tomato) were grown in experimental field, where mineral fertilizers have not been used for more than 30 years. The same varieties of crops were used in both farming system. Basic soil cultivation, sowing, and harvesting dates and methods were identical for organic and conventional experimental plot. Organic crops were only irrigated while conventional crops were fertilized with Plantella extra plus NPK (15:15:15), according to instructions given by the manufacturer, on the 14^{th}, 21^{st}, 28^{th}, 35^{th} and 42^{nd} day of growth period. The climatic conditions, variety, irrigation, ripening time, and storage conditions were the same for the vegetables and herbs grown organically and conventionally.

Samples of same variety of fruits (apple, cherry, pear and raspberry) were obtained from known

organic and conventional farms in Slovenia. Organic fruits derived from certified organic productions and had certification for organic farming, according to the Institute of Certification (KON-CERT Maribor, Slovenia).Conventional fruit samples were from conventional farms.

2.2 Methods

The samples were nondestructively analysed by EDXRF (Energy Dispersive X-ray Fluorescence) in order to determine P, S, Cl, K and Ca. From 0.5 to 1.0 g of powdered sample material pellets were prepared using a pellet die and hydraulic press. As primary excitation sources, the radioisotope excitation sources of Fe-55 (25 mCi), and Cd-109 (20 mCi) from Eckert and Ziegler Isotope Products (Germany) were used. The emitted fluorescence radiation was measured by the EDXRF spectrometer composed of a Si(Li) detector (Canberra), a spectroscopy amplifier (Canberra M2024), ADC (Canberra M8075) and PC based MCA (S-100, Canberra). The spectrometer was equipped with a vacuum chamber. The energy resolution of the spectrometer was 175 eV at 5.9 keV. The measuring time was 4000 s. The analysis of complex X-ray spectra was performed by the AXIL (Van Espen, 1993) spectral analysis program. The evaluated uncertainty of this procedure included the statistical uncertainty of measured intensities and the uncertainty of the mathematical fitting procedure. The overall uncertainty of spectral measurement and analysis was in most cases better than 1 %. Quantification was then performed utilizing QAES (Quantitative Analysis of Environmental Samples) software developed in J. Stefan Institute (Nečemer, 2011). The estimated uncertainty of the analysis was around 5 % to 10 %. Rather high total estimated uncertainty is mainly due to contributions of matrix correction and geometry calibration procedures, which include errors of tabulated fundamental parameters, and also contributions of spectrum acquisition and analysis.

The results of elemental content were expressed as mg per kg of dry matter (mg/kg DM).

2.3 Statistical analysis

The data were analysed using SAS/STAT statistical software (SAS, 2012). Differences in mineral contents among three groups of crops and two farming systems were graphically presented using SGPLOT procedure. Pearson correlation coefficients were estimated using the CORR procedure.

3 RESULTS

3.1 Calcium content in organic and conventional crops

Calcium content in crops was in the most cases quite uniform regarding different methods of farming. Basil, parsley, rocket and celery had higher content of Ca in organic samples as compared to conventional one (Fig. 1). On the other hand, calcium content was higher in the conventionally grown broccoli and cucumber as compared to organic ones. Likewise, Ca content of eggplants, tomatoes and beetroots were quite higher in conventionally grown crops. The highest calcium content was found in the organic rocket with the value of 46,700 mg/kg. The lowest Ca content belonged to conventionally produced pear (350 mg/kg).

When we grouped samples, the order from the highest to the lowest contents of calcium was as follows: organic herbs> conventional herbs> conventional vegetable> organic vegetable> conventional fruits> organic fruits. This are not in accordance with many other studies (Worthington, 2001; Perez-Lopez et al., 2007; Roussos in Gasparatos, 2009; de Souza Araújo et al., 2014), where calcium content were higher in the organically produced fruits and vegetables as compared to conventionally produced.

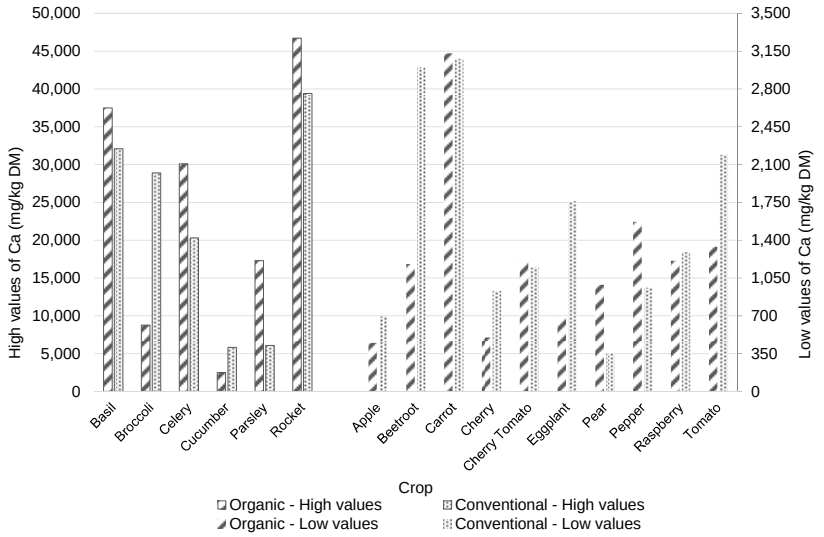

Figure 1: The comparison of calcium content (Ca) in the crops from two production systems

3.2 Potassium content in organic and conventional crops

Taking into consideration different farming systems potassium content was higher in organic broccoli, eggplant, parsley, rocket and celery compared to conventional the same crops (Fig. 2). On the other hand, potassium content was higher in conventionally grown basil, cherry, pear, apple, carrot, cucumber, raspberry, pepper, tomato, cherry tomatoes and beetroot. The highest potassium content was measured in the conventional beetroot with the value of 51,300 mg/kg. The lowest content had organic apple (5,310 mg/kg).

When we grouped samples the order from the highest to the lowest potassium content was as follows: organic herbs> conventional herbs> conventional vegetable> organic vegetables> conventional fruits> organic fruits. Most crops in this study had higher potassium content produced in conventional farming what is in accordance with Kristl et al. (2013) but it is not in agreement with the findings of Roussos and Gasparatos (2009). Higher calcium and potassium contents in the conventional green pepper as compared to organic one, is in accordance with Perez-Lopez et al. (2007).

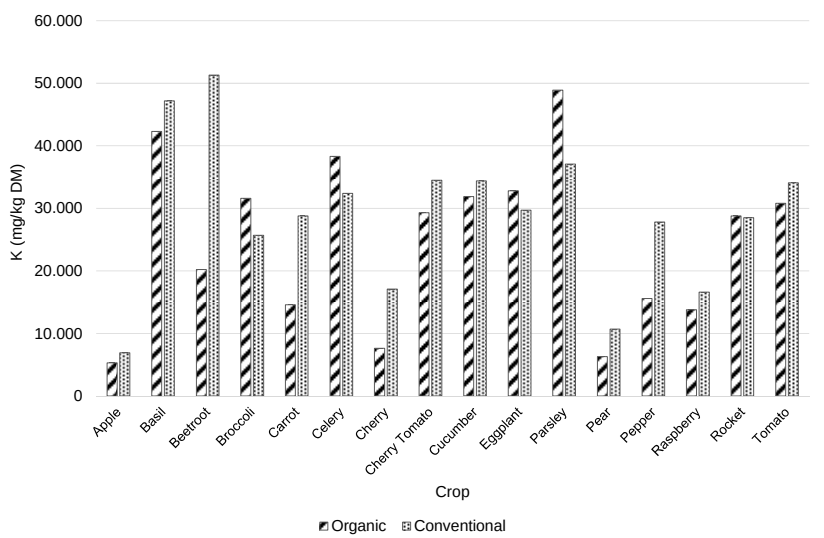

Figure 2: Comparison of potassium content (K) in the crops from two production systems

3.3 Phosphorus content in the organic and conventional crops

Phosphorus content was in most cases higher in conventionally grown crops as compared to organic crops. Only pepper, tomato, rocket, celery and beetroot had higher content of phosphorus in the organic samples as compared to conventional (Fig. 3) what is in accordance to Dangour et al. (2009). The highest content of phosphorus was determined in conventionally grown broccoli (7,270 mg/kg) and the lowest content in the organic apples (355 mg/kg).

Among three groups of crops, the highest average phosphorous content belonged to herbs, followed by vegetables and fruits. All conventional crops had higher phosphorous content as compared to organic crops.

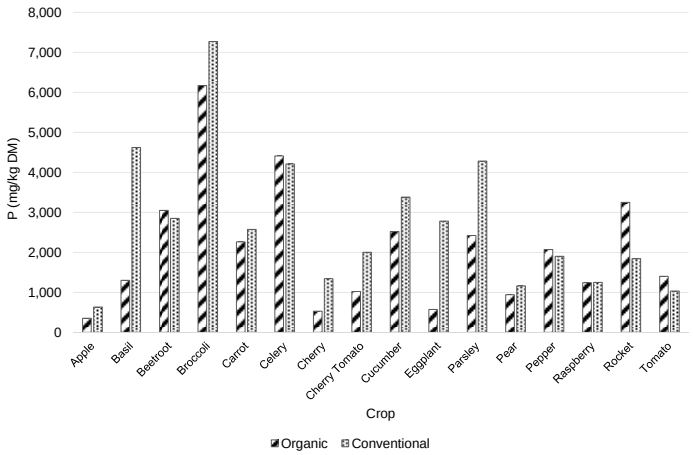

Figure 3: Phosphorus content (P) in the crops from two production systems

3.4 Sulphur content in the organic and conventional crops

Sulphur content was higher in conventional crops, except parsley, rocket and celery, where higher values were determined in organic crops (Fig. 4). The highest sulphur content was determined in conventional broccoli with the value of 13,600 mg/kg and the lowest content in the organic apple (181 mg/kg).

Among all three groups of crops, the highest average sulphur content belonged to herbs, followed by vegetables and fruits. All groups of conventionally grown products had higher sulphur contents than organic.

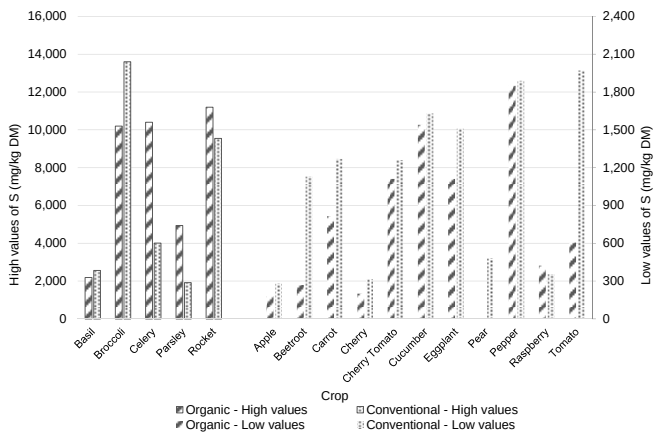

Figure 4: Sulphur content (S) in the crops from two production systems

3.5 Chlorine content in the organic and conventional crops

For chlorine content there the highest value in the beetroot from conventional farming and in the celery from organic production (Fig. 5). Cherry tomatoes, rocket, parsley, eggplant, broccoli and basil had a higher content of chlorine in organic production; while others in conventional farming.

The highest chlorine content was determined in organic celery with value 14,100 mg/kg. The lowest content had organic apple (70.3 mg/kg).

When we grouped samples in crops the order from the highest to the lowest chlorine content was as follows: organic herbs > conventional vegetables > conventional herbs > organic vegetable > conventional fruits > organic fruits.

On the non-selective medium, 3.0% of regenerants failed (Table 3).

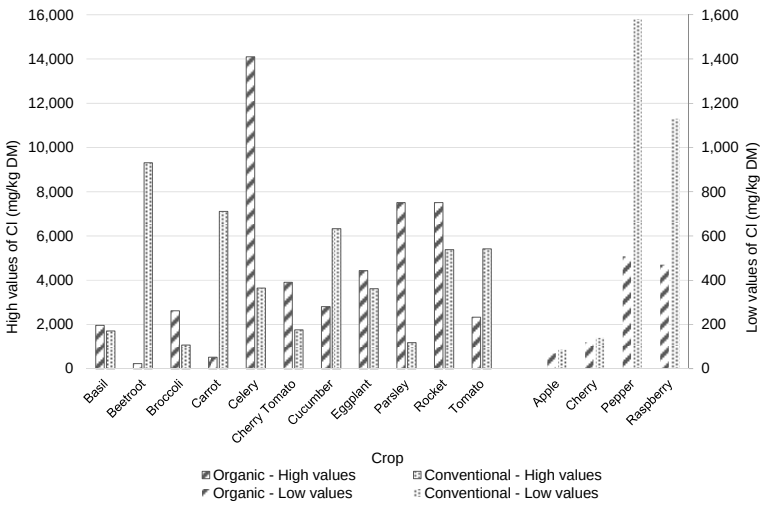

Figure 5: Chlorine content (Cl) in the crops from two production systems

3.6 Correlation between the contents of determined minerals

Table 1 presents the correlations between minerals. Estimated correlations were high and statistically significant between sulphur and calcium (r = 0.75), between phosphorus and sulphur (r = 0.70) and between chlorine and potassium (r = 0.61). However all other correlations were statistically significant, but with low Pearsons correlations coefficients, with the exception of estimated correlations between phosphorus and chlorine as well as between sulphur and potassium, where correlations were not statistically significant.

Table 1: Pearson correlation coefficients among minerals contents

			S	Cl	Ca	K
Phosphorus	(P)	Correlation	**0.697**	0.218	**0.456**	**0.445**
		p-value	<0.001	n.s.	0.01	0.01
		n	31	30	32	32
Sulphur	(S)	Correlation		**0.427**	**0.750**	0.280
		p-value		0.02	<0.001	n.s.
		n		30	31	31
Chlorine	(Cl)	Correlation			**0.381**	**0.611**
		p-value			0.04	<0.001
		n			30	30
Calcium	(Ca)	Correlation			1.	**0.425**
		p-value				<0.02
		n				32

n-number of observation; Correlation-Pearson correlation coefficient; n.s. – not statistical significant.

4 DISSCUSSION AND CONCLUSIONS

Worthington (2001) compared results from 1240 studies and shown that organic fruits and vegetables contained more minerals than conventionally grown crops. Likewise, Wszelaki et al. (2005) found more K, Mg, P, S and Cu in organically grown potatoes in comparison to the conventional potatoes. Comparing the effect of organic and conventional farming systems on fruit quality is inherently difficult due to the wide range of factors that can potentially affect crop composition such as climate, soil conditions, cultivar, soil type, planting date, harvesting time and growing seasons (Lopez in sod., 2013). Widely excepted reason is that organic matter in soil make minerals due to slower release less prone to leaching and thus more available to be absorbed by the roots (Brandt in Molgaard, 2001). Soil's pH have been shown to modulate the uptake of the macronutrients Ca and Mg and micronutrients zinc, manganese and iron (Lammerts van Bueren in sod., 2011).

Regardless many authors (Schuphan,1975, Roussos and Gasparatos, 2009; de Souza Araújo et al., 2014) reported higher contents of minerals (Ca, K and P) in the crops from organic farming in our study higher mineral contents were determined mostly in conventional crops. Among organically grown samples more calcium was determined in basil, parsley, pear, pepper, rocket and celery. Higher potassium contents had broccoli, eggplant, parley, rockets and celery while higher phosphorus contents had pepper, rocket, celery, beetroot and tomato from organic compared to conventional farming. The mineral content of these crops were in accordance with literature mentioned above.

Higher sulphur content in the organic samples from this study was determined only in parsley, rocket, celery and raspberries.

Dangour et al. (2009) compare 46 studies and found no evidence of a difference between organic and conventional production method for contents

of minerals (magnesium, potassium, calcium, zinc and copper).Only phosphorus was significantly higher in organically produced crops.

In conclusion we could say that our results demonstrated mineral content varied considerably depending on the cultivars and species. Differences in mineral content among three groups of crops were higher than differences in mineral content between organic and conventional farming system. Generally we could not confirm higher content of minerals in the organic crops, neither in the conventional crops.

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Antioxidant activity in selected Slovenian organic and conventional crops

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ABSTRACT

The demand for organically produced food is increasing. There is widespread belief that organic food is substantially healthier and safer than conventional food. According to literature organic food is free of phytopharmaceutical residues, contain less nitrates and more antioxidants. The aim of the present study was to verify if there are any differences in the antioxidant activity between selected Slovenian organic and conventional crops. Method of DPPH (2,2-diphenyl-1-picryhydrazyl) was used to determine the antioxidant activity of 16 samples from organic and conventional farms. The same varieties of crops were analysed. DPPH method was employed to measure the antioxidant activity of polar antioxidants (AA_p) and antioxidant activity of fraction in ethyl acetate soluble antioxidants (EA AA). Descriptive statistics and variance analysis were used to describe differences between farming systems. Estimated differences between interactions for the same crop and different farming practice were mostly not statistically significant except for the AA_p for basil and beetroot. Higher statistically significant values were estimated for conventional crops. For the EA AA in broccoli, cucumber, rocket and cherry statistically significant higher values were estimated for organic production.

Key words: antioxidant activity, organic farming, conventional farming, fruits, herbs, vegetables

IZVLEČEK

ANTIOKSIDATIVNA UČINKOVITOST V PRIDELKIH IZ SLOVENSKE EKOLOŠKE IN KONVENCIONALNE PRIDELAVE

Povpraševanje po ekološko pridelanih živilih se povečuje. Ekološki proizvodi veljajo za bolj zdrave v primerjavi s konvencionalnimi. Po navajanju drugih virov ne vsebujejo fitofarmacevtskih sredstev imajo manjšo vsebnost nitratov in vsebujejo več antioksidantov. Namen študije je bil preveriti ali obstajajo razlike v antioksidativni učinkovitosti med slovenskimi pridelki pridelanimi na ekološki in konvencionalni način. Za določevanje antioksidativne učinkovitosti smo uporabili metodo DPPH (2,2-difenil-1-pikrilhidrazil). Analiza je bila opravljena na 16 vrstah iste sorte pridelkov iz ekološke in konvencionalne pridelave. Izmerili smo polarno antioksidativno učinkovitost (AA_p) in antioksidativno učinkovitost v etil-acetatu topnih antioksidantov (EA AA). Izračunali smo osnovne statistične parameter po vrstah pridelkov in načinu kmetovanja in z analizo variance ocenili razlike med načinoma kmetovanja za posamezne pridelke. Ocenjene razlike med interakcijami pridelka in načina kmetovanja večinoma niso statistično značilne. Izjema za AAp sta bazilika in rdeča pesa, kjer so bile večje vrednosti za konvencionalne pridelke. Za vrednost EA AA so bile statistično značilno večje vrednosti ocenjene za ekološki brokoli, kumaro, rukolo in češnjo.

Ključne besede: antioksidativna učinkovitost, ekološko kmetijstvo, konvencionalno kmetijstvo, sadje, zelišča, zelenjava

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1 INTRODUCTION

For a variety of reasons all crop species vary in their composition of antioxidants, and other nutritional relevant substances. Fruits, herbs and vegetables are very important for human nutrition. Besides providing energy, food of plant origin, is a rich source of dietary fibres and minerals. Food of plant origin is thus the most important source of antioxidants (Hertog and Hollman, 1996; Pietta, 2000; Chandrasekara and Shahidi, 2011).

Antioxidant activity depends on different chemical attributes and may be specific to variety. It depends mainly on phenolics, which are considered more potent antioxidants as compared to vitamins (Koleva et al., 2002; Usenik et al., 2008). In foods of plant origin, there are numerous compounds that contribute to antioxidative activity and function in different ways. The most important antioxidants include asorbic acid, carotenoids and phenolic compounds. Group of phenolic compounds include monophenols with a single benzene ring, hydroxycinnamic acids, flavonoids and their glycosides which include catechins, proanthocyanidins, anthocyanins and flavonols (Gulcin, 2012). The most complex and poorly defined are high molecular weight tannins regularly present in fruit. In the above mentioned groups there are altogether few thousand of active substances which in different ways contribute to the total antioxidant activity (Hribar and Simčič, 2000).

Phenols are considered polar compounds and are soluble in polar solvents like water, methanol, etc. However some of them predominantly phenols with more aromatic rings, less OH groups or deglycosylated phenols are partially soluble in nonpolar solvents. Phenolic compounds also show partial solubility in less polar solvents like n-butanol and ethyl acetate (Dhingra et al. 2014). Higher EA AA means more above mentioned phenol compounds are present in ethyl acetate fraction (Rice-Evans et al., 1996) .

Soil, climate, variety, degree of ripeness and also the freshness, storage conditions can all affect the content of biologically active compounds. Type of farming system may also affect the chemical composition of foodstuffs, especially on those that originate from the use of chemical fertilizer and pesticides (Dangour et al., 2009). Organic farming represents a production system, looking for harmony between the environment and the agriculture production (Casado and de Molina, 2009; Bavec et al., 2010). It excludes the use of synthetic fertilizers and pesticides, plant growth regulators and genetically modified organisms (Singh et al., 2009). The use of pesticides was perceived to be associated with effects on health, but it was also associated with benefits like cheaper foods and higher yield (Huang, 1996; Miles and Frewer, 2001; Torjusen et al., 2001).

It is widely believed that mineral fertilizers reduce antioxidant levels in plants, while organic fertilizers enhance the antioxidant levels (Dumas et al., 2003; Aldrich et al., 2010; Oliveira et al., 2013). However literature show mixed data regarding the phytochemical status of organic and conventional vegetables (Faller and Fialho, 2010; Sinkovič et al., 2015).

In this study the antioxidant activity in selected organic and conventionally produced fruits, herbs and vegetables in Slovenia was measured. The aim of the study was to check if there are differences in antioxidant activity between analyzed organic and conventional crops.

2 MATERIALS AND METHODS

2.1 Plant material

Herbs (basil, parsley, celery) and vegetables (broccoli, beetroot, carrot, cherry tomato, cucumber, eggplant, tomato and rocket) were grown in experimental field, where mineral fertilizers had not been used for more than 30 years. The same varieties of crops were used in both farming systems. Crop varieties used in this study are presented in Table 1 and Table 2. Basic soil cultivation, sowing, and harvesting dates and methods were identical for organic and conventional experimental plot. Organic crops

were only irrigated while conventional crops were fertilized with Plantella extra plus NPK (15:15:15), according to instructions given by the manufacturer, on the 14th, 21st, 28th, 35th and 42nd day during growth period. The climatic conditions, variety, irrigation, ripening time, and storage conditions were the same for the crops grown organically and conventionally.

Samples of same variety of fruits (apple, cherry, pear and raspberry) were obtained from known organic and conventional farms in Slovenia. Organic fruits derived from certified organic productions possessed certification for organic farming, according to the Institute of Certification (KON-CERT Maribor, Slovenia). Conventional fruit samples were from conventional farms. Samples were harvested in the year 2012 when they were in commercial maturity stage. Samples were cleaned, each plot stored separately in a cooling room at + 4 °C and 95% relative humidity until the analyses in laboratory were performed.

2.2 Sample preparation

Samples were prepared in three repetitions no later than 12 h after harvest. They were washed, dried out in air and cut into small pieces. Ten gram of each sample were homogenised with 20 g of 2 % methaposphoric acid, using an Ultra-turrax T 25 (IKA, Germany). Methaposphoric acid was used to get low pH which stabilises ascorbic acid in samples (Osborn-Barnes and Akoh, 2003). The obtained homogenates were immediately frozen at – 20 °C till further use.

Samples were thawed before use and centrifuged at 1700 ×g for 5 minutes (Rotanta 460R; Hittich, Germany). The supernatants were transferred into micro centrifuge tubes and centrifuged again at 16 × g for 5 minutes (Centrifuge 5415c; Eppendorf, Germany). Finally, supernatants were filtered through 0.45 µm filter and used as a sample.

2.3 Determination of antioxidant activity

The antioxidant activity of fruits, herbs and vegetables from organic and conventional farming was measured by means of free radical DPPH (2,2-diphenyl-1-picryhydrazyl) as previously reported by Brand-Williams et al. (1995) and Shyu and Hwang (2002) with some modifications. This method is based on the reduction of the stable DPPH radical (2,2-dipenyl-1-picrylhydazyl) in to $DPPH_2$. A solution of 4 mg DPPH /100 ml of ethyl acetate was used to determine antioxidant activity of fraction in ethyl acetate soluble antioxidants (EA AA) and a solution of 4 mg DPPH /100 ml of methanol was used for polar antioxidant activity (AA_p).

For the determination of EA AA 5 ml of supernatant and 5 ml of the ethyl acetate were thoroughly mixed and upper layer (ethyl acetate fraction) was used for the essay. For AA_p 60 or 100 µl of supernatant was mixed with 1.5 ml freshly prepared DPPH. After stirring, the tubes were left in the dark for 30 minutes. The absorbance of the samples was measured at 517 nm after the reaction time. All samples were analysed as triplicate. For EA AA ethyl acetate was used as a blank and for AA_p methanol was used as a blank. The antioxidant activity was expressed as millimol DPPH equivalents per 100 g of fresh weight (mmol DPPH/100 g FW).

2.4 Statistical analysis

The data were analysed using SAS/STAT statistical software (SAS, 2012). Analysis of variance was performed using the GLM (General Linear Models) procedure. Statistical model included farming system, crop species and interaction between farming system and crop species as fixed effects (statistical model (1)). In statistical model y_{ijk} is dependent variable (AA_p or AE AA), µ is estimated overall mean, F_i is farming system fixed effect i=1,2 (organic farming, conventional farming), C_j is crop fixed effect j=1,2,3,…,16 (basil, apple, …), FC_{ij} is interaction between F_i and C_j and e_{ijk} is residual.

Differences between LSMs (Least Square Means) for the same crop in different farming system were estimated using t-test and significance level was set at $p < 0.05$.

$$y_{ijk} = \mu + F_i + C_j + FC_{ij} + e_{ijk} \quad \ldots(1)$$

3 RESULTS AND DISCUSSION

3.1 Descriptive statistic

The descriptive statistic for antioxidant activities of fraction in ethyl acetate soluble antioxidants and antioxidant activities of polar antioxidants of crops grown according to organic farming system are shown in the Table 1 and according to conventional farming system in the Table 2.

Table 1: Descriptive statistics for antioxidant activities of fraction in ethyl acetate soluble antioxidants (EA AA) and polar antioxidants' activities (AA_p) of crops produced in organic farming

Farming	Crop	Variety	EA AA (mmol/100 g FW)				AA_p (mmol/100 g FW)			
			M	**SD**	**Min**	**Max**	**M**	**SD**	**Min**	**Max**
Organic	Apple	*Malus domestica* 'Idared'	0.33	0.018	0.31	0.35	0.35	0.008	0.34	0.36
crops	Basil	*Ocimum basilicum*	0.33	0.024	0.30	0.34	0.93	0.007	0.92	0.93
	Beetroot	*Beta vulgaris* 'Rote Kugel'	0.06	0.032	0.04	0.10	0.82	0.492	0.26	1.17
	Broccoli	*Brassica oleracea* 'Corvet'	0.14	0.020	0.12	0.16	0.86	0.046	0.83	0.92
	Carrot	*Daucus carota* 'Kuroda'	0.14	0.040	0.11	0.19	0.18	0.003	0.18	0.18
	Cherry	*Prunus avium* 'Burlat'	0.29	0.024	0.26	0.31	0.44	0.015	0.43	0.46
	Cherry tomato	*Lycopersicon esculentum* var. *cerasiforme* 'Gardener`s Delight"	0.13	0.013	0.12	0.14	0.31	0.008	0.31	0.32
	Cucumber	*Cucumis sativus* 'Darina'	0.10	0.011	0.09	0.11	0.13	0.004	0.12	0.13
	Eggplant	*Solanum melongena* 'Halflange Violette'	0.19	0.020	0.17	0.21	0.80	0.018	0.78	0.81
	Parsley	*Petroselinum crispum* 'Italian Parsley'	0.11	0.010	0.10	0.12	0.58	0.044	0.53	0.62
	Pear	*Pyrus communis* 'Conference'	0.14	0.044	0.11	0.17	0.23	0.008	0.22	0.23
	Pepper	*Capsicum anuum* 'California Wonder'	0.11	0.051	0.05	0.15	0.38	0.017	0.36	0.40
	Raspberry	*Rubus idaeus* 'Willamette"	0.30	0.023	0.27	0.31	0.80	0.009	0.79	0.81
	Rocket	*Eruca sativa*	0.13	0.009	0.12	0.13	0.73	0.055	0.67	0.76
	Tomato	*Lycopersicon esculentum* 'Volovsko srce'	0.08	0.027	0.05	0.10	0.34	0.015	0.32	0.35
	Subtotal		**0.18**	**0.092**	**0.04**	**0.35**	**0.56**	**0.314**	**0.12**	**1.17**

AA_p – polar antioxidants' activity; EA AA – antioxidant activity of fraction in ethyl-acetate soluble antioxidants; FW – fresh weight; M – average value; SD – standard deviation; Min – minimum; Max - maximum

The AA_p of the organic samples varied from the lowest at 0.13 mmol DPPH/100 g FW (cucumber) to 0.93 mmol DPPH/100 g FW (basil), while the EA AA ranged from 0.08 mmol DPPH/100 g FW (tomato) to 0.33 mmol DPPH/100g FW (basil and apple).

Table 2: Descriptive statistics for antioxidant activities of fraction in ethyl acetate soluble antioxidants (EA AA) and polar antioxidants' activities (AA_p) of crops produced according to conventional farming

Farming	Crop	Species/Variety	EA AA (mmol/100 g FW)				AA_p (mmol/100 g FW)			
			M	SD	Min	Max	M	SD	Min	Max
Conventional	Apple	*Malus domestica* 'Idared'	0.30	0.023	0.28	0.32	0.51	0.039	0.47	0.55
crops	Basil	*Ocimum basilicum*	0.36	0.009	0.35	0.37	1.68	0.072	1.60	1.73
	Beetroot	*Beta vulgaris* 'Rote Kugel'	0.07	0.009	0.06	0.08	1.25	1.326	0.10	2.70
	Broccoli	*Brassica oleracea* 'Corvet'	0.09	0.011	0.08	0.10	0.74	0.020	0.71	0.75
	Carrot	*Daucus carota* 'Kuroda'	0.11	0.022	0.09	0.13	0.15	0.011	0.14	0.16
	Cherry	*Prunus avium* 'Burlat'	0.20	0.018	0.18	0.22	0.45	0.002	0.45	0.45
	Cherry tomato	*Lycopersicon esculentum* var. *cerasiforme* 'Gardener`s Delight"	0.12	0.021	0.11	0.15	0.42	0.034	0.38	0.45
	Cucumber	*Cucumis sativus* 'Darina'	0.05	0.035	0.02	0.08	0.08	0.003	0.07	0.08
	Eggplant	*Solanum melongena* 'Halflange Violette'	0.24	0.006	0.23	0.24	0.55	0.005	0.55	0.56
	Parsley	*Petroselinum crispum* 'Italian Parsley'	0.10	0.032	0.06	0.12	0.45	0.017	0.43	0.46
	Pear	*Pyrus communis* 'Conferense'	0.14	0.037	0.10	0.17	0.23	0.021	0.21	0.25
	Pepper	*Capsicum anuum* 'California Wonder'	0.14	0.026	0.12	0.16	0.34	0.018	0.32	0.35
	Raspberry	*Rubus idaeus* 'Willamette"	0.26	0.029	0.24	0.30	0.68	0.014	0.66	0.69
	Rocket	*Eruca sativa*	0.06	0.022	0.04	0.08	0.50	0.009	0.49	0.51
	Tomato	*Lycopersicon esculentum* 'Volovsko srce'	0.08	0.016	0.07	0.10	0.17	0.014	0.16	0.18
	Subtotal		**0.16**	**0.094**	**0.02**	**0.37**	**0.56**	**0.489**	**0.07**	**2.70**

AA_p – polar antioxidant activity; EA AA – antioxidant activity of fraction in ethyl-acetate soluble antioxidants; FW – fresh weight; M – average value; SD – standard deviation; Min – minimum; Max - maximum

The AA_p of the conventional samples varied from the lowest value of 0.07 mmol DPPH/100 g FW (cucumber) to 2.70 mmol DPPH/100 g FW (beetroot), while the EA AA ranged from 0.02 mmol DPPH/100 g FW (cucumber) to 0.37 mmol DPPH/100g FW for basil.

The mean AA_p for cherry was very similar between the farming systems (organic, 0.44 mmol

DPPH/100 g FW; conventional, 0.45 mmol DPPH/100 g FW). The mean AA_p for pear were equal (0.23 mmol DPPH/100 g FW).

The mean EA AA for pear (0.14 mmol DPPH/100 g FW) and tomato (0.08 mmol DPPH/100 g FW) were unchanged between the farming system.

There are a number of reviews that compare nutritional quality of organically versus conventionally grown foods (Weibel et al., 2000; Brandt and Molgaard, 2001; Bourn and Prescott, 2002). Weibel et al. (2000) showed that fruit quality of organic apples were either similar or slightly better than that of conventional ones. According to literature data, the organic crops presented higher antioxidant activity than conventional ones (Worthington, 2001; Wang et al., 2008; Aldrich et al., 2010; Arbos et al., 2010; Crinnion, 2010; Lairon, 2010; Borguini et al., 2013). This can be attributed to the fact that organic farming uses less phytopharmacheuticals and plant therefore develop several defense mechanisms (Koleva et al., 2002; Borguini et al., 2013). On the other hand, Lamperi et al. (2008) and Cardoso et al. (2011) argue that there is no difference in the antioxidant activity between products from different farming systems.

3.2 Analysis of variance

In AA_p 75 % and for EA AA 94 % of total variability were explained with statistical model (1). Effect of farming system was not statistically significant in AA_p but it was in EA AA. In both antioxidant activity measurements crop was highly statistical significant. Interaction between farming system and crop was statistically significant for EA AA, but in AA_p interaction has a trend that approached significance (Table 3). This shows that variability between crops was higher than variability between farming systems.

Table 3: *F*-values and *p*-values for effects included in statistical model (1) for AA_p and EA AA

Effect	AA_p		EA AA	
	F-value	*p*-value	*F*-value	*p*-value
Farming system	*0.00*	*0.949*	*7.65*	*0.008*
Crop	*10.74*	*<0.001*	*60.84*	*<0.001*
Farming system and crop interaction	*1.76*	*0.061*	*2.54*	*0.005*

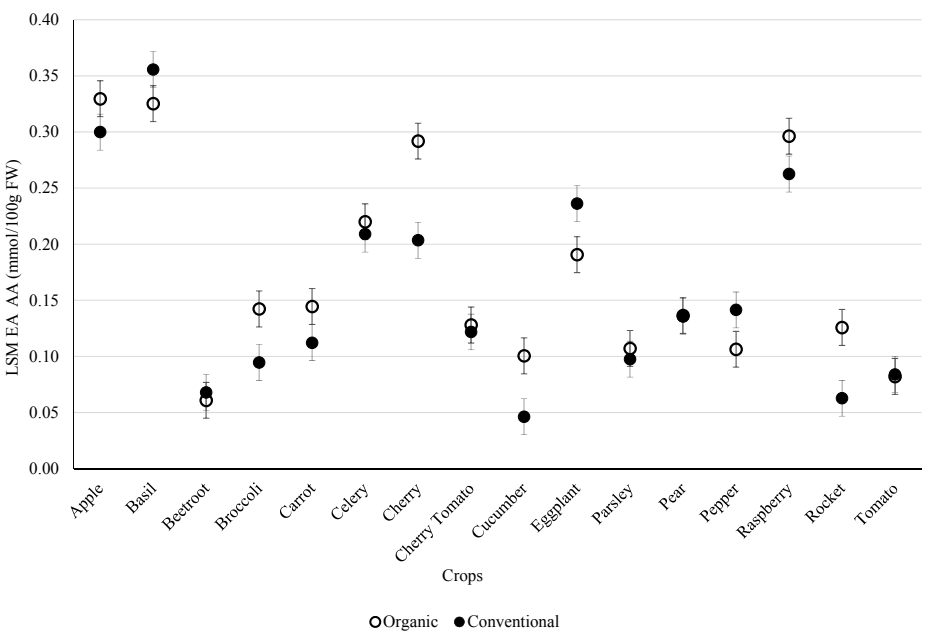

Figure 1: LSM (least square means) values for antioxidant activity of fraction in ethyl acetate soluble antioxidants (EA AA)

Estimated LSMs for interaction between farming system and crop in EA AA show that all observed types of fruits from organically farming (cherries, apples, raspberries and pears) had higher estimated EA AA compared to conventional ones (Figure 1) but only for cherry difference was statistically significant ($p < 0.001$). Higher values of antioxidant activity in organic fruit were also reported by Wang et al. (2008) and Vrček et al. (2011).

Bavec et al. (2010) and Borguini et al. (2013) found higher antioxidant activity in organic vegetables as compared to conventional. In our study statistically significant differences in the EA AA of LSMs between farming systems for vegetables were estimated only for broccoli ($p < 0.05$), cucumber ($p < 0.05$) and rocket ($p < 0.01$) (Figure 1).

Estimated LSMs for interaction between farming system and crop for AA_p were higher for conventionally produced basil, cherries, apples, cherry tomatoes and beetroots had higher AA_p compared to organic (Figure 2). Other crops had higher estimated LSMs for AA_p from organic production compared to conventional. But statistically significant differences in AA_p LSMs between farming practices were estimated only for basil ($p < 0.001$) and beetroot ($p < 0.05$).

In the study of Faller and Failho (2010) broccoli stems also demonstrated higher antioxidant activity from conventional production. In our study broccoli had higher estimated LSMs for AA_p in samples from organic production but differences were not statistically significant.

Cardoso et al. (2011) and Lamperi et al. (2008) also found no statistically significant differences for antioxidant activity between organic and conventional crops. Nevertheless some researchers found higher antioxidant activity values in organic compared to conventional crops (Aldrich et al., 2010; Arbos et al., 2010; Borguini et al., 2013).

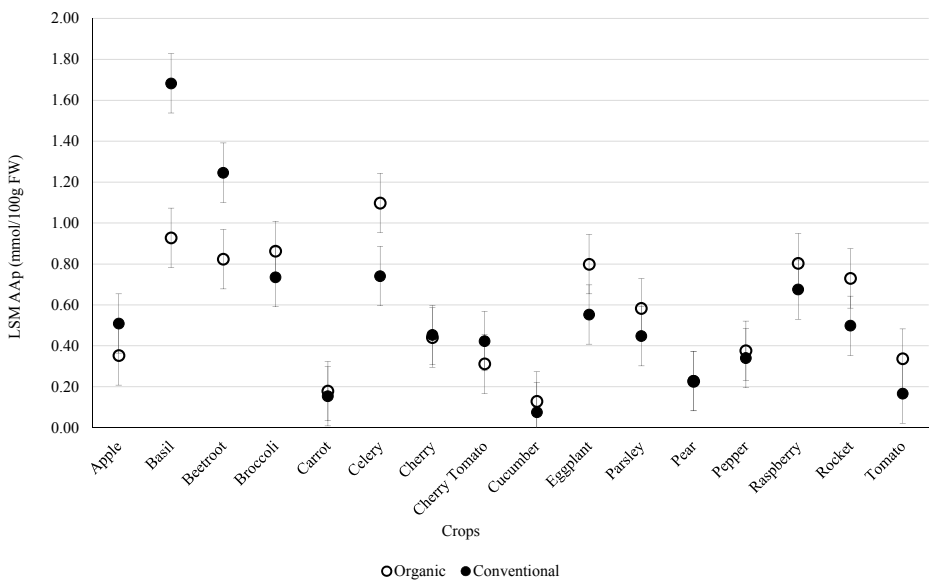

Figure 2: LSM values for polar antioxidants' activity (AAp)

We expected that the highest antioxidant activity, among fruits, will be measured in raspberries. As reported by Kahkonen et al. (1999) berries, had clearly higher antioxidant activities compared to other fruits predominantly due to high content of anthocyanins which is reflected in profound red colour. In our study AA_p of raspberry was the highest among fruits, but when comparing fruits EA AA, just pear had lower values than raspberry. Obviously no antioxidants soluble in ethyl acetate were present in pear and raspberry. As reported by Kahkonen et al. (1999) herbs possess strong

antioxidant activity which is in accordance with our study. In this study the highest antioxidant activity (AA_p and EA AA) was measured for basil. Analysis of variance showed no statistically significant differences between farming practise in general, while estimated differences between crop species were very high (till 0.3 for EA AA and 1.6 for AA_p).

4 CONCLUSIONS

Although some literature report that the exposure of crop plant to stress conditions during growth could modulate the synthesis of defence substances such as antioxidants. Often reported benefits of organic agriculture are an increased concentration of antioxidants due to stress provoked by nitrogen deficiency. That was not clearly demonstrated in this study. Our results showed that antioxidant activity of water extract of plant material varied among organic and conventional crops with no prevalence from either production type. Estimated differences between interactions for the same crop and different farming practice were mostly not statistically significant except for the AA_p for basil and beetroot. Higher statistically significant values were estimated for conventional crops. For the EA AA in broccoli, cucumber, rocket and cherry statistically significantly higher values were estimated for organic production. We can conclude that, except for some crops, there are no statistically significant differences in antioxidant activity between organic and conventional fruits, herbs and vegetables, which were included in this study.

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Does paternal sterility impact on progeny germination and survivorship, case study in strawberries

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ABSTRACT

Studies on the parental role on progeny performance have mostly focused on the maternal parent, while less attention was given to the paternal parent. This study investigated the impact of paternal pollen sterility (ranging from 3.1 – to 77.2%) on F_1 seed germination and progeny survivorship in *Fragaria* (strawberry, Rosaceae) using controlled crosses. In crosses within *F. vesca* ssp. *vesca* the paternal pollen sterility was not correlated with F_1 seed germination (N = 14, p > 0.074) and progeny survivorship (N = 14, p > 0. 0.710). Paternal sterility in crosses between *F. vesca* ssp. *vesca* and *F. vesca* ssp. *monophylla* did not affect on F_1 seed germination (N = 7, p > 0.295) and progeny survivorship (N = 6, p > 0.812). Similarly, no correlation was found between father pollen sterility and F_1 seed germination (N = 6, p > 0.924) and progeny survivorship (N = 6, p > 0.215) in crosses between *F. vesca* ssp. *americana* and *F. vesca* ssp. *vesca*. Furthermore, crossing different maternal plants by pollen of the same paternal plant in all three cross types produced progeny with variable levels of F_1 seed germination and survivorship. These results indicate the crucial role of maternal plant on progeny performance and support the general idea of the importance of maternal rather than paternal parent on progeny performance.

Key words: maternal impact, paternal impact, progeny performance, reproductive fitness, parental impact, *Fragaria*, strawberry

IZVLEČEK

ALI MOŠKA STERILNOST VPLIVA NA KALITEV IN PREŽIVETJE POTOMCEV? PRIMER RAZISKAVE NA JAGODNJAKIH (*Fragaria*)

Raziskave vloge staršev na uspevanje potomcev so pri rastlinah pogosto osredotočene na ženskega straša, manj pozornosti je posvečeno vlogi moškega starša. Ta raziskava je preučevala vpliv moške sterilnosti peloda (v razponu od 3,1 do 77,2 %) na kalitev in preživetje F1 potomcev jagodnjaka (*Fragaria,* Rosaceae) po kontroliranih križanjih. Po križanju gozdnega jagodnjaka (*F. vesca* L. ssp. v*esca*) moška sterilnost peloda ni korelirala s kalitvijo F1 semen (N = 14, p > 0.074) in preživetjem potomcev (N = 14, p > 0. 0.710). Enako moška sterilnost po križanjih med taksonoma *F. vesca* ssp. *vesca* in *F. vesca* ssp. *monophylla* ni imela vpliva na kalitev F1 semen (N = 7, p > 0.295) in preživetje potomcev (N = 6, p > 0.812). Podobno ni bila ugotovljena korelacija med moško pelodno sterilnostjo in kalitvijo F1 semen (N = 6, p > 0.924) ter preživetjem potomcev (N = 6, p > 0.215) po križanjih med taksonomoma *F. vesca* ssp. *americana* in *F. vesca* ssp. *vesca*. Še več, po križanjih različnih materinskih rastlin s pelodom iste očetovske rastline je imelo po vseh treh vrstah križanj potomstvo spremenljivo kalivost F1 semen in spremenljivo preživetje. Ti rezultati kažejo na odločilno vlogo materinskega starša na uspevanje potomcev in podpirajo splošno idejo o ključni, večji vlogi materinske rastline primerjalno z očetovsko.

Ključne besede: materinski vpliv, očetovski vpliv, uspevanje potomcev, reproduktivni potencial, starševski vpliv, *Fragaria*, jagodnjak

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1 INTRODUCTION

The variations in F_1 phenotype are thought to be affected mainly by maternal parents, although the parental genotype and environment do also affect of progeny performance (Roach and Wulff, 1987). The maternal effects on progeny performance are carried out via the different mechanisms including through cytoplasmic genetic, since organelles such as plastids and mitochondria can be directly transferred from the maternal plant to the offspring (Raghavan, 2005). The maternal parent can also affect on progeny via the endosperm because the endosperm contains more doses of maternal than paternal genes, and also contains enzymes and the nutrients resources important for germination and developing of the embryo (Bernasconi, 2003). In addition, the other maternal effects on offspring are caused by the structural or physiological effects of maternal tissues e.g. integuments of the ovule and the wall of the ovary surrounding the developing embryo and endosperm. These structures which form the seed coat, fruit, and accessory seed structures determine seed dormancy, dispersal, and germination (Raghavan, 2005). The impact of maternal parent on the progeny is different from the equal chromosomal contribution by both paternal and maternal parents (Roach and Wulff, 1987).

The level of progeny performance e.g. F_1 seed germination, survivorship and fertility are crucial in the natural selection in plants and therefore, in speciation (Niklas, 1997). In addition, based on "biological species concept" assessing the components of reproductive fitness in F_1 progeny in plants is important for understanding species delimitation and patterns of speciation (Kay, 2006), and has been used to delimit species boundaries in several plant congeneric species e.g. *Coreopsis* (Archibald et al., 2005), *Glycine*, *Silene*, *Streptanthus* (Moyle et al., 2004) and *Zigiber* (Kay, 2006).

There have been wide studies on the impact of maternal parent on the variations of the F_1 progeny performance in plants (e.g. Lloyd, 1980; Bernasconi 2003; Obeso, 2004; Halpern 2005; Raghavan, 2005; Gehring and Delph, 2006; Bayer et al., 2009; Holland et al., 2009; Diggle et al., 2010). These studies indicated that the levels of offspring performance in plants are mainly controlled by maternal parent, while the impact of paternal parent on the progeny performance has been less investigated (Holland et al., 2009). Therefore, it is important to understand the effects of pollen donor diversity on progeny performance in plants (Leishman et al., 2000; Westoby et al., 2002; Beaulieu et al., 2007).

In the current study the impact of paternal pollen sterility on F_1 seed germinations and progeny survivorship was investigated through controlled cross pollination in diploid species of *Fragaria* L. (strawberry, Rosaceae). The genus *Fragaria* is a perennial herb and comprises of about 22 species some of which have several subspecies (Staudt 1989, 2009).

2 MATERIALS AND METHODS

2.1 Species study

Several genotypes of each of the following diploid taxa within *Fragaria vesca* were used in the study. 27 controlled cross-pollinations were made among *F. vesca* ssp. *vesca*, *F. vesca* ssp. *vesca* var. *alba*, *F. vesca* ssp. *vesca* f. *monophylla*, and *F. vesca* ssp. *americana* using different genotypes (Table 1). 14 intra-subspecific crosses were made within *F. vesca* ssp. *vesca* by including *F. vesca* ssp. *vesca* var. *alba*. 6 crosses were carried out between *F. vesca* ssp. *vesca* and *F. vesca* ssp. *vesca* f. *monophylla*, and 6 crosses were performed at inter-subspecific level between *F. vesca* ssp. *vesca* and *F. vesca* ssp. *americana*.

2.2 Controlled crosses

In making controlled crosses, the floral buds on seed parents were emasculated approximately 3-4 days before anthesis by removing the stamens, and then the stigmas were pollinated by directly rubbing the anthers from the paternal parent. The cross-pollinated flowers were covered by a double-layer of fibre fleece to avoid uncontrolled open-pollination and desiccation. The cross-pollination

was repeated within 3-4 days to ensure the pollination. After 3 weeks, the originating berries were collected and the seeds (achenes) were separated from the berries. The seeds were treated by absolute sulphuric acid for 2 minutes for removing dormancy, and then were germinated in growth cabinets under 16/8 h light/dark regime at 16 °C on peat for a week. The levels of F_1 seed germination was calculated on the basis of the percentage of germinated seeds to the total number of seeds sown. The seedlings were transferred to the glasshouse. The level of F_1 progeny survivorship was obtained from the percentage of the progeny survived to the total seedlings by the end of the year.

To select the pollen donor plants with variable levels of pollen sterility, a large number of plants were examined, and subsequently, several plants with a wide range of pollen sterility (3.1 to 77.2%) were select for conducting crossing experiments. The levels of pollen sterility of pollen parents were measured on the basis of proportion of the unstained pollen using 0.05% cotton blue in lactophenol by randomly studying a minimum of 500 pollen grains under light microscope. The pattern of nutrients and water supplies for all plants under study were kept consistent to eliminate the impact of resource variations on the progeny performance.

2.3 Statistical analyses

Relationship of the paternal parent pollen sterility with F_1 seed germination and progeny survivorship were tested on the basis of Pearson Rank Correlation test (SPSS, ver. 11.2). Kruskal-Wallis Test (SPSS, ver. 11.2) was used to test the significance level of pollen sterility variation in each cross type.

Table 1: Plant materials used in this study.

Species	Source & determination	Geographical distribution
F. vesca ssp. *vesca* L.	Royal Botanic Garden, Kew, UK	Europe (England, UK)
F. vesca ssp. *vesca* var. *alba* L.	NCGR*/197.000 PI 551572	Europe
F. vesca ssp. *vesca* L.	NCGR/478.000 PI 551826	Europe
F. vesca ssp. *vesca* var. *alba* L.	NCGR/198.000 PI 551573	Europe
F. vesca ssp. *vesca* L.	University of Joensuu, Finland	Europe (Finland)
F. vesca ssp. *vesca* L.	Poyntzfieldherbs, Nursery, Inverness, UK	Europe (Scotland, UK)
F. vesca ssp. *vesca* f. *monophylla*	NCGR/612.000 PI 551909	Europe
F. vesca ssp. *americana* (Porter) Staudt	NCGR/554.001 PI 551881	USA, East

*National Clonal Germplasm Repository, Corvallis, Oregon, USA.

3 RESULTS AND DISCUSSION

The variations in the levels of the paternal parent pollen sterility and F_1 seed germination and progeny survivorship originating from controlled cross-pollinations in *Fragaria* are shown in Table 2. In intra-subspecific crosses within *F. vesca* ssp. *vesca* paternal parent with significant variation in pollen sterility (ranging from 3.1 to 77.2%; N =14, $df = 5$, $p < 0.023$, Kruskal-Wallis Test) produced great variation in F_1 seed germination (ranging from 38.5 to 100%) and progeny survivorship (60-100%). However, pollen parent sterility was not correlated with F_1 seed germination (N =14, $p > 0.074$) and progeny survivorship (N =14, $p > 0.710$; Figure 1, A).

Reciprocal crosses between *F. vesca* ssp. *vesca* and *F. vesca* ssp. *vesca* f. *monophylla* using pollen donors with dramatic male sterility variation ranging from 6.8 to 77.2 (N =7, $df = 5$, $p < 0.05$, Kruskal-Wallis Test) resulted in highly variable levels of F_1 seed germination and progeny survivorship (5.9 - 87.5% and 85.7 - 96.6%, respectively). However, there was no correlation between pollen parent sterility and F_1 seed

germination (N = 7, $p > 0.295$) and progeny survivorship (N = 6, $p > 0.812$, Figure 1, B).

Although crossing several plants of *F. vesca* ssp. *americana* by pollen of *F. vesca* ssp. *vesca* with highly variable pollen sterility (3.1 to 77.2%) though not significant (N = 6, $df = 5$, $p > 0.082$, Kruskal-Wallis Test) gave rise to large variations in F_1 seed germination (48.3 – 92.4%) and progeny survivorship (42.9 - 100%). There was no correlation between father pollen sterility with seed germination (N = 6, $p > 0.924$) and progeny survivorship (N = 6, $p > 0.215$; Figure 1, C).

Within each cross type, crossing different maternal plants by pollen of the same paternal plant produced progeny of variable levels of performance. For example, within *F. vesca* ssp. *vesca* crossing three different individual plants by pollen of a single pollen donor with 3.1% sterility produced progeny with 38.5 to 54.5% F_1 seed germination and 60 to 100% progeny survivorship.

The results of the current investigation showed that the pollen parent sterility does not affect on F_1 seed germination and progeny survivorship in *Fragaria*. These data are consistent with the general view that the levels of progeny performance in plants are mostly influenced by maternal parent (Lloyd, 1980; Obeso, 2004; Gehring and Delph, 2006). In addition, the results of the current study do agree with previous reports from other taxa e.g. *Hydrophyllum appendiculatum* (Wolfe, 1995), *Lesquerella fendleri* (Mitchell, 1997) and *Mirabilis jalapa* (Niesenbaum, 1999; Davis, 2004), and *Pachycereus schottii* (Holland et al., 2009), which have shown that pollen donor diversity did not affect on seed and fruit set nor on later components of life history. In contrast, the pollen donor diversity in *Rhamnus alpinus* has been reported to affect on different reproductive components of F_1 progeny e.g. embryo size (Nakamura and Stanton, 1989), seed size, germination rate, seedling vigor and survivorship (Banuelos and Obeso, 2003), and the embryos developments (Diggle et al., 2010).

Contrary to above-mentioned reports, several other studies indicated the impact of both maternal and paternal parent on the progeny performance. For instance, in *Raphanus sativus* seed growth rate (Diggle et al., 2010), and embryo/seed development rate (Marshall and Diggle, 2001) were shown to be affected by both maternal and paternal parents. This simultaneous impact of both paternal and maternal on progeny performance indicates genotypic interactions (Marshall et al. 2000). While the impact of paternal plant on progeny performance depends on the identity of the pollen-receiving individual and also on interaction between recipient and donor plants (Bernasconi, 2003).

Table 2: Variations in the levels of paternal pollen sterility and originating F_1 seed germination and progeny survivorship in controlled cross-pollinations in *Fragaria*.

Controlled crosses (Seed parent X pollen parent)	%Paternal sterility	No. seeds sown	No. seeds germinated	% F_1Seed germination	No.F_1seedlings	No. F_1 plants survived	% F_1progeny survivorship
F. vesca ssp. *vesca* X *F. vesca* ssp. *vesca*	3.1	13	5	38.5	9	9	100.0
	3.1	11	5	45.5	5	3	60.0
	3.1	11	6	54.5	5	5	100.0
	17.06	20	14	70.0	14	13	92.9
	17.06	11	9	81.8	6	5	83.3
	18.14	22	16	72.7	16	13	81.3
	18.14	17	13	76.5	14	12	85.7
	21.6	22	18	81.8	16	15	93.8
	21.6	24	21	87.5	21	16	76.2
	21.6	14	14	100	13	10	76.9
	34.63	36	29	80.6	35	34	97.1
	34.63	55	49	89.1	49	38	77.6
	77.2	24	16	66.7	18	16	88.9
	77.2	35	35	100	29	29	100.0
F. vesca ssp. *monophylla* X *F. vesca* ssp. *vesca*	6.8	60	49	81.7	49	46	93.9
	6.8	24	21	87.5	21	18	85.7
	6.8	21	15	71.4	15	13	86.7
F. vesca ssp. *vesca* X *F. vesca* ssp. *monophylla*	34.6	67	42	62.7	42	40	95.2
	34.6	33	25	75.8	29	28	96.6
	77.2	101	6	5.9	25	24	96
	77.2	35	29	82.9	ND	ND	ND
F. vesca ssp. *vesca* X *F. vesca* ssp. *americana*	3.1	58	28	48.3	41	41	100.0
	3.1	20	16	80.0	16	11	68.8
	18.14	85	71	83.5	71	66	93.0
	18.14	66	61	92.4	61	59	96.7
	77.2	41	28	68.3	28	12	42.9
	77.2	52	41	78.8	28	23	82.1

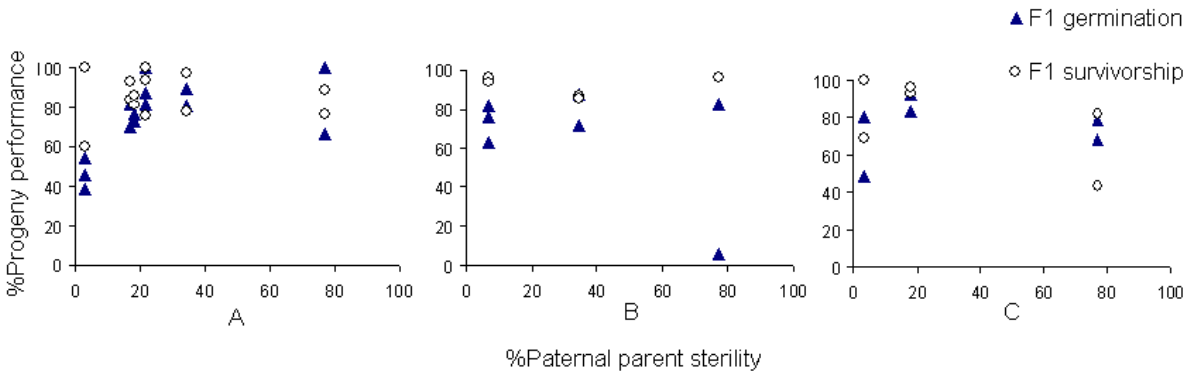

Figure 1. Lack of correlation between paternal pollen sterility and progeny performance in controlled crosses in *Fragaria*. A) Intra-subspecific crosses within *F. vesca* ssp. *vesca*, B) *F. vesca* ssp. *vesca* and *F. vesca* ssp. *vesca* f. *monophylla*, C) Inter-subspecific crosses between *F. vesca* ssp. *vesca* and *F. vesca* ssp. *americana*.

4 CONCLUSION

The results of the current study obtained from *Fragaria* along side those data reported from other plant taxa indicate that patterns of parental impact on offspring performance and reproductive fitness appear to be very diverse. Understanding the pattern of parental plants on the progeny performance could be used in improving the crop quantities and qualitative.

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Impact of different culture media on hairy roots growth of *Valeriana officinalis* L.

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ABSTRACT

Transformed hairy root cultures of *Valeriana officinalis* were established by infection with *Agrobacterium rhizogenes* strain ATCC 15834. To determine the effect of different media on the growth of *V. officinalis* hairy roots, MS, B5 media (1.0X and 0.5X strength), N6 medium and a modified MS medium without phytohormones were used. In addition, different NH_4^+ to NO_3^- ratios in MS medium were studied. The effects of these treatments were evaluated after 21 days of culture in relation to hairy root growth. B_5 and ½ B_5 media were the best basal media for hairy root growth. MS medium supplemented with a 20:20 ratio (mM) of NH_4^+ to NO_3^- displayed highest growth rates and biomass yield in hairy root cultures. The present study demonstrated that the composition of culture medium and the ratio of different nitrogen sources have significant impact on the growth of *V. officinalis* hairy roots.

Key words: *Valeriana officinalis,* hairy root, medium composition

IZVLEČEK

VPLIV RAZLIČNIH GOJITVENIH GOJIŠČ NA RAST TRANSFORMIRANIH KORENIN ZDRAVILNE ŠPAJKE (*Valeriana officinalis* L.)

Transformirana kultura korenin zdravilne špajke je bila vzpostavljena z bakterijsko okužbo *Agrobacterium rhizogenes*, sev ATCC 15834. Preučevana so bila različna gojišča MS, B5 (1,0X in 0,5X koncentracija), N6 in modificiran MS brez fitohormonov. Dodatno so bila v MS gojišču preučevana različna razmerja med NH_4^+ in NO_3^-. Učinki teh tretmajev na rast transformiranih korenin so bili ovrednoteni po 21 dneh. B_5 in ½ B_5 sta bili najboljši osnovni gojišči za rast transformiranih korenin. MS gojišče dopolnjeno z dušikovimi spojinami v razmerju 20:20 (mM) NH_4^+: NO_3^- je vplivalo na največjo rast in biomaso korenin. Raziskava je pokazala, da imata sestava rastnega gojišča in različna razmerja dušikovih spojin značilen vpliv na rast transformiranih korenin zdravilne špajke.

Ključne besede: *Valeriana officinalis,* transformirane korenine, sestava rastnega gojišča

1 INTRODUCTION

Valeriana officinalis L. (valerian) is a perennial herbaceous and rhizomatous medicinal plant native to Europe and Asia which has been naturalized in eastern North America and cultivated on a commercial scale in these regions (Cronquist, 1981). *V. officinalis* has been longley used as an important source of pharmaceutical compounds in traditional medicine (Straube, 1968; Morazzoni and Bombardelli, 1995; O'Hara et al., 1998) e.g., clinical trials have shown that valerian extract is effective in treatment of mild to moderate sleeping disorders and it can encourage sleep and improve sleep quality (Leathwood and Chauffard, 1985; Schulz et al., 1994). Furthermore, the valerian root is considered as mild anodyne, anticonvulsant, antispasmodic, carminative and hypotensive (Capasso and DeFeo, 1996; Hiller, 1996). Potential mechanisms for the pharmacological activity of

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valerian extracts may include increased release of γ-aminobutyric acid (GABA) and agonistic activities on the GABA receptors (Marder et al., 2003).

Sesquiterpenes of the volatile oil (valerenic acid and its derivatives, valeranone, valeranal) and valepotriates (valtrate, didrovaltrate, acevaltrate, iso-valeroxyhydroxyvaltrate) are two main groups of compounds in the subterranean organs of valerian(Goppel, 2004). The valerenic acids have been reported for the sedative activity of *V. officinalis* and they are often used as an indicator of medicinal quality (Hendriks et al., 1981). The main representative of these compounds are valerenic acid (VA), acetoxyvalerenic acid and hydroxyvalerenic acid (Bos et al., 1996).

Genetically transformed hairy roots obtained by infection of plants with *Agrobacterium rhizogenes* are suitable source for production of bioactive molecules due to their genetic stability and generally show fast growth in culture media free of growth hormones (Shanks and Morgan, 1999). The hairy roots often exhibit about the same or greater biosynthetic capacity for secondary metabolite production as compared to their parent plants, hence hairy roots have been used as an alternative and attractive method for the production of several plant metabolites (Zhou et al., 2011). Several attempts have also been made to enhance hairy root growth and their production of important bioactive compounds (Yu et al., 1996; Yu et al., 2006; Satdive et al., 2007; Shinde et al., 2009). Optimizing the composition of inorganic nutrients of the media for hairy root cultures is essential to gain high production of secondary metabolites (Condori et al., 2010; Shinde et al., 2010). It has been reported that concentration of nitrogen and appropriate ratio of nitrogen sources in the culture medium greatly affected the growth and production of secondary metabolites in hairy root cultures (Oksman-Caldentey et al., 1994; Yu et al., 1996; Lourenco et al., 2002).

In the present study, the effect of different basal media and various NH_4^+ to NO_3^- ratios in the culture medium on the growth of hairy root cultures of *V. officinalis* are discussed.

2 MATERIALS AND METHODS

2.1 Hairy root induction

Cotyledons of in vitro grown sterile *V. officinalis* L. plants were cut into pieces of approximately 1 cm in length. The explants were immersed in a suspension of *A. rhizogenes* strain ATCC 15834 containing 100 µM acetosyringone for 10 min and then blotted on sterile filter papers. All explants were then placed in petri dishes containing 25 ml of half strength solidified MS medium for co-cultivation. After 48 hours incubation in the dark at 28 ˚C, explants were transferred to fresh solidified ½ MS medium containing 500 mg l^{-1} cefotaxime and were subcultured at two week intervals to eliminate the bacteria. Tips of hairy roots were exsected and transferred to 50 ml, liquid ½ MS medium and were incubated at 25 ˚C on a rotary shaker at 110 rpm in darkness. Among several hairy root lines established, line No. 9 was selected for its vigorous and sustained growth and used for further experiments.

2.2 PCR analysis of hairy roots

Genomic DNA was extracted from the hairy roots of line No. 9 and from the roots of a non-transformed plant, to serve as a negative control, using the DNeasy Plant Mini kit (Qiagen, USA). Two 20-mer oligonucleotide primers, 5́gctcttgcagtgctagattt3́ (forward) and 5́gaaggtgcaagctacctctc3́ (reverse), were used for PCR amplification of the *rolB* and *rolC* genes. In addition, primers 5́atgtcgcaaggcagtaagccca3́ (forward) and 5́ggagtctttcagcatggagcaa3́ (reverse), amplifying a fragment of *vir*D2 gene were used for detecting bacterial contamination in hairy roots. The PCR reactions were carried out in a total 25 µl volume and consisted of 100 ng of genomic DNA, 10 µM each primers, 0.2 mM dNTP mix, 1 unit of *Taq* DNA polymerase and 2 mM $MgCl_2$. PCR condition was as follows: 94 ˚C for 3 min (initial denaturation), 35 cycles of 94 ˚C for 45 s, 56 ˚C for 1 min and 72 ˚C for 1 min and a final extension at 72 ˚C for 7 min. The PCR products were

separated by electrophoresing on a 1.2 % agarose gel in TBE buffer.

2.3 Media composition

Standard Gamborg's (B5), Murashige and Skoog's (MS) basal media, half strength medium of these media and N6 medium were used in the experiments. To determine the effects of different NH_4^+ to NO_3^- ratios on hairy root growth, conventional MS medium was modified in such a way that total NO_3^- and NH_4^+ ions were supplied from KNO_3 and $(NH_4)_2SO_4$ salts, respectively. Various combinations of this modified medium with different NH_4^+ to NO_3^- ratios (0:20, 10:20, 20:20, 20:10, 20:0 and 20:40 mM) were prepared. For investigating the effect of substituting NH_4NO_3 with $(NH_4)_2SO_4$, the modified medium containing 20:40 ratio and its half strength were compared with conventional MS (half and full strength) in all experiments. The pH values of the media were adjusted to 5.8±0.2 prior to autoclaving and the concentration of sucrose was 30 g l^{-1} for all media.

2.4 Growth measurement

Two hundred miligrams of hairy roots were added to 50 ml of each media as primary inoculum. After 21 days of cultivation at 25 °C at 110 rpm in darkness, hairy root clones were harvested to determine the fresh and dry weights. All experiments were replicated three times and four erlenmeyer flasks were used for each treatment.

2.5 Statistical analysis

In all of the experiments, the layout was totally randomized. Analyses were performed using SAS V. 9 software package (SAS Institute Inc., Cary, NC, USA.). For comparing different treatments, a one-way analysis of variance (ANOVA) and Duncan test with a critical value of $P \leq 0.05$ were applied.

3 RESULTS AND DISCUSSION

3.1 Establishment of hairy root clones

Hairy roots induced by *A. rhizogenes* provide an alternative system for the production of valuable bioactive compounds because of their genetic and biochemical stability (Zhou et al., 2011). In the present study, hairy roots were successfully induced by infection of cotyledon segments of *V. officinalis* with *A. rhizogenes* strain ATCC15834. Hairy roots emerged from the infected sites within 12-15 days with 30% transformation frequency and maintained on hormone free ½ MS medium. Among the hairy root clones, clone No. 9 was selected on the basis of its growth rate. The selected transformed clone showed rapid growth rate and tendency for profuse branching and active elongation, whilst untransformed roots did not show similar growth, elongation or branching pattern (Figure 1).

Figure 1: A) Hairy root formation on *V. officinalis* explant after 7 days of inoculation. B) non-transformed root after 4 weeks culture in ½ MS medium. C) Hairy roots after 4 weeks culture in ½ MS medium.

3.2 Molecular analysis

PCRs were performed with specific primers to determine presence of T-DNA segment of Ri plasmid in the genomic DNA of *V. officinalis* hairy roots. The PCR with primers specific for *rol*B and *rol*C genes and template DNA from hairy roots amplified the expected bands of 450 and 700 bp, respectively (Figure 2) confirming the successful integration of T-DNA, while DNA templates from untransformed roots (used as control) did not show any amplification. The PCR analysis of hairy root clones also revealed that no band was amplified for *vir*D2 gene (Figure 2c), indicating absence of *A. rhizogenes* ATCC 15834 contamination in the cultures.

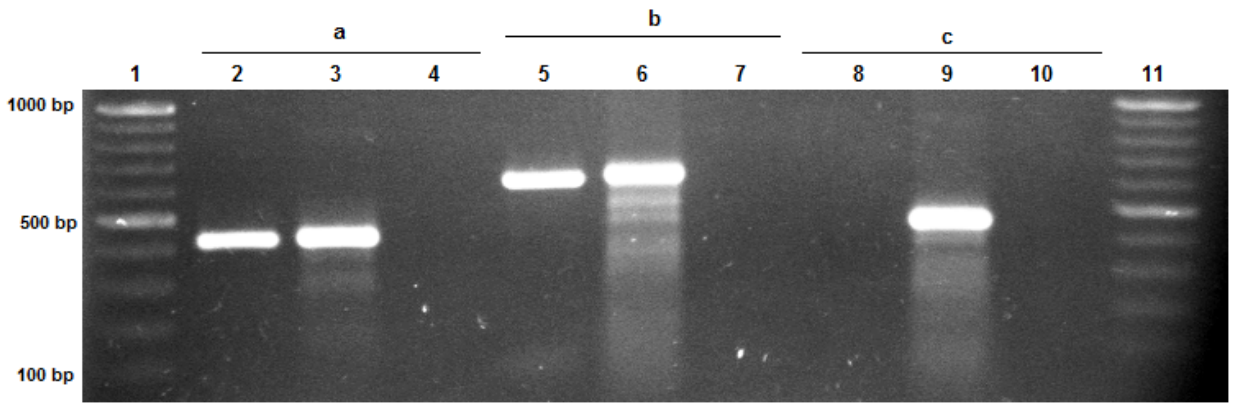

Figure 2: a) PCR amplification of *rol*B gene, lane 2: DNA from hairy roots; lane 3: *A. rhizogenes* DNA (positive control); lane 4: DNA from non-transformed root (negative control). b) PCR amplification of *rol*C gene, Lane 5: DNA from hairy root; lane 6: *A. rhizogenes* DNA; lane 7: DNA from non-transformed root. c) PCR amplification of *vir*D gene, Lane 8: DNA from hairy root; lane 9: *A. rhizogenes* DNA; lane 10: DNA from non-transformed root. Lanes 1and 11: Molecular size marker (100 bp ladder, Fermentas Co., Germany).

3.3 Effect of basal medium on hairy root growth

Based on previous studies, media composition could have a significant impact on hairy root growth in culture systems (Yu et al., 1996; Lourenco et al., 2002; Sivakumar et al., 2005). In the present study, the growth rate of hairy root cultures in different media was measured after three weeks. B5 and ½ B5 media produced the highest dry weight of hairy roots, 3.85 and 3.67 g l^{-1}, respectively (Figure 3). Although, biomass production in B5 medium was higher than ½ B5 medium, this difference was not statistically significant. The N6 medium was the weakest medium for root growth of *V. officinalis* with just 0.86 g l^{-1} dry weight after 21 days. Both ½ MS and ½ MSV media had similar effects on the growth of hairy roots and this result was also observed for full concentration of these two media. These results indicated that substituting NH_4NO_3 with $(NH_4)_2SO_4$ in MSV medium had no significant effect on root growth. Furthermore, increased K^+ ion concentration in the medium due to this replacement had not substantial alteration on the hairy root cultures. As shown in the figure 3, concentration of MS and MSV basal media influenced the biomass production. In both media higher dry weight values were obtained by roots grown in half strength MS and MSV media, 2.22 to 1.62 g l^{-1} and 2.658 to 2.01 g l^{-1}, respectively. These results are in contrast with the results of Russowski et al. (2006) that studied the growth of whole plant in liquid culture and Chen et al. (2003) for taxol production in cell cultures of *Taxus yunnanensis*. High concentrations of inorganic nutrients in the full strength MS and MSV media may be the cause of these results. However, hairy roots dry weight in ½ MS and MSV were not significantly different (Figure 3).

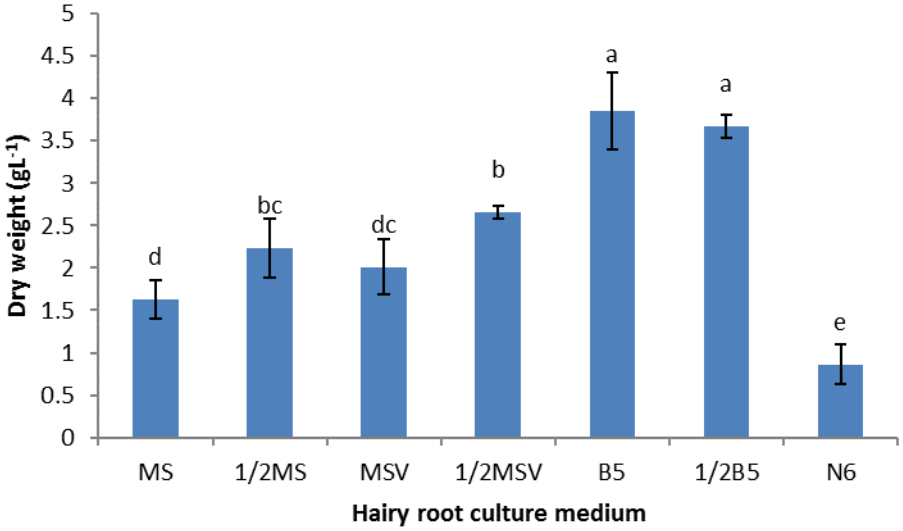

Figure 3: Effect of different culture media on hairy root growth of *V. officinalis*. Results are the mean of three replicates ± SD. Means with the same letter are not significantly different ($p > 0.05$).

3.4 Effect of NH_4^+ to NO_3^- ratio on hairy root growth

Different ratios of NH_4^+ to NO_3^- in MS medium were used for supplying the nitrogen requirement of the hairy roots. Figure 4 shows the effect of different nitrogen forms ratios on hairy root growth of *V. officinalis*. MS media supplemented with a 20:20 mM and 20:40 mM ratio of NH_4^+ to NO_3^- produced the maximum biomass after 21 days, 1.80 and 1.62 g l^{-1}, respectively. MS medium with a 20:20 ratio of NH_4^+ to NO_3^- slightly had greater influence on the growth of hairy roots than basal MS medium (20:40 mM), but this difference was not statistically significant. On the other hand, Shinde et al (2010) observed the highest biomass of *Psoralea corylifolia* hairy roots when MS medium was supplemented with NH_4^+ and NO_3^- at a ratio of 20:10. Decreasing the NH_4^+ concentration to 10 and 0 mM in the medium significantly reduced the hairy root growth. Similarly, decreasing the NO_3^- concentration had the same effect on the hairy root growth. Therefore, a balanced ratio of NH_4^+ to NO_3^- is an important factor for enhancing the growth of hairy root cultures in the *V. officinalis*.

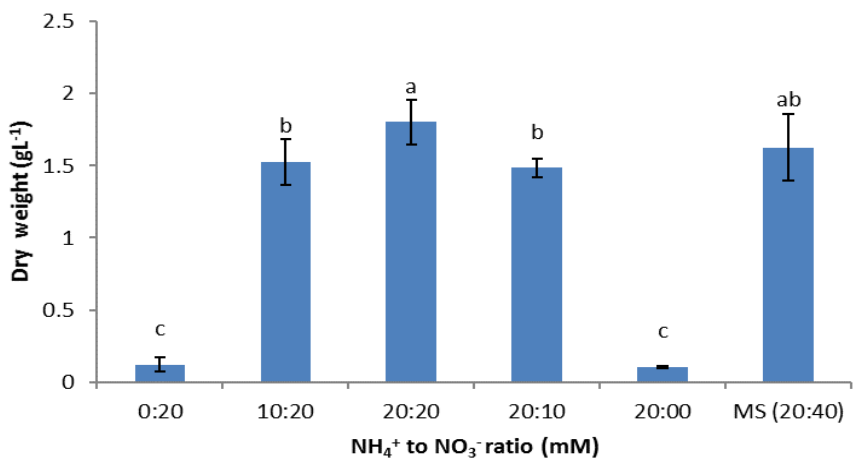

Figure 4: Effect of NH_4^+ and NO_3^- ratio on hairy root growth of *V. officinalis*. Results are the mean of three replicates ± SD. Means with the same letter are not significantly different ($p > 0.05$).

4 CONCLUSION

The present study highlights the importance of optimizing the culture media composition for *V. officinalis* hairy roots. The growth of valerian hairy roots was greatly affected by the composition and concentration of the culture media. The valerian hairy root system provides a promising platform that preserves the production of valuable sesquiterpenoids such as Valerenic acid.

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Agris category code: f30,f62

The effect of gamma radiation on seed germination of barley (*Hordeum vulgare* L.)

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ABSTRACT

The aim of the study was to determine the effect of gamma radiation on barley (Hordeum vulgare L.) seed germination and changes of seed viability associated with aging. The seed samples of the variety 'Astor' were irradiated at the Jožef Štefan Institute, in the Reactor Centre at Podgorica near Ljubljana, in 2006. The samples were irradiated with three different doses: 0.1, 0.2 and 0.4 kGy. After the irradiation, the seeds were stored in a refrigerator at 4 °C. The percentage of seed germination was tested each year after irradiation until 2014, except in the second and the third year. The experiments were based on the Latin square design with 4 replications of each treatment, and were conducted in a greenhouse under controlled temperature at 20 °C. In all years, the samples irradiated with the highest dose (0.4 kGy) exhibited a significantly lower percentage of germination. The germination rate of the samples irradiated with 0.2 and 0.4 kGy decreased significantly with aging, when compared to the control and the samples irradiated with 0.1 kGy. In all years of testing, the percentage of germination of seeds irradiated with 0.1 kGy did not differ from the control, and in the fifth year after irradiation, it was even significantly higher than the control.

Key words: gamma radiation, *Hordeum vulgare* L., seed germination, induced mutations

IZVLEČEK

VPLIV OBSEVANJA Z GAMA ŽARKI NA KALIVOST JEČMENA (*Hordeum vulgare* L.)

Cilj raziskave je bil ugotoviti vpliv obsevanja zrnja ječmena z gama žarki na kalivost ter na spremembo kalivosti s staranjem zrnja po obsevanju. Vzorci zrnja ječmena sorte 'Astor' so bili obsevani na Inštitutu Jožef Stefan, Ljubljana v Reaktorskem centru Podgorica v letu 2006. Vzorci so bili obsevani s tremi različnimi dozami: 0,1, 0,2 in 0,4 kGy ter po obsevanju hranjeni v hladilni omari pri temperaturi 4 °C. Po obsevanju smo vsako leto, razen v 2. in 3. letu, do l. 2014, v rastlinjaku preizkušali kalivost obsevanih vzorcev. Poskus je bil postavljen po metodiki latinskega kvadrata v 4 ponovitvah, poleg treh različnih doz obsevanja še kontrola z neobsevanim zrnjem. Vzorec z največjo dozo obsevanja (0,4 kGy) je v vseh letih preizkušanja kalivosti imel statistično značilno najmanjšo kalivost. Pri dozi obsevanja 0,2 in 0,4 kGy se je kalivost s staranjem zrnja statistično značilno zmanjšala glede na kontrolo in dozo sevanja 0,1 kGy. Seme obsevano z dozo 0,1 kGy je v vseh letih preizkušanja imelo enako kalivost kot kontrola, v 5. letu po obsevanju pa celo značilno večjo kalivost kot kontrola.

Ključne besede: gamma žarki, *Hordeum vulgare* L., kalivost zrnja, inducirane mutacije

1 INTRODUCTION

In plant breeding, mutations play an important role in the development of genetic variations and new varieties. Despite of a large number of undesirable mutants, the use of induced mutations has been found to be very useful not only in practical plant breeding but also in theoretical genetic research. The indications are numerous 'mutant' varieties which are very popular and have high economic impact on agriculture and food production (Ahloowalia et all, 2004). As reported by Kurowska et al. (2012) more than 3000 'mutant' varieties belonging to 200 species have been

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documented and officially released. All officially released 'mutant' varieties are documented in the FAO/IAEA Mutant Varieties Database (http://mvgs.iaea.org/ Search. aspx). Besides the artificially induced mutants, in agriculture production there are also some well-known spontaneous mutants such as sweet maize (Zea mays saccharata L.), pumpkins with non-lignified seed coat (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Grebensc.) and various dwarf genotypes in many plant species. For the induction of mutations, the most frequently used are gamma and X radiation, fast neutrons and some chemical agents like ethyl methane-sulphonate and methyl- or ethyl-nitroso urea. The radiation treatments became very popular in late 1920s when Stadler (1928) discovered and described some of the mutagenic effects of X-rays on barley and maize.

The aim of this study is to investigate the effect of gamma radiation (three different doses) on the percentage of seed germination, and changes of seed viability associated with aging (i.e., how seed germination changes in the years after irradiation).

2 MATERIAL AND METHODS

The study included barley (*Hordeum vulgare* L.), the variety 'Astor', seeds treated with gamma radiation in 2006, in the Jožef Štefan Institute, the Reactor Centre at Podgorica near Ljubljana. Three samples of barley seeds, 0.5 kg each, were treated with gamma rays using three different doses: 0,1, 0.2 and 0.4 kGy. As a control, we used a sample which was not irradiated.

After the treatment, the samples were stored in plastic bags in a refrigerator under the temperature 4-6 °C. Such conditions are required for the storage of the seed gene bank material (seed germination can be retained up to 20 years). The germination tests were conducted repeatedly each year after the irradiation. Each time, after the seeds had been taken for the experiment, the remaining seeds were put immediately back to the refrigerator.

The experiments were conducted in a greenhouse under the controlled temperature of 20 °C and were based on the Latin square design with 4 replications of each treatment. On each of the plots, 100 seeds were sown. Seeds of each treatment were sown in a special plateau filled with special germination substrate. To enable normal germination, regular watering was provided. Ten days after sowing we counted the number of all the seedlings that germinated. The same procedure was repeated each year, except in the second and the third year, until 2014.

The data were statistically analysed. For the analysis of variance the program Statgraphics Centurion XV.II and Microsoft Excel 2010 was used. The differences among the mean values of all treatments (doses, years) were compared using the Duncan's multiple rang test.

3 RESULTS

The analysis of variance showed that there were statistically significant differences in seed germination among years and among different doses of gamma radiation (Table 1). Considering all years, only the seeds treated with 0.4 kGy had a significant lower germination rate than other three treatments (control, 0,1 and 0.2 kGy). These treatments showed statistically equal seed germination (Table 2). Different percentage of seed germination was also found in different years after the treatment.

Regardless the irradiation dose, the lowest percentage of seed germination was found in the seventh and the ninth year after the irradiation, while the percentage of seed germination tested in the eighth year after the irradiation was not statistically different from rest of the years.

Table 1: The analysis of variance for the germination of seeds treated with different doses of gamma radiation, and in different years after irradiation

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-value
Doses	7186.38	3	2395.46	42.71	0.0000
Year	1407.67	6	234.61	4.18	0.0009
Row	101.59	3	33.86	0.60	0.6142
Column	62.32	3	20.77	0.37	0.7745
Residual	5159.44	92	56.08		
Total	13788.1	107			

Table 2: The percentage of germinated seeds treated with different irradiation doses

Irradiation dosses (kGy)	Seed germination (%)
0.4	70.5 a*
0.2	86.8 b
0.1	90.6 b
Control	90.5 b

* - the same letter indicate the non-significant difference at p = 0.05

Table 3: The percentage of germinated seeds after different number of years after irradiation

Years after irradiation	Seed germination (%)
9	77.6 a*
7	81.3 ab
8	84.3 bc
4	84.9 bc
5	86.6 bc
1	88.6 c
6	88.7 c

* - the same letter indicate the non-significant difference at p = 0.05

For the irradiation dose of 0,1 kGy there were significant differences in germination only between the sixth and the ninth year after the treatment (Table 4). For the irradiation dose of 0.2 kGy only in the ninth year after the treatment the germination rate was significantly different from the rest of the years (among which there were no significant differences).

Similar results were obtained at the highest dose of irradiation (0.4 kGy, Table 4). The lowest percentage of germinated seeds was found in the ninth year after the treatment. Regarding the control (without irradiation), there were significant differences in germination only between the first (the highest percentage of seed germination) and the fifth year (the lowest percentage of seed germination) after the treatment. However, there was no significant difference between the first and the ninth year after irradiation.

Table 4: The percentage of germinated seeds treated with different irradiation doses in different years after treatment

Years of testing	Irradiation doses (kGy)			
	0.1	0.2	0.4	Control
1	90.5 ab	90.0 b	80.0 c	94.0 b
4	93.2 ab	90.8 b	64.5 b	91.3 ab
5	90.7 ab	89.2 b	80.9 c	85.8 a
6	96.1 b	89.6 b	77.2 bc	93.1 ab
7	90.1 ab	85.0 b	68.3 bc	92.5 ab
8	89.6 ab	86.4 b	72.0 bc	89.0 ab
9	84.3 a	75.6 a	46.3 a	87.8 ab

* - the same letter indicate the non-significant difference at p = 0.05

In all years, only seeds treated by the highest dose of irradiation exhibited significantly lower germination rate than other doses and control. In the first, fourth, eighth, and ninth year after the irradiation no significant differences among irradiation doses of 0.1 and 0.2 kGy) and control were found. In the seventh year after irradiation, the non-irradiated seeds (control) germinated significantly better than irradiated with 0.2 kGy, while in the fifth year, the germination of the seeds of the control was lower than those irradiated with 0.1 kGy (Table 5). In the last two years after treatment, all the irradiation doses resulted in obvious decreasing percentage of germination (Fig. 1).

Table 5: The percentage of seed germination in different years after treatment at different irradiation doses

Irradiation doses (kGy)	Year of germinating test after irradiation						
	1st	4th	5th	6th	7th	8th	9th
0.1	90.5 b	93.2 b	90.7 c	98.8 c	90.2 c	89.6 b	84.3 b
0.2	90.0 b	90.8 b	89.2 bc	89.6 b	85.0 b	86.4 b	74.4 b
0.4	80.0 a	64.5 a	80.9 a	77.3 a	68.4 a	72.0 a	48.6 a
Control	94.0 b	91.3 b	85.8 b	93.1 bc	92.5 c	89.0 b	87.8 b

* - the same letter indicate the non-significant difference at p = 0.05

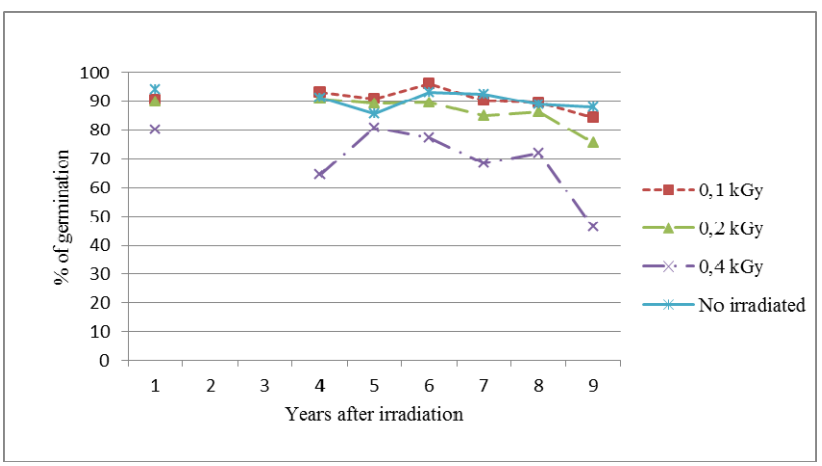

Figure 1: Germination of barley seeds in different years after treatment with three different doses of gamma rays

4 DISCUSION AND CONCLUSIONS

In plant breeding the variability of the genetic material is very important. Higher variability may also be created by mutagenesis. There are numerous mutagenic agents such as irradiation, treatments with various chemical substances, temperature, etc. Recently, very popular are also treatments with the laser radiation. The mutagenic treatment should not be drastic because it may cause lethal damages of the organism. It is very important to determine the right dose. As reported by several authors (Rybinski, 2002; Gladyszewska, 2009; Hernandez-Aguilar et al., 2009; Sacala et al., 2012) the laser irradiation leads to a lower frequency of very severe or milder injuries. Serious injuries can be prevented by various, mostly low doses of irradiation (Qing-he Li et al., 2012). In literature, it is possible to find data about the effects of different doses of irradiation. Wang et al. (2010) reported that low-dose laser irradiation induced low-frequency but significant alterations in DNA methylation level and pattern in sorghum plants, while Horn and Shimelis (2013) found that the effects of different doses of gamma radiation depended also on genotypes. The percentage of cowpea germination dropped from 100 % (at 0 Gy) to 0 % when applying 0.3 and 0.4 kGy on the genotypes 'Nakare' and 'Shindimba', but the germination of the genotype 'Bira' eposed to 0.6 kGy was 47 %. In our study, we established that the maximum dose of irradiated barley (0.4 kGy) has a strong influence on the reduced germination rate and rapid aging of seeds (i.e., lower germination in later years). The lowest irradiation dose (0.1 kGy) did not exhibit lower germination than the control, even more, in the fifth year after irradiation it had a significantly higher germination rate than the control. The seeds treated with higher doses (0.2 and 0.4 kGy) exhibited fast decrease of germination rate in last two years and this may be very important for plant breeders and especially for the maintainers of the gene banks material. Irradiated seeds should not be stored for a long time.

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Comparative study of diagnostic methods used for monitoring of common grape vine (*Vitis vinifera* L.) crown gall (*Agrobacterium vitis* Ophel & Kerr*)* in Slovenia

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ABSTRACT

Agrobacterium vitis causes common grape vine (*Vitis vinifera* L.) crown gall disease that destroyed a lot of Slovenian vineyards more than a decade ago. Eighty isolates of *Agrobacterium* spp. collected during monitoring in 2006 were identified as *A. vitis* and *A. tumefacies* by *peh*A and multiplex PCR method. Tumor-inducing capacity of these strains was assessed on test plants and with PCR methods for detection of the Ti plasmid responsible for tumor induction. With VCF3/VCR3 primer pair six false negatives and no false positives were detected. The high genetic diversity of pathogenic *Agrobacterium* spp. strains affects the performance of molecular methods, thus biological test should be performed where results from molecular methods are doubtful.

Key words: *Agrobacterium vitis*, common grape vine, host plants, *peh*A, multiplex PCR, VCF3/VCR3

IZVLEČEK

PRIMERJAVA DIAGNOSTIČNIH METOD SPREMLJANJA POJAVA RAKA (*Agrobacterium vitis* Ophel & Kerr) ŽLAHTNE VINSKE TRTE (*Vitis vinifera* L.) V SLOVENIJI

Bakterija *Agrobacterium vitis* je povzročitelj raka žlahtne vinske trte (*Vitis vinifera* L). Ta bolezen je uničila mnogo slovenskih vinogradov pred več kot desetimi leti. V sklopu spremljanja pojava bolezni smo leta 2006 izolirali 80 izolatov, ki smo jih s PCR metodama *peh*A in multipleks določili kot vrsti *A. vitis* in *A. tumefaciens*. Sposobnost sevov, da izzovejo nastanek tumorjev, smo ocenjevali na gostiteljskih rastlinah. Z molekularnimi metodami pa smo določali prisotnost plazmida Ti, povzročitelja nastanka tumorjev. Z metodo PCR smo ugotovili šest lažno negativnih patogenih sevov in nobenega lažno pozitivnega. Velika genetska raznolikost patogenih sevov *Agrobacterium* spp. vpliva na zanesljivost določanja z molekularnimi metodami, zato se v primeru dvomljivih rezultatov priporoča dodatna izvedba bioloških testov na rastlinah.

Ključne besede: *Agrobacterium vitis*, vinska trta, gostiteljske rastline, *peh*A, multipleks PCR, VCF3/VCR3

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1 INTRODUCTION

Crown gall disease occurs worldwide and causes major economical losses in fruit and grapevine production (De Cleene and De Ley, 1976; Kennedy and Alcorn, 1980; Pulawska, 2010). The major part of income loss is attributed to crown gall on young grafted plants in nurseries. The disease is characterized by a tumor which is usually formed on a plant stem just above the ground. Still, the disease is rarely fatal. Mainly young or stressed plants develop more pronounced symptoms: loss of plant vigour, reduction in crop yield, or even plant death (Poncet *et al.*, 1996; Epstein *et al.,* 2008). The disease is problematic on perennial horticultural crops, such as grapevines, stone and pome fruit trees, and ornamental plants, where tumors weaken the plant year after year. The causal agents of the disease are pathogenic *Agrobacterium* spp. carrying the Ti plasmid (pTi) (Van Larebeke *et al.*, 1974; Watson *et al.*, 1975). The ability to cause tumors is encoded on a portion of the pTi (T-DNA) that integrates into the host genome. Upon expression, the T-DNA genes alter the level of plant hormones resulting in uncontrolled plant cell proliferation and tumor formation (reviewed in Escobar and Dandekar, 2003).

Traditional identification of *Agrobacterium* spp. is based on biochemical tests (Holt *et al.*, 1994). The INCO-DC European program ERBIC18CT970198, "Integrated Control of Crown Gall in Mediterranean Countries" has presented an identification scheme for agrobacteria with minimal biochemical tests (reviewed in Shams *et al.*, 2012). Additionally, accurate identification can be achieved by molecular methods. Eastwell *et al.* (1995) developed a PCR method for detecting *A. vitis* (Ophel and Kerr, 1990) – causative agent of crown gall of grapevines. The method targets chromosomal polygalacturonase gene that is found in *A. vitis*, but not in *A. tumefaciens* (Smith & Townsend, 1907) Conn 1942 or *A. rhizogenes* (Riker *et al.* 1930) Conn 1942, which are rarely found in grapevine tumors. A decade later, Pulawska *et al*. (2006) developed a multiplex PCR for classification of *Agrobacterium* strains into *A. tumefaciens*, *A. rhizogenes* and *A.vitis*. This method amplifies the specific fragment on 23S rRNA and enables rapid diagnosis of *Agrobacterium* species. These molecular techniques are based on bacterial DNA and are more specific, sensitive, rapid and suitable for diagnostics. The genetic diversity within genus *Agrobacterium* has recently led to reclassification of *A. rhizogenes* into genus *Rhizobium* (Young *et al.*, 2006). On the other hand, *A. tumefaciens* and *A. vitis* were reported to differ from the members of the genus *Rhizobium* and therefore can remain in the same genus (Farrand *et al.*, 2003, Lindström and Young, 2011). Additionally, genetically variable strains of *A. tumefaciens*, now termed *A. tumefaciens* species complex group, were clustered into genomospecies that will progressively be reclassified into new species (Mougel *et al.,* 2002¸ Portier *et al.,* 2006; Lindström and Young, 2011; Pulawska and Kalužna, 2012).

Effective detection of tumor-inducing agrobacteria in plant material is crucial in propagating material and efficient management of crown gall disease. Traditionally, bacteria are isolated from plant or soil material by cultivation on selective media followed by testing their tumor-inducing capacity on test plants. According to Schroth *et al*. (1971), this protocol is not sensitive or robust enough in comparison to molecular methods. Most molecular methods for detection of tumorigenic isolates target tumorigenicity genes on a conserved *vir* region on the pTi. Sawada *et al.* (1995) developed VCF/VCR primers that target pTi-encoded *vir*C1 and *vir*C2 genes. Suzaki *et al*. (2004) improved the specificity of the primers (VCF3/VCR3) for pathogenic *Agrobacterium* strains from apple seedlings, but the primers work on *A. vitis* strains as well (Kumagai and Fabritius, 2008).

Grape crown gall caused substantial damage to vineyards in the winegrowing regions of Slovenia in 1999 (Šabec-Paradiž *et al.,* 2002). Much of the following *Agrobacterium*-based research in Slovenia was dedicated to control and prevention of *A. vitis* and *A. tumefaciens* infections on grapevine plants and propagating material, and also to characterization of *A. vitis* isolates in Slovenia (Fabjančič and Milevoj, 2003). In the present study we compared the *Agrobacterium* identification methods used for grape crown gall disease monitoring in Slovenia.

2 MATERIALS AND METHODS

2.1 Bacterial strains and isolates

A crown gall monitoring was conducted in 2006. Eighty-seven symptomatic grapevine grafts (Figure 1) were collected from nurseries and vineyards from across various winegrowing regions of Slovenia. Eighty isolates of *Agrobacterium* spp. were obtained from plant material on 3DG medium semi-selective for *A. vitis* (Brisbane and Kerr, 1983). All 80 strains were subcultured on King's B medium (KB), pure cultures preserved in meat peptone broth with glycerol, and stored at -80 °C until further use. All 80 strains were analysed in the diagnostic laboratory at Agricultural Institute of Slovenia.

Reference *A. tumefaciens* C58 (INRA, France), *A. vitis* 339-26 (IVIA, Spain) and *Rhizobium rhizogenes* K84 (IVIA, Spain) strains were used as controls in molecular and biological diagnosis.

2.1.2 Preparation of bacterial DNA

The bacterial DNA used in PCR reactions was extracted from 24 h-old colonies grown on KB medium at 27 °C. We used a standard alkaline lysis method (Sambrook *et al*., 1989), diluted the DNA (1:1000) in sterile distilled water and stored it at -20 °C.

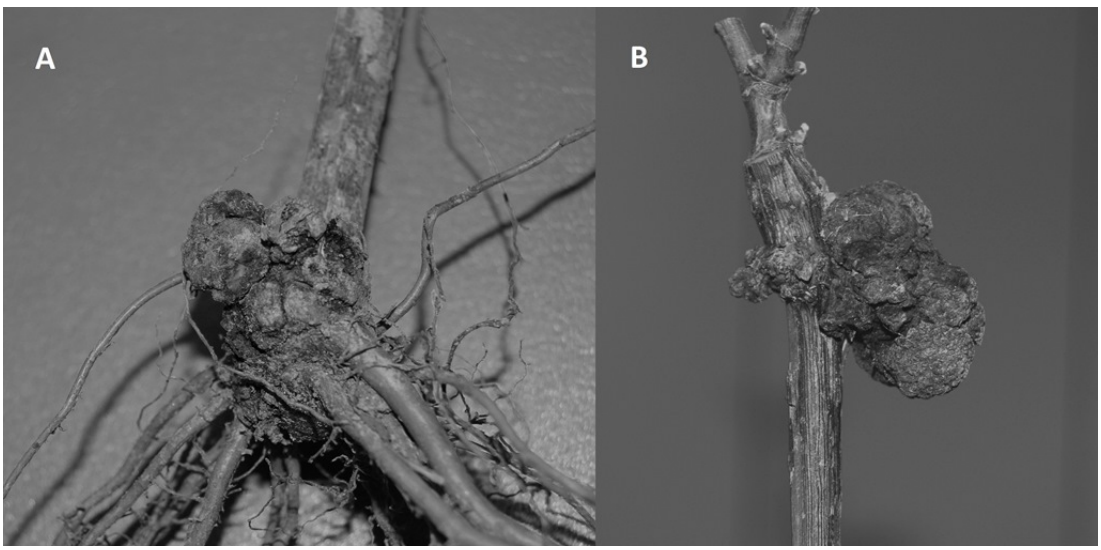

Figure 1: Grapevine grafts showing crown gall symptoms on a heel (A) and on a graft union (B) (Photos: I. Zidarič).

2.2 Identification of *A. vitis*

A. vitis isolates were identified based on polygalacturonase gene amplification (*peh*A) and multiplex PCR (Pulawska *et al*., 2006). Repeatability of the PCR results on the same DNA samples was verified in 2013.

2.2.1 Polygalacturonase gene amplification

The *peh*A PCR was performed in a total volume of 25 µl applying the protocol of Eastwell *et al*. (1995). For the PCR reaction, 1 µl of bacterial DNA template was used for PCR amplification in 1× PCR Buffer (Promega), 2.0 mM $MgCl_2$, 0.1 µM each *peh*A primer (Table 1), 0.2 mM dNTPs, 0.25 U GoTaq Flexi DNA Polymerase (Promega). The thermal cycler was programmed for an initial denaturation at 95 °C for 3 min followed by 40 cycles of amplification (95 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min) with 5 min of final elongation at 72 °C. The amplified fragments of 205 bp were visualized on 2 % agarose gel.

2.2.2 Multiplex PCR

The multiplex PCR was performed in a 15 µl reaction volume applying the protocol of Pulawska *et al*. (2006). All reactions were performed in 1× PCR buffer (Promega), 1.5 mM $MgCl_2$, 1 µM each primer (UF, B1R, B2R and AvR) (Table 1), 0.2 mM dNTPs and 1.0 U GoTaq Flexi DNA

Polymerase (Promega). The amplification conditions comprised an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 67 °C for 1 min, extension at 72 °C for 1.5 min and a final extension step at 72 °C for 10 min. The amplified PCR fragments were visualized on a 2 % agarose gel. Strains belonging to *A. tumefaciens* gave a 184 bp product and those belonging to *A. vitis* gave a 478 bp product (Figure 2).

2.3 Assessing tumor-inducing capacity

Diagnosis of pathogenic strains of *Agrobacterium* spp. is carried out biologicaly on wounded test plants and molecularly through detection of bacterial tumour-inducing plasmid (pTi) responsible for uncontrolled plant cell growth.

2.3.1 Pathogenicity tests

The pathogenicity of *Agrobacterium* strains was determined on tomato, sunflower and kalanchoe plants. Young, four-week-old seedlings were punctured three times in the stem using a sterile entomological needle dipped in pure culture colonies grown on KB medium for 24 hours at 27 °C. Tests were performed in triplicates. Inoculated seedlings were maintained in a glasshouse at 20 – 30 °C with natural lighting conditions. In the period of 3 to 6 weeks post inoculation, the plants were visually inspected for tumor formations every few days. The strains C58 and 339-26 were used as positive controls; strain K84 and water served as negative controls. The test was completed in 2007.

2.3.2 pTi detection

The PCR was performed in a 25 µl reaction volume applying the protocol of Suzaki *et al*. (2004). For the PCR reaction, 2 µl of bacterial DNA template (diluted 1000 ×) were used for PCR amplification in 1× PCR Buffer (Promega), 1.5 mM $MgCl_2$, 0.5 µM VCF3 and VCR3 primers (Table 1), 0.2 mM dNTPs, 0.5 U GoTaq Flexi DNA Polymerase (Promega). The thermal cycler was programmed with an initial denaturation at 94 °C for 5 min followed by 35 cycles of amplification (94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min) with 5 min of final elongation at 72 °C. The amplified fragments of 414 bp were visualized on 2 % agarose gel (Figure 2).

Table 1: Primers pair sequences used in our study.

Primer	Sequence	Reference
pehAF	5'-CGATGGCGGCGAGGATTT-3'	Eastwell *et al*., 1995
pehAR	5'-ATCGGGCGTGAAACAAGT-3'	
UF f	5'-GTAAGAAGCGAACGCAGGGAACT-3'	Pulawska *et al*., 2006
B1R r	5'-GACAATGACTGTTCTACGCGTAA-3'	
B2R r	5'-TCCGATACCTCCAGGGCCCCTCACA-3'	
AvR r	5'-AACTAACTCAATCGCGCTATTAAC-3'	
VCF3	5'-GGCGGGCGYGCYGAAAGRAARACYT-3'	Suzaki *et al*., 2004
VCR3	5'-AAGAACGYGGNATGTTGCATCTYAC-3'	

2.4 Data analysis

Agreement between PCR and pathogenicity test was evaluated by calculating positive and negative percent agreement with respect to imperfect reference standard, in our case the *peh*A method and the biological pathogenicity test. The agreement indices were calculated from two-dimensional contingency table shown in Table 2.

Table 2: Two-dimensional contingency table for calculating agreement indices between two methods.

	Standard method A	
Method B	positive	negative
positive	a	b
negative	c	d
Total	(a+c)	(b+d)

Positive percent agreement with respect to imperfect reference standard was calculated according to equation (1) and was interpreted as sensitivity (Se) of the method. Similarly, negative percent agreement with respect to imperfect standard was calculated according to equation (2) and was interpreted as specificity (Sp) of the method. For estimation of confidence limits the 95 % confidence interval (CI) was calculated where appropriate.

$$\mathrm{Se} = \left(a/a + c\right) \cdot 100\% \quad (1)$$

$$\mathrm{Sp} = \left(d/b + d\right) \cdot 100\% \quad (2)$$

3 RESULTS AND DISCUSSION

3.1 Identification of *A. vitis* by *peh*A or multiplex PCR method

Morphologically, most rhizobial colonies appeared similar to one another on a general media. Therefore, it is imperative to use selective media for isolation of *A. vitis*. On 3DG medium, *A. vitis* colonies were visible sooner (after 3 days at 27 °C) than colonies of *A. tumefaciens* and *R. rhizogenes,* which also had different colony morphologies on 3DG medium. Where no typical *A. vitis* colonies were found, we selected for colonies that predominated on 3DG medium.

From 87 grapevine grafts we obtained 80 *Agrobacterium* isolates. According to multiplex PCR (Pulawska *et al.*, 2006) 75 isolates were identified as *A. vitis* and five as *A. tumefaciens*. The number of identified *A. vitis* strains was compared to the number of *peh*A positive (*A. vitis*) strains. There was a perfect agreement (100 %) between the two methods. All *peh*A positive isolates had an *A.vitis*-diagnostic band of 478 bp in multiplex PCR. The results were verified in 2013 on the same DNA samples stored at - 20 °C.

Our diagnostic laboratory has completely replaced the *peh*A identification method with multiplex PCR as it distinguishes between *A. vitis* and *A. tumefaciens* and differentiates them from other rhizobia in the Rhizobiaceae family in one reaction.

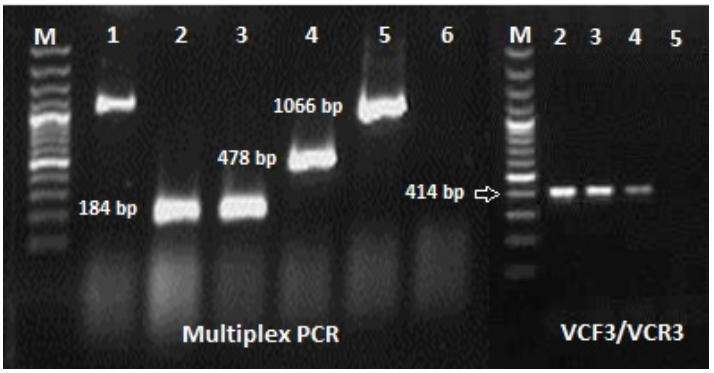

Figure 2: Agarose gel electrophoresis of diagnostic fragments from multiplex and VCF3/VCR3 PCR; M (ladder), 1 (unknown soil isolate), 2 (grapevine isolate), 3 (C58, *A. tumefaciens*), 4 (339-26, *A. vitis*), 5 (K84, *R. rhizogenes*), and 6 (water).

3.2 Pathogenicity status and pTi detection

All 80 isolates were tested for pathogenicity and abilitiy to cause tumors on stems of inoculated plants. This is a standard method for diagnosis of tumor-inducing strains and detection of latent infections (Janse, 2005). Almost 70 % of the strains were found pathogenic. One pathogenic strain was identified as *A. tumefaciens* causing tumors on all three test plants. In 2013 we analysed the same strains for the presence of pTi. We used a PCR method for identification of pathogenic and non-pathogenic strains of agrobacteria using primers VCF3/VCR3 with improved specificity (Suzuki *et al.*, 2004). All strains with detected pTi were identified as *A. vitis* by multiplex PCR. The agreement between results from pathogenicity tests and pTi detection method was not exact (Table 3). The sensitivity of VCF3/VCR3 primer pair was 89.1 % with six false negatives (Table 4). The only pathogenic *A. tumefaciens* strain was one of them. However, the specificity was 100 % with no false positives. The VCF3/VCR3 results were most compatible with pathogenicity assessment on kalanchoe and tomato test plants, though specificity was higher on kalanchoe plants (Table 4).

Table 3: Summary of results by two methods for *Agrobacterium spp.* pathogenicity assessment.

	Pathogenicity test		
VCF3/VCR3	+	-	Total
+	49	0	49
-	6	25	31
Total	55	25	80

It is not uncommon for *A. vitis* strains to have different host range even with identical physiological and biochemical characteristics (Tolba and Zaki, 2011). Interestingly, results from Tolba and Zaki (2011) indicate tomato as an unreliable test plant giving positive results on pathogenic *A. vitis* strains in only 5 of 12 isolates. At the same time, test on tomato proved specific for certain strains that caused tumors only on grapevines. In our case, none of the grapevine strains caused tumors specifically on tomato. On the other hand, six strains caused tumors solely on sunflower, and two solely on kalanchoe test plants. The presence of pTi was diagnosed on only half of these strains (three and one). One possible explanation is the sensitivity of the primers. These might be affected by high genetic diversity within pathogenic agrobacteria which could result in false negatives.The use of a set of three plants proved crucial in pathogenicity determination, as few of the pathogenic strains exhibited preference toward one host plant. If we had used only tomato or sunflower test plants, we would have observed fewer pathogenic strains and determined lower specificity of the VCF3/VCR3 PCR method in comparison to pathogenicity test results (Table 4).

Table 4: Sensitivity (Se) and specificity (Sp) of VCF3/VCR3 primer pair with respect to pathogenicity test on test plants.

VCF3/VCR3	Test plant tomato	sunflower	kalanchoe	Overall
Se	95.1	89.1	95.7	89.1 CI [82.27; 95.93]
Sp	74.4	76.5	85.3	100

The biological pathogenicity test is laborious and time-consuming. Pathogenicity is affected by environmental factors like temperature (Hamilton and Fall, 1971) and plant age (Binns and Thomashow, 1988). Also, the absence of tumors does not necessarily imply the absence of pTi. This is where molecular methods provide an additional confirmation. We repeated pathogenicity tests three times in three different seasons (spring, summer and autumn) using young to mature plants (results not shown). The most consistent results were obtained on young plants in late spring and early autumn when the average air temperature in the greenhouse was around 25 °C. Also, the interpretation can be doubtful when the PCR shows the absence of pTi, but the test plants develop tumors. Although PCR techniques for simultaneous identification of pathogenic and non-pathogenic *A. vitis* are available (Kawaguchi *et al.*, 2005), the traditional pathogenicity test is still a standard technique in strain pathogenicity determination.

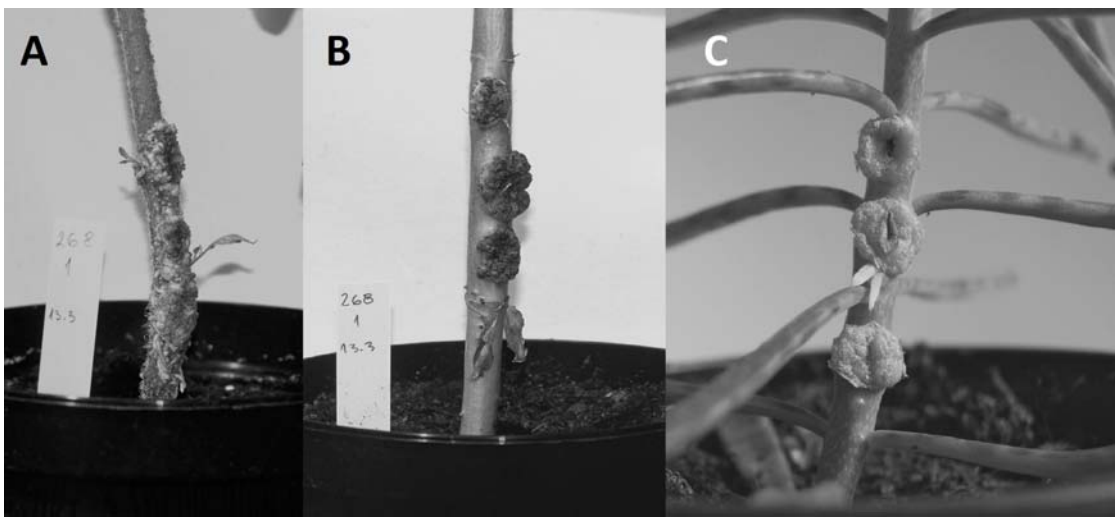

Figure 3: Pathogenicity tests on tomato (A), sunflower (B) and kalanchoe (C) plants (Photos: I. Zidarič).

4 CONCLUSION

Pathogenic *A. vitis* strains predominated among isolates of *A. vitis* from Slovenian grapevine grafts. Only one strain of *A. tumefaciens* was found pathogenic. In identification of *A. vitis* we obtained matching results using *peh*A or multiplex PCR primers. Therefore, we suggest using multiplex PCR (Pulawska *et al.*, 2006) for reliable identification of *A. vitis* and *A. tumefaciens* on grapevine. Further, we detected most of the pathogenic strains with VCF3/VCR3 primers. Based on our results, one might conclude that VCF3/VCR3 PCR could replace pathogenicity tests, but due to the false negatives, we conclude that biological pathogenicity test is still an invaluable tool in plant bacteriology.

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Agris category code: h10

Zatiranje plodove vinske mušice (Drosophila suzukii [Matsumura], Diptera, Drosophilidae) s poudarkom na okoljsko sprejemljivih načinih

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IZVLEČEK

Plodova vinska mušica (*Drosophila suzukii*) spada med gospodarsko pomembne škodljive žuželke, ki povzročajo škodo na gojenih in divjih sadnih vrstah. Škodljivec, ki je uvrščen na seznam A2 EPPO, se v Sloveniji pojavlja od leta 2010 in se postopoma širi. Od prve najdbe, leta 2008 v Španiji in Italiji, jo danes najdemo že v večini sredozemskih držav. V pričujočem preglednem članku so predstavljeni najpomembnejši načini varstva sadnih rastlin pred plodovo vinsko mušico. V nekaterih delih sveta škodljivca učinkovito zatirajo s sintetičnimi insekticidi, ki pa so pri nas problematični zaradi karenčne dobe, saj se mušica pojavlja na zorečih in predvsem na zrelih plodovih tik pred obiranjem. Tako je v svetu vse več raziskav usmerjenih v preizkušanje alternativnih (okoljsko sprejemljivih) načinov zatiranja škodljivca. Biotično zatiranje plodove vinske mušice je v Aziji zaenkrat bolj uspešno kot v Evropi, zato velja na Stari celini raziskavam razširjenosti in učinkovitosti domorodnih naravnih sovražnikov škodljivca v prihodnje nameniti več pozornosti. Med njimi predlagamo raziskave učinkovitosti plenilske stenice *Orius majusculus* in entomopatogenih ogorčic, v trenutni vsakdanji praksi pa priporočamo še uporabo vab z atraktanti za masovno lovljenje odraslih osebkov, rastlinsko higieno v nasadih, prekrivanje rastlin z gosto mrežo in podobno.

Ključne besede: plodova vinska mušica, *Drosophila suzukii*, kemično zatiranje, semiokemikalije, biotično varstvo rastlin

ABSTRACT

CONTROL OF SPOTTED WING DROSOPHILA (*Drosophila suzukii* [Matsumura], Diptera, Drosophilidae) WITH THE EMPHASIS ON ENVIRONMENTALLY ACCEPTABLE METHODS

Spotted wing drosophila (*Drosophila suzukii*) is an economically important insect pest, which causes damage on cultivated and wild-growing fruit plants. The pest, which is placed in A2 EPPO list, occurred in Slovenia since 2010 and it is spreading progressively. Since its first record in Spain and Italy (2008), it is now present in the majority of Mediterranean countries. In the review paper the most important control methods against the spotted wing drosophila are presented. In some parts of the world the pest is efficiently controlled with synthetic insecticides, however their use is questionable owing to waiting period, since the insect occurs on fruits in the time of ripening and before harvesting. Thus, more and more researches are focused in the studies of alternative (environmentally sound) control methods of this pest. So far, biological control of spotted wing drosophila is more effective in Asia than in Europe. In the upcoming years it is therefore important to investigate the distribution and efficiency of indigenous biological control agents in the Old continent. Among them we suggest to investigate how effective are the predatory bug *Orius majusculus* and entomopathogenic nematodes against this pest. For everyday practice, we recommend the use of traps filled with attractants for the massive trapping of adults, plant hygiene in plantations, covering the plants with dense net etc.

Key words: spotted wing drosophila, *Drosophila suzukii*, chemical control, semiochemicals, biological control

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1 UVOD

Plodova vinska mušica (*Drosophila suzukii* [Matsumura], Diptera, Drosophilidae) spada med pomembne invazivne vrste in ogroža pridelavo sadnih rastlin v Evropi (Arnó in sod., 2012).

Ta žuželčja vrsta je uvrščena na seznam A2 EPPO (EPPO Standards, 2012) in izvira iz Azije. V Evropi se je najprej pojavila v Kataloniji (Španija) in Italiji leta 2008, že naslednje leto pa so jo našli v Franciji (Arnó in sod., 2012; Cini in sod., 2012). V Sloveniji je bila žuželka ugotovljena leta 2010 (Seljak, 2011), v obdobju 2010-2011 pa so njeno zastopanost potrdili v Švici, Avstriji, Nemčiji, Belgiji in na Hrvaškem. Doslej so škodljivca potrdili že v večini sredozemskih držav (Cini in sod., 2012). V letu 2012 se je mušica v ZDA pojavljala v 28 državah in v dveh provincah v Kanadi (Lee in sod., 2012).

Odrasli osebki plodove vinske mušice, za katero je sicer značilen spolni dimorfizem, merijo v dolžino 2-3 mm (Cini in sod., 2012). Njen razvojni krog traja od 8 do 14 dni, pri 25°C traja razvoj škodljivca 10 dni. Odrasli osebki spolno dozorijo 1-2 dneva po pojavu. Samica lahko v en plod odloži do 3 jajčeca. Število plodov, kamor lahko samica dnevno odloži jajčeca pa se giblje med 7-16. Ovipozicija traja 10-59 dni, kar pomeni, da lahko samice v življenju v povprečju odložijo 400 jajčec (Cini in sod., 2012). Vrsta, ki prezimi kot odrasel osebek, sicer slabše prenaša temperature pod 10°C (Cini in sod., 2012) in nad 32°C (Calabria in sod., 2012). V ugodnih razmerah lahko ta škodljiva mušica razvije 7-15 rodov (Cini in sod., 2012), njihovo število pa je odvisno predvsem od okoljskih dejavnikov in razpoložljive hrane (Seljak, 2011). Plodova vinska mušica je posebna predvsem zaradi oblike leglice (Cini in sod., 2012), ki je sestavljena iz dveh močno hitiniziranih polovic sabljaste oblike, z nizom močnih in skoraj črnih trnov po robu (Seljak, 2011). Poleg vrste *D. subpulchrella* Takamori and Watabe je plodova vinska mušica ena od dveh vrst plodovih vinskih mušic, ki lahko poškodujejo zdrave, nepoškodovane plodove. Poškodbe in ovipozicija (Mitsui in sod., 2006) plodove vinske mušice so bolj pogoste na zorečem sadju kot na zrelih in gnilih plodovih (Cini in sod., 2012). Ličinke (žerke) se prehranjujejo z mehkim tkivom v notranjosti plodov, katerih vsebina postane zdrizasta. Napadeni plodovi so neuporabni (*Drosophila suzukii*, EPPO, 2013).

Škodljivec je bil prvič najden na češnjah, in sicer leta 1916 na Japonskem (Lee in sod., 2011), do danes pa se je krog njegovih gostiteljskih rastlin zelo razširil. Tako ga lahko najdemo na številnih gojenih in samoniklih rastlinah, med bolj znanimi gostitelji pa so vrste *Fragaria ananasa*, *Rubus* spp. (*Rubus idaeus*, *Rubus fructicosus*, *Rubus lacinatus*, *Rubus armeniacus*, *Rubus ursinus*,…), *Prunus* spp. (*Prunus avium*, *Prunus armeniaca*, *Prunus persica*, *Prunus domestica*), *Eriobotrya japonica*, *Vaccinium* spp. in hibridi borovnic, *Ribes* spp., *Ficus carica*, *Morus* spp., *Rhamnus alpnia* spp. *fallax*, *Rhamnus frangula*, *Cornus* spp., *Actinidia arguta*, *Diospyros kaki*, *Eugenia uniflora*, *Murraya paniculata*, *Myrica rubra*, *Lonicera* spp., *Elaeagnus* spp., *Sambucus nigra*, *Vitis vinifera*, *Vitis labrusca* (Seljak, 2011; Arnó in sod., 2012; Cini in sod., 2012; Landolt in sod., 2012; Poyet in sod., 2013). Širok krog gostiteljskih rastlin, tako divjih kot gojenih, škodljivcu omogoča hitro širjenje. Ugotovljeno pa je tudi bilo, da je ima plodova vinska mušica veliko sposobnost ohranjanja populacij (Cini in sod., 2012).

Namen našega dela je predstaviti doslej znane načine zatiranja omenjene gospodarsko pomembne škodljive vrste, ki povzroča vse več težav v sadjarstvu.

2 NAČINI ZATIRANJA PLODOVE VINSKE MUŠICE

2.1 Kemično zatiranje

Zatiranje plodove vinske mušice z insekticidi je bilo doslej najbolj intenzivno v ZDA (Beers in sod., 2011; Lee in sod., 2011). V dveh raziskavah so tako insekticidi iz skupin organskih fosforjevih estrov (OFE) in piretroidov ter pripravka na podlagi spinosada in piretrina uspešno zmanjšali populacijo plodove vinske mušice v nasadih češenj v Kaliforniji, nanos piretroidov pa je vplival

predvsem na večjo smrtnost jajčec in ličink v nasadih jagodičevja. Avtorji raziskave ugotavljajo, da je potrebno za vzdrževanje vrste *Drosophila suzukii* pod pragom gospodarske škode v nasadih češenj vsaj dvakratno škropljenje med rastno dobo, pri čemer priporočajo insekticid malation. Zaradi znanih negativnih vplivov avtorji proti škodljivcu odsvetujejo uporabo neonikotinoidov, za ekološko pridelavo sadja pa predlagajo uporabo spinosada v kombinaciji s piretrinom (Beers in sod., 2011; Bruck in sod., 2011). Za razliko od zgoraj omenjenih avtorjev pa Grassi in sod. (2011) poročajo o zmernem delovanju insekticidov na plodovo vinsko mušico, pri čemer je bil najbolj učinkovit pripravek na podlagi lambda-cihalotrina (Grassi in sod., 2011; Cini in sod., 2012).

Države, ki uvažajo jagode iz Kalifornije, med drugim tudi Avstralija, so sprejele zelo stroge ukrepe pri zatiranju plodove vinske mušice. Tako uvožene plodove tretirajo z metilbromidom, pri čemer izpostavljenost >80 mg L^{-1} za 1 uro povzroči 100 % smrtnost škodljivca (Walse in sod., 2012).

2.2 Uporaba semiokemikalij

Med uspešnimi tehnikami privabljanja vrst iz rodu *Drosophila* se v strokovni literaturi največkrat navaja uporaba vodne suspenzije kvasa (Seljak, 2011; Cha in sod., 2012). Pri razgradnji kvasa kvasovke in mlečnokislinske bakterije povzročijo nastanek ocetne kisline in etanola, ki delujeta privabilno na plodovo vinsko mušico. Pri preizkušanju vab, ki so vsebovale kis in vino, pa so ugotovili, da takšne vabe veliko bolj privabljajo škodljivca kot tiste, ki so vsebovale ocetno kislino in etanol (Cha in sod., 2012). Uspešnejše privabljanje mešanice kisa in vina v primerjavi z ocetno kislino in etanolom ugotavljajo tudi Landolt in sod. (2011). Zanimiva je ugotovitev, da je število odraslih osebkov plodove vinske mušice, ki se ujamejo v pasti z mešanico vina in kisa, odvisna tudi od vrste vina in kisa. Tako sta za image najbolj privabilna 'Merlot' in rižev kis (Landolt in sod., 2012). Kot učinkovit atraktant plodove vinske mušice pa velja tudi vonj razkrajajoče hrane, v katero lahko samice odlagajo jajčeca (Landolt in sod., 2011). Predstavljene načine lahko uspešno izkoriščamo v sistemih masovnega lovljenja škodljivca, med katerimi je lahko učinkovit tudi način »privabi in ubij« (angl. »attract and kill«) (Cini in sod., 2012).

Na Kitajskem, kjer so na enem hektaru razporedili 24-40 pasti, je to vplivalo na zmanjšanje populacije plodove vinske mušice. Velja pa omenjeni način zatiranja za delovno zahtevnejši postopek in je posledično ustreznejši za manjše nasade (Lee in sod., 2011). Uspešnost lovljenja plodove vinske mušice je pogojena tudi z barvo, obliko in strukturo vabe. Med barve, ki najbolj privabljata škodljivca, so tako uvrstili črno in rdečo (Cini in sod., 2012; Basoalto in sod., 2013), najpogosteje uporabljena vaba pa je bila sestavljena iz plastične steklenice s 5-10 mm velikimi luknjami (Cini in sod., 2012). Vabe so lahko prekrite tudi z mrežo, ki ima luknje omenjene velikosti (Walsh in sod., 2013). V vabi je tekočina, ki privablja odrasle osebke, z namenom preprečitve pobega pa je vabi dodana še lepljiva plošča (Cini in sod., 2012). Učinkovitost vab je največja, če so postavljene v senčno, hladno lego, na primer na talno površje ali na rastline v bližino plodov (Walsh in sod., 2011; Cini in sod., 2012).

Nekateri avtorji med atraktanti, ki so uporabni za privabljanje plodove vinske mušice, navajajo tudi tiste, ki so namenjeni zatiranju oljčne muhe (*Bactrocera oleae* Gmelin) (Seljak, 2011) in breskove muhe (*Ceratitis capitata* [Wiedemann]) (Escudero Colomar in sod., 2011).

2.3 Biotično zatiranje

Informacije o biotičnem varstvu rastlin pred plodovo vinsko mušico so še vedno zelo redke. Vse več raziskav se osredotoča na iskanje naravnih sovražnikov, predvsem parazitoidov, ki bi lahko zmanjšali številčnost populacij te gospodarsko pomembne škodljive vrste (Chabert in sod., 2012). Kožekrilca *Pachycrepoideus vindemmiae* (Rondani) (Pteromalidae) in *Trichopria drosophilae* Perkins (Diapriidae) sta doslej v Evropi edini potrjeni vrsti ektoparazitoidov, ki se lahko uspešno razvijata na bubah plodove vinske mušice. Medtem ko se lahko vrsta *P. vindemmiae* uspešno razvija tako na ameriški kot na francoski populaciji škodljivca, pa je vrsta *T. drosophilae* dovzetna le za francosko populacijo *D. suzukii* (Chabert in sod., 2012; Cini in sod., 2012). Parazitoid *P. vindemmiae* sicer velja tudi za učinkovitega naravnega sovražnika mušice *D. melanogaster* Meigen (Cini in sod., 2012).

Raziskave potrjujejo, da je večina populacij plodove vinske mušice odporna na larvalne parazitoide. Vrste *Asobara tabida* Nees, *Leptopilina heterotoma* Thomson in *Leptopilina boulardi* Barbotin *et al*. (Hymenoptera: Eucoilidae) so vrste larvalnih parazitoidov, ki so v Evropi zelo pogoste. Njihov razvoj je vezan na vinske mušice, vendar ne na populacije plodove vinske mušice. Kljub temu pa sta vrsti *Asobara tabida* in *Asobara japonica* Belokobylskij sposobni uspešno parazitirati vrsto *D. suzukii*, ki se pojavlja na Japonskem (Cini in sod., 2012; Chabert in sod., 2012). Med parazitoide, ki se lahko uspešno razvijajo na populacijah mušice *D. suzikii* na Japonskem uvrščamo tudi paraziotidne ose iz reda *Ganaspis* (Figitidae) (Cini in sod., 2012; Kasuya in sod., 2013). Omenjeni biotični agensi predstavljajo na Daljnem vzhodu relativno pomembnega naravnega sovražnika plodove vinske mušice, saj njihova učinkovitost niha med 2 in 7 % (Cini in sod., 2012).

Med uspešne naravne sovražnike vrste *D. suzukii* pa nekateri prištevajo tudi plenilske stenice iz družin Anthocoridae in Miridae (Arnó in sod., 2012; Cini in sod., 2012). V Španiji se stenice *Orius laevigatus* (Fieber), *Cardiastethus nazarenus* Reuter, *C. fasciventris* (Garbillietti) in *Dicyphus tamaninii* Wagner pojavljajo na sadnih vrstah, kjer je škodljiva plodova vinska mušica, vendar so podatki o njihovi biotični učinkovitosti zelo skopi (Arnó in sod., 2012) in ocenjevanja še potekajo (Cini in sod., 2012). V eni od laboratorijskih raziskav je bila tako dokazana uspešnost prehranjevanja stenice *Orius laevigatus* z ličinkami vrste *D. suzukii* na borovnicah (Walsh in sod., 2011).

Glede na doslej zbrane podatke je dovzetnost posameznih populacij vrste *D. suzukii* za naravne sovražnike pogojena predvsem z vsebnostjo hemocitov (Kacsoh in Schlenke, 2012). Omenjene krvne celice so namreč pomemben dejavnik imunskega sistema plodove vinske mušice in predstavljajo veliko oviro v razvoju parazitoidov. Zgradba hemocitov v vrsti *D. suzukii* je podobna kot pri vrsti *D. melanogaster*, vendar je njihovo število pri prvi vrsti veliko večje. Prav tako se večje število hemocitov pojavlja v francoskih populacijah kot v populacijah iste vrste na Japonskem (Poyet in sod., 2013). Razlago za to lahko ponudi dejstvo, da na Japonskem obstaja veliko več vrst parazitoidnih os, ki lahko uspešno omejujejo plodovo vinsko mušico kot v Franciji. V Evropi sta zaenkrat potrjeni le dve parazitoidni osi, ki lahko uspešno kljubujeta imunskemu sistemu vrste *D. suzukii* (Poyet in sod., 2013).

2.4 Drugi zatiralni ukrepi

Plodova vinska mušica je bila v Evropo zanesena s transportom napadenega sadja, zato je dejstvo, da lahko z učinkovitejšim pregledom uvoženega sadja njeno širjenje precej otežimo (Cini in sod., 2012). Na Japonskem grme borovnic prekrivajo z mrežo z velikostjo lukenj 0,98 mm in s tem učinkovito zmanjšujejo napad preučevane škodljive vrste (Lee in sod., 2011; Cini in sod., 2012).

Eden od pomembnih ukrepov zatiranja plodove vinske mušice je tudi rastlinska higiena, pri čemer je potrebno poškodovane plodove odstraniti in uničiti. Posebno kritični so tisti plodovi, ki so že padli na tla (Lee in sod., 2011). Takšnih plodov namreč ne skladiščimo na kompostu, saj predstavljajo vir za potencialni prenos škodljivca v nasad. V slednji zvezi so pomembne tudi samonikle vrste gostiteljev plodove vinske mušice (Cini in sod., 2012).

Za potrebe varstva rastlin pred plodovo vinsko mušico pa lahko izkoriščamo tudi razmerje med škodljivcem in endosimbionti. Bakterije iz rodu *Wolbachia* lahko na več načinov delujejo na svojega gostitelja in na njegove naravne sovražnike. Poleg povzročanja citoplazmatske inkompatibilnosti (CI) pri njihovem gostitelju in posledično sterilnosti samcev, lahko pri naravnih sovražnikih (iz reda Hymenoptera) spodbuja partenogenezo. Najbolj pogosto pride do pojava CI, pri čemer CI preprečuje okuženim samcem, da bi se uspešno parili z samicami, ki vsebujejo drugačen sev omenjene bakterije oziroma s samicami, ki sploh niso okužene z bakterijo. CI bi lahko izkoristili za potrebe SIT (angl. »sterile insect technique«) (Cini in sod., 2012), vendar je potrebno še veliko izboljšav omenjene metode (Mazzi in Dorn, 2012). Pri vrsti *Drosophila mauritiana* Tcacas et David pa zastopanost bakterij iz roda *Wolbachia* stimulativno vpliva na njeno plodnost. Delovanje bakterij je odvisno od njihovega seva, genotipa gostitelja in od interakcij med njima (Cini in sod., 2012).

3 SKLEPI

Plodova vinska mušica, ki je bila leta 2010 v Sloveniji prvič ugotovljena na območju Nove Gorice (Seljak, 2011), je bila v letih 2012 in 2103 potrjena še na drugih lokacijah, predvsem na SV, V in JV države (Fitosanitarni prostorski portal, 2013). V prihodnje pričakujemo, da se bo ta škodljiva vrsta v Sloveniji še razširila. Zaenkrat pri nas za zatiranje plodove vinske mušice še nimamo registriranih insekticidov (Seznam registriranih …, 2013), zato velja toliko več pozornosti nameniti iskanju drugih, zlasti okoljsko sprejemljivih načinov zatiranja tega škodljivca. To je še posebno pomembno zato, ker pri uporabi insekticidov za zatiranje obravnavanega škodljivca lahko pričakujemo težave zaradi njihove karence, glede na to, da vrsta *D. suzukii* povzroča poškodbe na zorečih in predvsem na zrelih plodovih tik pred obiranjem (Seljak, 2011; Cini in sod., 2012).

Pridelovanje sadnih vrst v manjših nasadih lahko bolj natančno nadzorujemo s pregledovanjem in uničevanjem napadenih plodov ter odstranjevanjem divjih gostiteljev plodove vinske mušice (Cini in sod., 2012). Na večjih zemljiščih pride v poštev uporaba drugih okoljsko sprejemljivih načinov zatiranja, med katerimi je tudi uporaba pasti, v katere so nameščeni različni atraktanti. Ob splošno znanem dejstvu, da so atraktanti na podlagi kisa in vina zelo učinkoviti za lovljenje odraslih osebkov plodove vinske mušice, pa so nekatere novejše raziskave usmerjene v preizkušanje atraktantov, ki poleg oljčne muhe (Seljak, 2011) in breskove muhe (Escudero Colomar in sod., 2011) privabljajo tudi plodovo vinsko mušico.

Uporaba naravnih sovražnikov za potrebe zatiranja *D. suzukii* je v zadnjih letih vse bolj razvijajoče se raziskovalno področje, ki zaenkrat še ni privedlo do konkretnih rešitev v praksi. Populacije plodove vinske mušice iz različnih območij so različno dovzetne za napade naravnih sovražnikov (Cini in sod., 2012), zato bo potrebno v prihodnje na območjih, kjer je razširjen obravnavani škodljivec, še več energije usmeriti v iskanje domorodnih parazitoidov in plenilcev (Chabert in sod., 2012).

Med naravnimi sovražniki, ki jih kot potencialno učinkovite zglede za zatiranje plodove vinske mušice navaja tuja strokovna literatura, bi v Sloveniji v prihodnje veljajo preučiti bližnjo sorodnico vrste *Orius laevigatus*, in sicer domorodno plenilsko stenico *Orius majusculus* (Reuter), ki je sicer bolj znana kot plenilec resarjev (Bosco in sod., 2013), pa tudi ščitkarjev (Arnó in sod., 2008). Stenico *O. majusculus* je namreč mogoče tudi kupiti (Koppert, 2013) in jo na ta način vnašati v okolje. Dodatno možnost biotičnega zatiranja plodove vinske mušice pa predstavljajo v Sloveniji domorodne entomopatogene ogorčice (Laznik in Trdan, 2011), katerih učinkovitost proti temu škodljivcu doslej še ni bila preučena, so pa zato pokazale dobro delovanje na vrsto *D. melanogaster* (Dobes in sod., 2012).

4 ZAHVALA

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Ruralna sociologija v spoznavanju in spodbujanju razvoja kmetijstva in podeželja v Sloveniji**

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IZVLEČEK

Prispevek v prvem delu prikaže začetke in razvoj ruralno-sociološkega raziskovanja v Sloveniji ter vključevanje ruralne sociologije v pedagoški proces na univerzah v Sloveniji. V drugem delu je predstavljen model pristopa k celovitem razvoju podeželskih skupnosti ter na osnovi slovenskih in mednarodnih izkušenj izpostavljenih nekaj predlogov za spodbuditev razvoja (slovenskega) kmetijstva in podeželja. V sklepu pa je poudarjena potreba po interdisciplinarnosti, predvsem pa po večjem upoštevanje družbenih ved v razvojnem načrtovanju.

Ključne besede: ruralna sociologija, poučevanje ruralne sociologije, kmečko gospodarstvo, kmečka družina, kmečke ženske, podeželske skupnosti, prenos znanja v kmetijsko pridelavo, vzdržen razvoj, interdisciplinarnost

ABSTRACT

RURAL SOCIOLOGY IN STUDYING AND ENCOURAGING THE DEVELOPMENT OF AGRICULTURE AND RURAL AREAS IN SLOVENIA

The article in the first part describes beginnings and development of rural sociological research and teaching rural sociology at the universities in Slovenia. In the second part, a working model of integrated approach towards rural development is presented, and, on the basis of Slovenian as well as international experiences some suggestions for promoting the development of (Slovenian) agriculture and rural communities are specified. In the conclusion, the need for the interdisciplinary approach, especially greater consideration of social sciences in developmental planning is pointed out.

Key words: rural sociology, teaching rural sociology, agricultural holdings, farm family, farm women, rural communities, transfer of knowledge into agricultural practice, sustainable development, interdisciplinary approach

1 NAMEN

Namen prispevka je trojen: prvič, na kratko in zato morda nepopolno prikazati začetke in razvoj ruralno-sociološkega raziskovanja v Sloveniji s posebnim poudarkom na raziskovalnem delu v okviru Biotehniške fakultete Univerze v Ljubljani; drugič, vključevanje ruralno-sociooloških predmetov v pedagoški proces na Univerzi v Mariboru in Univerzi v Ljubljani; in tretjič,

* prof. dr.

[1] Čeprav sprejemam v Sloveniji uradno sprejet izraz *trajnostni razvoj*, v svojem pisanju praviloma uporabljam izraz *vzdržen razvoj,* ki mu pripisujem tako pomen vzdržljivosti v časovnem smislu, to je, v smislu dolgoročnega trajanja, kot v smislu (samo) omejevanja rabe virov in proizvodov ter s tem prispevanja k njihovemu daljšemu obstoju/trajanju.

** Besedilo je bilo kot izvirni znanstveni članek objavljeno v hrvaškem prevodu: Barbič, A. (2013). Ruralna sociologija u prepoznavanju i poticanju razvoja poljoprivrede i ruralnih prostora u Sloveniji. *Sociologija i prostor,* 51 (196):303-328; dostopno tudi na *http://hrcak.srce.srce/index.php?show=toc&id broj=8952*

predstaviti model celovitega pristopa k vzdržnemu razvoju[1] podeželskih skupnosti ter izpostaviti nekaj predlogov za njegovo spodbuditev v (slovenskem) kmetijstvu in na podeželju. Pomeni, da ne bom obravnavala širših sociooloških tem, povezanih s kmečkim/podeželskim prebivalstvom, podeželskim prostorom in lokalnimi skupnostmi na podeželju, z inovativnostjo kmečkega/podeželskega prebivalstva in njegovim vstopanjem v globalne povezave v informacijski dobi. Te in druge teme, pomembne tudi za ruralno sociologijo, poglobljeno obravnava Zdravko Mlinar v svojih objavah.

2 RURALNO-SOCIOLOŠKO RAZISKOVANJE V SLOVENIJI

2.1 Podeželje in kmetijstvo kot predmet družboslovnih raziskav

Ruralno-sociološke raziskave je potrebno najprej umestiti v širši okvir družboslovnega raziskovanja, predvsem pa opozoriti na njihovo povezanost s sociologijo lokalnih skupnosti in prostorsko sociologijo. Razloga za tak pristop sta vsaj dva. Prvič, prostor je podlaga in predmet človekovega bivanja in delovanja, ter drugič, preučevanje prostora terja interdisciplinarni pristop, ki povezuje znanstvene ugotovitve v celovito poznavanje prostora, njegove rabe in njegovega upravljanja. Potrebo po več disciplinarnem preučevanju podeželja v Sloveniji dodatno utemeljuje dejstvo, da po statističnih podatkih iz konca 20. stoletja podeželje pokriva 96 odstotkov slovenskega ozemlja, na katerem prebiva dobra polovica prebivalcev Slovenije (Ravbar, 1997:84). Podatki iz leta 2012 sicer kažejo, da v podeželskih naseljih (urbanizirana podeželska naselja in podeželska naselja) živi zgolj tretjina (34,7% od skupaj 2,050.000) prebivalcev Slovenije, hkrati pa je delež tovrstnih naselij (83,4% od skupaj 6031 vseh naselij v Sloveniji) (Kozina, 2013) zelo visok.

Z opredeljevanjem in razmejevanjem podeželskega in mestnega prostora so se v Sloveniji v drugi polovici 20. stoletja ukvarjali predvsem geografi, ki so podeželski prostor praviloma opredeljevali kot »ne mestni prostor« (Klemenčič, 1960; Vrišer, 1965; Kokole, 1976). Ravbar pa je svoje raziskovalno delo usmeril v opredeljevanje in preučevanje podeželskih in semi-podeželskih naselij ter njihovih vplivnih območij ter opozoril vsaj na tri vrste sprememb, ki jih prinaša urbanizacija: na spremembe v fizičnem razvoju naselij, na spremembe v socialni strukturi podeželskih območij in posredno še na spremembe v sistemu kulturnih vrednot, ki jih v podeželska naselja vnaša mestno naravnan način življenja, spodbujen z urbanizacijo (Ravbar, 1992). K povezovanju mestnega in podeželskega prostora v sodobnem času posebej prispeva več namenskost kmetijske dejavnosti, ki nima le proizvodnih, temveč ima tudi socialne in okoljske funkcije (Ravbar in Razpotnik Visković, 2010).

Ne glede na to, da je opredeljevanje deleža podeželskih in mestnih naselij odvisno od upoštevanih kriterijev podeželskosti oz. mestnosti naselja, se Slovenija po OECD tipologiji regij glede na stopnjo ruralnosti tako po deležu prebivalstva kot po površini območij v posameznih kategorijah (pretežno ruralne - 50%, pomembno ruralne – 30%, pretežno urbane- 20% regije) skupaj z Avstrijo, Finsko, Belgijo in Dansko, vendar za Švedsko in Norveško, uvršča v skupino s prevladujočim deležem pretežno in pomembno ruralnih regij (OECD, 1994:30).

S preučevanjem gospodarskih dejavnosti kmečkega prebivalstva in virov dohodkov podeželskih skupnosti se je v Sloveniji v 1960-ih in 1970-ih letih ukvarjal predvsem ekonomist Krašovec (1965; 1974), ki je preučeval pojav kmeta-delavca in ga takrat opredelil kot zgolj prehoden pojav, češ da je človeku težko delati na dve strani zlasti še, ko mu zaradi ustreznosti dohodka zunaj kmetije to ni več potrebno (Krašovec, 1974:144). Nasprotno pa je agrarni ekonomist Levstik že pred njim opozoril na dejstvo, da tako v razvitem kapitalizmu kot v socialističnih deželah polproletarec, kot so v 1960-ih letih in morda že prej imenovali kmeta-delavca, prihaja do relativno boljše mezde, ker hrano pridela doma, kar mu hkrati omogoča investiranje v nakup zemljišč in kmetijskih strojev (Levstik, 1964:III, cit po: Barbič, 1990a:17), pri čemer njegova učinkovitost na delovnem mestu ni prizadeta. Nasprotno! Trstenjak celo izpostavi, da delavec-kmet na delovno mesto (v industriji) prinaša s seboj tradicionalne vrednote delavnega kmeta, ki mu je jasno, da je učinkovitost

(produktivnost) njegovega dela odvisna od njega samega in za neuspeh ne more kriviti kolektiva (Trstenjak, 1982:93, cit. po: Barbič, 1990a:18; Barbič in sod.,1984b).

Sociološko raziskovanje podeželja in kmečkega prebivalstva se je v Sloveniji začelo leta 1959 na istega leta ustanovljenem Inštitutu za sociologijo in filozofijo z zasnovo raziskovalnega in pedagoškega področja sociologije lokalnih skupnosti, katerega nosilec je bil Z. Mlinar (Mlinar, 2005), ki je že v svojih prvih raziskavah ruralno-sociološke teme vključeval v širše disciplinarne in družbene okvire, še posebej glede na ekonomske, politične in kulturne neenakosti med mestom in podeželjem. Pri tem je opozarjal na protislovnost razvojne in kmetijske politike, ki je v imenu napredka dejansko zavirala razvoj kmetijstva in podeželja (zemljiški maksimum, oviranje mehanizacije kmetijstva).

Ko primerja razvite in manj razvite občine v Sloveniji Mlinar ugotavlja, da slednje praviloma pokrivajo najbolj kmetijska območja. V primeru odpiranja industrijskih obratov na manj razvitih območjih pa gre v večini primerov za prenos zastarelih tehnologij, ki reproducirajo nerazvitost (Mlinar, 1986:131-146). Njeno vsaj delno preseganje v sodobnosti omogočajo procesi globalizacije in vse hitrejši razvoj informacijske družbe, v okviru katerih tudi odmaknjena (rovtarska) območja »z inovativnim nadgrajevanjem tradicije vstopajo v svet«, kar Mlinar (2011) pojasnjuje na primeru izbranih krajev. Podeželsko prebivalstvo in podeželske skupnosti Mlinar upošteva tudi v okviru svojih novejših teoretičnih razmišljanj in empiričnih raziskav kot eno od življenjskih okolij v globalni informacijski družbi (2008a, 2012).

Mlinar je že v svojih prvih raziskavah preučeval tudi delovanje vaških skupnosti z vidika vključevanja prebivalcev (participacije) v procese odločanja na lokalni/občinski ravni (1965, 1969, 1970, 1971a, 1971b). Iskal je odgovore na vprašanje, kje se zaustavljajo pobude in kritike ter razkrival veliko neskladje med neformalnim in formalno-institucionalnim delovanjem ljudi (Mlinar, 1966).

Za preseganje tedanjega sociološkega ločevanja med mestom in podeželjem (dihotomni koncept) je Mlinar dajal prednost teoriji kontinuuma ter poenoteno obravnaval in nadgrajeval vsebine ruralne in urbane sociologije pod imenom sociologija lokalnih skupnosti. Z upoštevanjem več nivojske prostorske organizacije družbe pa je v svojem nadaljnjem delu raziskovalno in pedagoško zasnoval področje prostorske sociologije (Mlinar, 1990), ki jo poleg Mlinarja razvijajo sodelavci Centra za prostorsko sociologijo Fakultete za družbene vede Univerze v Ljubljani.

Golob, Mlinarjev sodelavec na Inštitutu za sociologijo in filozofijo, je s kombinacijo antropoloških in sociooloških pristopov med drugim preučeval sodelovanje in oblike medsebojne pomoči na vasi (1967), sorodstvene (klandestine) skupine (1976, 1978) kot tudi že takrat aktualne probleme nasledstva na kmečkih gospodarstvih (1982).

2.2 Ruralno-sociološke raziskave v okviru Biotehniške fakultete Univerze v Ljubljani

Raziskovanje ruralno-sociooloških tem se je v 1980-ih letih nadaljevalo na Katedri za agrarno ekonomiko Oddelka za agronomijo Biotehniške fakultete Univerze v Ljubljani s stalno zaposlitvijo takrat docentke Ane Barbič kot predavateljice predmetov Temelji sociologije in Temelji politologije. Glede na naravo študija na omenjeni fakulteti je izvajalka obeh predmetov že na samem začetku raziskovalno delo skupaj s sodelavci katedre in študenti usmerila v preučevanje ruralno sociooloških tem, katerih izsledke je vseskozi vključevala v pedagoško delo.

Raziskovalno delo v okviru omenjene katedre je bilo v naslednjih desetletjih usmerjeno v tri glavna področja:

- preučevanje družinske kmetije, kmečke družine/gospodinjstva in kmečke ženske v okviru kmečkega gospodinjstva in kmečkega gospodarstva ter podeželske skupnosti;
- povezovanje sociooloških in tehnoloških vidikov pridelovanja živeža na družinskih kmetijah (prenos znanja v kmetijsko pridelavo);
- izvajanje projektov celovitega razvoja podeželskih skupnosti.

Družinska kmetija, kmečka družina/gospodinjstvo in kmečka ženska

Z namenom preučiti stanje na področju družinskega kmetovanja v Sloveniji je bila v letih 1982/83 opravljena obsežna empirična raziskava z naslovom »Zasebne kmetije kot dejavnik razvoja kmetijstva in podeželja« v 13 občinah na 10 odstotnem vzorcu gospodinjstev s kmečkim gospodarstvom, ki je imelo v lasti najmanj 1 hektar obdelovalnih zemljišč. Tako obsežen vzorec za vsako občino je bil določen zaradi zagotovitve reprezentativnosti podatkov za vsako občino, ki je na svojem območju financirala raziskavo (Barbič, 1990a: 55-58), hkrati pa je skupno število 2052 v raziskavo vključenih kmetij zagotovilo splošnejšo veljavnost zbranih podatkov. Na osnovi podatkov o virih dohodkov (zaposlitev, delo na kmetiji) članov jedra kmečke družine (gospodar/mož, gospodarica/žena, naslednik/naslednica) je bila izdelana tipologija, ki je družinske kmetije razvrstila v štiri skupine: ostarele kmetije (noben član jedra kmečke družine ni mlajši od 65 let) (9,0%), mešane kmetije 2 (vsi člani jedra kmečke družine kmetujejo ob redni zaposlitvi zunaj kmetije (12,8%), mešane kmetije 1 (vsaj en član jedra kmečke družine dela samo na kmetiji) (56,2%) in čiste kmetije (vsi člani jedra kmečke družine delajo samo na kmetiji) (22,0%) (Barbič, 1990a: 74-74). Poleg agrarno-ekonomskih obeležij kmetij in njihove (ne)tržne usmerjenosti je raziskava preučila tudi nekatere sociološke in psihološke značilnosti članov jedra kmečke družine (njihovo politično delovanje, prostočasne dejavnosti ter vlogo znanja in kmetijske pospeševalne službe v kmetijski pridelavi (Barbič, 1990a:193-242). V raziskavi je bila posebna pozornost namenjena kmečkim ženskam, tako tistim, ki delajo samo na kmetiji (Barbič, 1985a), kot tistim, ki so zaposlene zunaj kmetije (Barbič in sod., 1984c), dopolnilnim dejavnostim na kmetijah kot dopolnilnemu viru dohodka znotraj kmečkega gospodarstva in gospodinjstva (Barbič in Tomšič, 1987) ter vlogi žensk v procesih odločanja o zadevah družine/gospodinjstva in kmečkega gospodarstva (Barbič, 1988). K ozaveščanju o vlogi žensk v razvoju kmetijstva in podeželja pa poleg raziskovalnih ugotovitev o položaju žensk v kmetijstvu in na podeželju pomembno prispeva njihova organiziranost (aktivi kmečkih žensk v Sloveniji) ter njihovo mednarodno povezovanje (Rus, A. in Rupena-Osolnik, M., 2001).

V isti tematski sklop sodijo tudi poznejše raziskave kot so: raziskava o kmečki družini (Barbič, 1993a; Barbič, 1993b) in o možnostih mladih kmetov/kmetic, da si družino ustvarijo (Barbič, 1989) ter raziskava o razvojnih možnostih (kmečkih) gospodinjstev v Triglavskem narodnem parku (Barbič, 1993c).

Med rezultati omenjenih raziskav kaže posebej izpostaviti tipologijo kmečkih družin/gospodinjstev na osnovi podatkov raziskave Kmečka družina-socialna sestavina družinske kmetije, opravljeni leta 1991. Obdelava podatkov o družini/gospodinjstvu in kmečkem gospodarstvu z metodo voditeljev je 780 preučevanih enot raziskave razvrstila v tri skupine, ki so bile poimenovane kot propadajoče tradicionalne (25,5%), vitalne tradicionalne (36,8%) in vitalne demokratične (37,7%) kmečke družine. Prve označuje predvsem majhno število članov praviloma ene (starejše) generacije, skromna opremljenost gospodinjstva s predmeti trajne potrošnje, odločitve sprejema gospodar. Oba tipa vitalnih kmečkih družin pa se razlikujeta predvsem po tem, da v vitalnih tradicionalnih o nakupih praviloma odloča gospodar, ki tudi zastopa družino in kmetijo zunaj doma, medtem ko se v vitalnih demokratičnih družinah vsi družinski člani odločajo skupaj, skupaj pa se tudi dogovorijo o opravkih zunaj doma (roditeljski sestanki, opravki v krajevni skupnosti, na občini, v kmetijski zadrugi) (Barbič, 2000a).

Prikaz značilnosti kmečke družine pomembno dopolnjujejo raziskave, ki primerjajo kmečke, nekmečke podeželske in mestne družine. Primerjava lastnosti treh skupin družin je pokazala, da se v stališčih do delitve vlog kmečke in nekmečke podeželske družine približujejo mestnim družinam, v dejanski delitvi vlog med spoloma pa se še vedno razlikujejo (Černič Istenič, 2007; Černič Istenič in Kveder, 2008). Se pa v kmečkih družinah po tradiciji še vedno rodi več otrok kot v nekmečkih podeželskih in v mestnih družinah (Černič Istenič, 2007). Raziskava z naslovom Odnosi med generacijami in spoloma na kmetijah v Sloveniji, opravljena v letih 2006-2008, pa je podrobno preučila delitev dela in odločanja na kmečkem gospodarstvu in v kmečkem gospodinjstvu, usposobljenost (predvsem) prevzemnikov/prevzemnic kmetije za kmetijsko dejavnost, odnose med generacijami v kmečkem

gospodinjstvu in dojemanje medgeneracijske solidarnosti (Kneževič Hočevar in Černič Istenič, 2010).

V empiričnih raziskavah so bile kmečke ženske preučevane tudi kot gospodarice na kmetiji. Kmetice so v največ primerih postale gospodarice šele po smrti moža in še to praviloma le v primeru, da kmetija ni imela naslednika/naslednice oz. ta še ni bil(a) določen(a), na kar opozarjajo raziskovalni podatki iz leta 1991, po katerih je delež gospodaric vdov (13,2%) občutno večji od deleža gospodarjev vdovcev (3,3%) (Barbič, 1993a:5). S splošnim gospodarskim in družbenim razvojem se je spreminjal/izboljševal tudi položaj kmečkih žensk. Če se zadržimo le pri njihovem deležu med gospodaricami na družinskih kmetijah, se je ta že v dobrem desetletju izboljšal. Tako Černič Istenič (2003:55) ugotavlja, da so bile po statističnih podatkih za leto 2000 na 27 odstotkov kmetij v Sloveniji gospodarice ženske, v državah članic EU pa le na 19 odstotkov kmetij. Je pa bilo po podatkih istega vira kar 68 odstotkov gospodaric v Sloveniji starejših od 55 let, v državah članic EU pa le 42 odstotkov. V začetku 21. stoletja je tudi slovenska kmetijska politika namenila posebno pozornost kmečkim ženskam oz. strategiji njihovega vključevanja v razvoj podeželja (Černič Istenič, 2002).

Kmečke ženske so (bile) tema več mednarodnih strokovnih srečanj v Sloveniji, med katerimi je bila posebej odmevna FAO Tehnična konzultacija o ženskah in pluriaktivnosti, 1983 leta (Barbič in sod., 1984d). Med številnimi mednarodnimi dogodki na temo kmečkih žensk s sodelovanjem slovenske udeležbe pa naj omenimo vsaj naslednje: Fifth International Seminar on International Research on Rural Communities. Vienna Cetre and European Society for Rural Sociology, Hungary, 14.-19.9.1985 (Barbič, 1985b), Scientific convention with international participation "Challenges to the Croatian Agriculture at the Verge of the 21st century. 8th - 11th November, 2000. Poreč, Croatia (Barbič, 2000b), The New Chalelenge of Womens' Role in Rural Europe". International Conference, Nicosia, Cyprus, 4-6 October 2001 (Barbič, 2001) in The European Conference »Women and Sustainable Rural Development in Europe«. Nicosia, Cyprus, June 7-10 (Černič Istenič, 2006).

Ob priliki 8. marca 2013 je vlogo kmečkih žensk v pridelavi omenil celo John Kerry, zunanji minister ZDA, z navedbo ugotovitve FAO (Organizacije Združenih narodov za prehrano in kmetijstvo), ki ocenjuje, »da kmetice, če imajo enak dostop do semen, gnojil in tehnologije kot moški, lahko zmanjšajo število nedohranjenih ljudi v svetu za 100 do 150 milijonov« (Kerry, 2013:5).

Omenjene in druge ruralno-sociološke raziskave v okviru Biotehniške fakultete Univerze v Ljubljani se vse od njihovih začetkov v 1980-ih letih izvajajo na pobudo in za potrebe konkretnih naročnikov, ki podatke in njihove analize potrebujejo za načrtovanje in izvajanje svojih dejavnosti, hkrati pa prispevajo k poglabljanju posameznih raziskovalnih tem in dodajanju novih, ki širijo spoznanja o kmečkem prebivalstvu, posebej o kmečkih ženskah, ter o kmečkih družinah in družinskih kmetijah v Sloveniji. Tovrstne empirične podatke pa pomembno dopolnjujeta vsaj dve temeljni raziskavi in sicer: magistrsko delo Jerice Tomšič Lušin z naslovom Slojevska struktura slovenskih kmetov, in doktorska disertacija Franca Hribernika z naslovom Tradicije kot element (dez)integracije na vasi: družbena mobilnost kmetov skozi prizmo izobraževalnega sistema.

Tomšič Lušin ugotavlja, da se »po podatkih za Slovenijo kmetje uvrščajo med nižje sloje slovenske družbe. Tudi znotraj podeželskih skupnosti je njihov položaj na spodnjem delu družbene strukture. Analiza znotrajslojevske strukture kmetov (upoštevani so člani jedra kmečke družine ne glede na njihovo osnovno delovno aktivnost), ki temelji na petih dimenzijah družbene slojevitosti (materialno-bivalni status, značilnosti kmetije, izobrazbeno-poklicni status, politična aktivnost in prostočasne aktivnosti) pa kaže, da se sloj kmetov notranje razslojuje na 10 skupin, ki hierarhično urejene tvorijo piramido s širokim dnom in koničastim vrhom. V spodnje sloje se uvrščajo predvsem kmetje delavci z manjših kmetij, v srednje kmetje s srednje velikih kmetij, njihov izobrazbeni status je podpovprečen. Sloj vitalnih kmetov, ki izhajajo iz večjih in dobro opremljenih kmetij in so tudi na ostalih dimenzijah slojevitosti nad povprečjem, pa se uvršča v sam vrh znotrajslojevske strukture. Poseben sloj zunaj hierarhično razvrščenih skupin predstavlja tretjina

kmetov, ki so na prehodu v nekmečke sloje« (Tomšič Lušin, 1993:II).

Hribernik v svoji doktorski disertaciji odkriva »pomen tradicij in tradicionalizma na podeželju, zlasti še med kmečkim prebivalstvom« in ugotavlja, da je po »nekaj desetletjih burnih procesov industrializacije, deagrarizacije, urbanizacije in modernizacije prebivalstva /…/ vse težje ohranjati specifično identiteto slovenskih vasi, še zlasti tistih, ko so v bližini urbanih centrov /…/. Socialna mobilnost, opazovana skozi prizmo izobraževalnega sistema, je relativno slaba in zadeva predvsem spodnji del izobraževalnega sistema /…/. Tudi analiza vključenosti starostnih generacij v izobraževalni sistem med 15. in 29. letom starosti je pokazala, da so prav kmečki otroci tisti, ki v odnosu do otrok zgornjega dela socialne strukture mnogo pogosteje prekinjajo šolanje po obveznem (v številnih primerih nedokončanem) šolanju« (Hribernik, 1993:I, II).

Povezovanje sociooloških in tehnoloških vidikov kmetovanja pri prenosu znanja v kmetijsko pridelavo

Ruralno-sociološko raziskovanje, poučevanje družboslovnih predmetov na nedružboslovnih fakultetah ter splošna ozaveščenost univerzitetnih učiteljev strokovnih predmetov o potrebi po sodelovanju tehnoloških in družboslovnih ved pri preučevanju in spodbujanju kmetijske pridelave v Sloveniji, so že v 1980-ih letih na Oddelku za agronomijo Biotehniške fakultete Univerze v Ljubljani spodbudili več akcij pod naslovom Sodobna pridelava poljščin na območju KZ Ljubljana, Zadružna enota Ig (Barbič in sod., 1987; Tajnšek, Barbič, Kocjan Ačko, 1988).

Tudi v okviru nekaterih raziskovalno-razvojnih projektov na konkretnem območju se je občasno »zgodil« prenos znanja v kmetijsko prakso. Tako se je na podlagi ugotovitev in predlogov projekta Program razvoja kmetijstva in dopolnilnih dejavnosti v Krajevni skupnosti Mirna peč, kmet, ki se je ukvarjal z mlečno proizvodnjo, preusmeril v ekološko sadjarstvo in postal eden največjih pridelovalcev jabolk sorte topaz v Sloveniji (Kovačič in sod., 1998).

Podobne akcije in projekti, ki so sledili, so potrdili uspešnost povezovanja družboslovnih in tehnoloških pristopov in znanj pri prenosu tehnoloških dosežkov v kmetijsko pridelavo, čeprav tovrstni pristopi še vedno niso vsakdanja praksa v raziskovalnem in pedagoškem delu. Morda je glavni vzrok za to v dejstvu, da naloge tehnološkega člena v navezi znanost - tehnologija - kmetijska pridelava v Sloveniji dokaj uspešno opravlja kmetijska svetovalna služba, ki s svetovanjem in izobraževanjem kmetov skrbi za uvajanje sodobnih tehnoloških, okoljskih in ruralno-sociooloških spoznanj v kmetijsko prakso. Pri tem so Kmetijski svetovalni službi pri Kmetijsko gozdarski zbornici Slovenije ustrezni raziskovalni podatki vsaj o tem, kaj kmetje od nje pričakujejo in kako ocenjujejo njeno delo, v pomembno oporo (Barbič, 1984a).

Kmetijskim svetovalcem pri svetovanju za uvajanje sodobnih načinov pridelave pomagajo predvsem podatki o konkretnih ravnanjih pridelovalcev kot tudi podatki o njihovem poznavanju znanstveno utemeljenih postopkov pridelave in rabe zaščitnih sredstev, kar spet zagotavljajo ustrezne raziskave, kakršna je bila na primer anketna raziskava o uporabi fitofarmacevtskih sredstev med ljubiteljskimi pridelovalci vrtnin (Fišer, Milevoj, Černič Istenič, 2009).

Načrtovanje razvoja kmetijstva je sicer naloga državne kmetijske politike, kmetijsko svetovanje pa mora upoštevati konkretno stanje tako na kmečkem gospodarstvu kot v kmečkem gospodinjstvu. Kot primer optimalnih strokovnih podlag za kmetijsko svetovanje konkretni kmetiji kaže omeniti tipologijo kmečkih gospodarstev in (kmečkih) gospodinjstev za vsako stanovanjsko enoto v naselju Podjelje na robu Triglavskega narodnega parka ob njeni hkratni umestitvi v prostorsko strukturo naselja (Barbič, 1992a).

Povezovanju tehnoloških, agrarno-ekonomskih in ruralno sociooloških znanj je bil na pobudo in v organizaciji Katedre za agrarno ekonomiko agronomskega oddelka Biotehniške fakultete Univerze v Ljubljani že v začetku 1990-ih let namenjen tudi Slovensko-britanski seminar na temo Ruralni razvoj in tržno gospodarstvo: družbeni in zasebni stroški in koristi (Barbič, 1990b).

Da je prenos znanja v kmetijsko pridelavo in prirejo še vedno aktualna raziskovalna tema, potrjuje leta 2012 končan ciljni raziskovalni projekt Izzivi in potrebe v prenosu znanja v kmetijsko prakso v Sloveniji (Černič Istenič in sod., 2012). Avtorji v sintezi rezultatov ugotavljajo, »da je prenos znanja v kmetijsko prakso oviran zaradi slabe medsebojne povezanosti akterjev in celo izključevanja nekaterih izmed njih. Obstoječi sistem kaže podobo ločenih in samozadostnih podsistemov (npr. raziskovalnega, izobraževalnega in svetovalnega)« (Černič Istenič in sod., 2012:109). Rešitev problema avtorji vidijo v izdelavi »celostne strategije prenosa znanja v kmetijsko prakso, v pripravo katere je treba vključiti akterje iz raziskovanja, izobraževanja, svetovanja, industrijske in kmetijske dejavnosti« (Černič Istenič in sod., 2012:112). Čeprav je predlagan pristop primerna pot k vzpostavitvi celovitega sistema prenosa znanja v kmetijsko prakso, pa zaradi časovne odmaknjenosti njegove opredelitve in poznejše vzpostavitve, če se bo to v bližnji prihodnosti sploh zgodilo, kaže sproti izvajati čim več konkretnih akcij prenosa znanja v kmetijsko prakso ter s tem na eni strani prispevati k ozaveščanju politične, strokovne in izvajalske javnosti o pomenu in nujnosti sodelovanja, po drugi strani pa nabirati izkušnje za oblikovanje in utemeljevanje posameznih sestavin in korakov predlagane strategije.

Projekti celovitega razvoja podeželskih skupnosti

Sredi 1980-ih let se je v okviru Biotehnične fakultete Univerze v Ljubljani področje ruralno-sociooloških raziskav in interdisciplinarnih akcij prenosa znanja v kmetijsko pridelavo razširilo na snovanje in izvajanje projektov celovitega razvoja podeželskih skupnosti. Prvi tovrstni projekt je bil Razvojni projekt »Trebnje«, ki se je začel l. 1984 (Barbič, Kovačič, Rupena-Osolnik, 1991) in predstavlja le nekajletni zamik za akcijo Evropske skupnosti, v kateri je bilo v šestih državah te skupnosti v letih 1980-1983 opravljenih dvanajst konkretnih projektov integralnega ruralnega razvoja (integrated rural development projects) (Ulbricht, 1986). V okviru tega projekta sta bila opredeljena idejna zamisel in delovni model celovitega razvoja podeželske skupnosti ter izdelana shema aktivnosti v njegovem uresničevanju (Barbič, 1985b).

Načrtovanje celovitega razvoja določene podeželske skupnosti terja interdisciplinarni pristop, ki hkrati upošteva prostorske danosti, gospodarske dejavnosti in človeški kapital, razvojne zamisli, oblikovane ob upoštevanju vseh treh razvojnih podlag, pa morajo temeljiti na principih vzdržnosti s ciljem trajnega varovanja okolja, naravne in kulturne dediščine območja ter zagotavljanju kakovostnega življenja lokalnim prebivalcem. Vodilno vlogo v posameznih projektih celovitega razvoja sicer prevzame stroka, katere področje je predmet konkretne pobude lokalne skupnosti, vendar se je v praksi izkazalo, da so sodelujoči strokovnjaki (prostorski načrtovalci, (krajinski) arhitekti, (agrarni) ekonomisti, ruralni in prostorski sociologi in strokovnjaki drugih področij) običajno enakovredni partnerji, od katerih v posameznih fazah izvajanja projekta vsak lahko prevzame vodilno vlogo. Kot primer takšne prakse naj omenimo projekt Koncept dolgoročnega razvoja Spominskega parka Trebče, ki ga je v letih 1988/89 vodil arhitekt Peter Gabrijelčič (Barbič, Gabrijelčič in Tomšič-Lušin, 1990), ko pa je projekt v naslednji fazi prerasel v Raziskovalno-razvojni projekt »Celovit razvoj občine Šmarje pri Jelšah«, pa ga je vodila ruralna sociologinja Ana Barbič (Barbič, Gabrijelčič in Hribernik, 1991).

Na Biotehniški fakulteti Univerze v Ljubljani so bili v okviru družboslovnih predmetov (ruralna sociologija, razvoj podeželja, metodologija družboslovnega raziskovanja) tako na dodiplomskem kot na podiplomskem študiju v raziskovalno-razvojne projekte vključeni tudi študentje. Tako je pod mentorstvom A. Barbič (v prvem letu ob somentorstvu I. Marušiča) v letih 1995-1998 potekal projekt Regijski park Škocjanske jame, v katerem so sodelovali študentje dodiplomskega in podiplomskega študija krajinske arhitekture in kmetijstva-smer agronomija. Rezultati dela vsakoletnega študijskega leta so bili objavljeni v šestih zvezkih publikacije pod naslovom Regijski park Škocjanske jame ter javno predstavljeni domačinom kot tudi upravni in strokovni javnosti (Barbič, 1999; Barbič, 2005). Ta in številni drugi raziskovalni projekti, v katere so bili vključeni študentje Biotehniške fakultete, so prispevali k ozaveščanju študentov o pomenu sociooloških znanj v spodbujanju razvoja kmetijstva in podeželskih skupnosti, kar posebej dokazuje uspešnost projekta Šmarje-Vrata v osrčje

slovenske Istre pod vodstvom diplomantov Oddelka za agronomijo Biotehniške fakultete in Ekonomske fakultete Univerze v Ljubljani. Pangea-Društvo za varovanje okolja Koper, ki je projekt spodbudilo, je skupaj s Krajevno skupnostjo Šmarje pri Kopru pritegnilo k sodelovanju strokovnjake različnih strok ter Mestno občino Koper ter v letih 2004-2005 v več delavnicah in raziskovalnih nalogah zagotovilo strokovne podlage in spodbudilo sodelovanje domačinov k snovanju in uresničevanju razvojnega projekta za naselje Šmarje in za vso krajevno skupnost. Projekt, ki je že prinesel vidne rezultate (Barbič, 2010a), še vedno poteka.

Glede na to, da je univerza prvenstveno usmerjena v pedagoško in temeljno raziskovalno delo, katerega rezultate sicer prenaša tudi v lokalno prakso, je njeno mednarodno sodelovanje usmerjeno predvsem v večje raziskovalne projekte kot so npr. okvirni programi EU. Ker pa tudi ti zahtevajo preverjanje teoretičnih konceptov v državnem/lokalnem okolju, so praviloma vezani na spodbuditev razvoja konkretne podeželske skupnosti in/ali posameznih (kmečkih) gospodinjstev v njihovem okviru. Tako je bil v Sloveniji empirični del v okviru projekta CEESA (Central and Eastern European Sustainable Agriculture) EU Petega programskega okvira (1999-2003) opravljen na območju načrtovanega Regijskega parka Trnovski gozd (Barbič, Udovč, Medved, 2001; Barbič, Udovč, Medved, 2004), zbrani ruralno-sociološki in agrarno-ekonomski podatki pa so bili uporabljeni tudi v enem diplomskem delu (Medved, 2001).

Mednarodno sodelovanje na področju razvojnih projektov podeželskih območij v Sloveniji je potekalo predvsem v povezovanju s sosednjimi državami. Med tovrstne projekte sodijo številni INTER-REG projekti, pa tudi tradicionalno sodelovanje med univerzami, kakršno že več desetletij gojita Univerza v Celovcu in Univerza v Ljubljani. V okviru tega sodelovanja je od 25. do 30. junija 1994 potekala v Atomskih toplicah mednarodna delavnica z naslovom Človek-upravljanje-obstojen razvoj (Barbič in Wastl-Walter, 1995; Verbole, 1999), ki so se je poleg strokovnjakov obeh univerz in nekaj drugih strokovnjakov udeležili tudi mladi domačini in zadnji dan delavnice družno obiskali Železno Kaplo/Eisenkappel, kjer so se seznanili z uspešnim prestrukturiranjem nekdaj industrijskega kraja v sodobno območje podeželskega zdravstveno-rekreacijskega turizma. Hkrati pa se je izkazalo, da imajo Pišece, ki so bile na delavnici predstavljene kot primer podeželskega kraja, ki »umira«, lokalne razvojne potenciale tako v turističnem društvu kot v drugih lokalnih društvih in v osnovni šoli, na katerih je možno graditi razvoj kraja in okolice. Z aktivnostmi za izvedbo osrednje slovenske proslave ob 100-letnici izida prve knjige Pleteršnikovega slovensko-nemškega slovarja je lokalno turistično društvo pričelo že v letu ali nekaj dalj pred omenjeno delavnico, proslavo pa uspešno izvedlo jeseni leta 1994 (Barbič, 1995). Skupina mladih Pišečanov in Pišečank pa se je pozneje vključila v državni program CRPOV (celosten razvoj podeželja in obnova vasi) in razvijala dejavnosti predvsem v smeri podeželskega turizma (Verbole, 1999).

S projekti celovitega razvoja podeželskih skupnosti in obnove vasi ali vsaj v njihovo zasnovo kot tudi z razvojnim projekti širših podeželskih območij se v Sloveniji poleg sodelavcev in študentov Biotehniške fakultete ljubljanske univerze ukvarjajo tudi nekatere druge fakultete oz. študijske smeri v njihovem okviru. Kot uspešen primer kaže omeniti podiplomski interdisciplinarni študij prostorskega planiranja na Fakulteti za gradbeništvo in geodezijo Univerze v Ljubljani, v okviru katerega je bil izdelan projekt razvoja in obnove vasi Žetale (Prosen, Vrabič, 1990).

Omenjeni kot tudi številni drugi domači in mednarodni raziskovalni projekti tako v okviru Biotehniške fakultete kot drugih raziskovalnih in razvojnih ustanov in podjetij v svoje programe v vedno večjem številu vključujejo sociologe, predvsem s področij ruralne in prostorske sociologije.

3 RURALNA SOCIOLOGIJA V PEDAGOŠKEM PROCESU NA DODIPLOMSKEM IN PODIPLOMSKEM ŠTUDIJU

Naj najprej poudarim, da se ruralna sociologija kot študijski predmet (v študijskih programih se pojavlja pod različnimi imeni) v Sloveniji predava samo na Biotehniški fakulteti Univerze v Ljubljani in na Univerzi v Mariboru v okviru študija kmetijstva.
Hkrati z zasnovo raziskovalnega področja sociologije lokalnih skupnosti na Inštitutu za sociologijo in filozofijo je njegov nosilec Z. Mlinar pripravil program za poučevanje tega predmeta na 1960. leta ustanovljenem Oddelku za sociologijo Filozofske fakultete Univerze v Ljubljani. Študijski predmet, katerega nosilec je bil njegov avtor, je vključeval sociološke vidike mesta in podeželja ter lokalne samouprave. S prenosom osnovnega sociološkega študijskega programa na današnjo Fakulteto za družbene vede pa se je oblikovalo raziskovalno in pedagoško področje prostorske sociologije kot posebne sociološke discipline.

Na Biotehniški fakulteti Univerze v Ljubljani se prvi družboslovni predmeti z izjemo agrarne ekonomike, ki se je predavala že prej, pojavijo v študijskem letu 1966/67 (politična ekonomija) in v študijskem letu 1972/73 (sociologija, politologija, filozofija). Sociologija in politologija se v študijskem letu 1976/77 združita v predmet z naslovom Temelji sociologije in politologije. Sociološki del predmeta je v študijskih letih 1976/77 do 1979/80 na vseh osmih oddelkih Biotehniške fakultete predavala Maca Jogan, ki je že takrat »vsebino predmeta prilagajala potrebam študijskih usmeritev ter posvečala pozornost okoljskim problemom, deagrarizaciji vasi in drugim problemom podeželja ter posebnostim medgeneracijskih odnosov (vključno z problematiko razlik med spoloma) v podeželskem okolju« (Jogan, 2013). Z redno zaposlitvijo A. Barbič na Oddelku za agronomijo Biotehniške fakultete pa so bili zagotovljeni tudi formalni pogoji za razvijanje ruralne sociologije kot matične sociološke discipline na tej fakulteti z A. Barič kot njeno utemeljiteljico (Enciklopedija Slovenije, 2002:8). Ne glede na to, da sta bili sociologija in politologija formalno združeni v predmetni sklop Temelji sociologije in politologije vse do vključno študijskega leta 1985/86, sta bila v praksi oba predmeta ločena. V študijskem letu 1986/87 sta se na večini oddelkov Biotehniške fakultete formalno še vedno združevala v predmetni sklop Sociologija in politologija, v študijskem letu 1989/90 pa sta bila oblikovala kot dva samostojna študijska predmeta. V tem študijskem letu sta se oba predmeta izvajala le še na oddelkih za agronomijo, veterino, živinorejo in živilsko tehnologijo, medtem ko sta Oddelek za gozdarstvo in Oddelek za lesarstvo ohranila samo predmet Sociologija. Tega je postopoma zamenjal predmet Ruralna sociologija in sicer najprej na Oddelku za gozdarstvo (v študijskem letu 1990/1991), v naslednjem študijskem letu pa tudi na Oddelku za agronomijo in Oddelku za zootehniko, v katerega se je preimenoval Oddelek za živinorejo. Na Oddelku za lesarstvo so ohranili predmet Sociologija do vključno študijskega leta 1991/1992.

Pod imenom Temelji sociologije se je sociologija v obsegu 30 ur v enem semestru predavala na vseh oddelkih Biotehniške fakultete tako na univerzitetnem kot na višjem in visokem strokovnem študiju vse do študijskega leta 1982/83, ko je bil obseg predmeta razdeljen na 15 ur predavanj in 15 ur seminarja, v katerem je vsak študent predstavil svoje seminarsko delo. Zaradi velikega števila študentov in posledično seminarskih del se je na Oddelku za agronomijo Biotehniške fakultete letu 1982 kot asistent zaposlil Franc Hribernik, ki je delo asistenta opravljal do vključno študijskega leta 1992/1993, ko so nekateri oddelki Biotehniške fakultete (Oddelek za biologijo, Oddelek za lesarstvo, Oddelek za živilsko tehnologijo) opustili predmet Sociologija ali Ruralna sociologija. Hkrati pa se je obseg predmeta na univerzitetnem, višjem in visokem strokovnem študiju oddelkov za agronomijo, gozdarstvo in zootehniko povečal s 30 na 45 ur v izbranem semestru.

V okviru bolonjskih študijskih programov, ki se na Biotehniški fakulteti Univerze v Ljubljani izvajajo od študijskega leta 2007/08 dalje, so sociološki predmeti pod imeni: sociologija podeželja, sociologija gozdnih virov, socialni projekti v gozdarstvu (nosilka vseh je M. Černič Istenič) in razvoj podeželja (nosilec predmeta je L. Juvančič) uvrščeni med izbirne predmete. Njihov obseg od 30 do 40 ur je praviloma razdeljen na predavanja,

seminarske vaje in terensko delo. Posamezni predmeti se izvajajo na treh oddelkih Biotehniške fakultete in sicer: na Oddelku za agronomijo, na Oddelku za zootehniko in na Oddelku za gozdarstvo.

Poseben primer vključevanja družboslovnih/sociooloških predmetov v študijske programe predstavlja študij krajinske arhitekture, od leta 1996 v okviru samostojnega oddelka Biotehniške fakultete Univerze v Ljubljani. Do študijskega leta 1992/93 se je na tem študiju poučeval predmet Temelji sociologije in politologije, po kratkem premoru pa od leta 1995/96 do leta 1998/99 predmet Urejanje podeželja, katerega izvajalca sta bila A. Barbič in J. Marušič. Hkrati pa se je na tem študiju občasno poučeval predmet Sociologija lokalnih skupnosti, od študijskega leta 1995/96 pa predmet Prostorska sociologija, katerega nosilec je bil najprej Zdravko Mlinar, od študijskega leta 2007/08 pa sta ga izvajala Pavel Gantar in Drago Kos. Na bolonjskem študijskem programu se ta predmet izvaja samo na drugi stopnji v okviru Magistrskega programa krajinska arhitektura z D. Kosom kot nosilcem predmeta.

Svojevrstno zgodbo predstavlja umeščanje predmeta (ruralna) sociologija v ustrezno katedro/inštitut kot organizacijsko enoto Oddelka za agronomijo Biotehniške fakultete Univerze v Ljubljani. Že od vključevanja prvih družboslovnih predmetov v študijske programe Biotehniške fakultete je bila za njihovo izvajanje »odgovorna« Katedra za agrarno ekonomiko v okviru agronomskega oddelka, kjer se je, kot že omenjeno, 1. februarja 1980 zaposlila kot prva sociologinja s polnim delovnim časom A. Barbič. V študijskem letu 1983/84 je bila Katedra za agrarno ekonomiko preimenovana v Katedro za agrarno ekonomiko in družboslovje, deset let pozneje pa se je z razširitvijo števila strokovnih predmetov, katerih nosilci so bili povezani v tej katedri, ta poimenovala kot Inštitut za agrarno ekonomiko, znotraj katerega so bile organizirane katedre za posamezna študijska in raziskovalna področja, med njimi Katedra za pospeševanje razvoja kmetijstva in podeželja, v katero je bilo uvrščeno tudi področje ruralne sociologije. To se je v študijskem letu 1998/99 oblikovalo v samostojno Katedro za ruralno sociologijo, ki se je nekaj let pozneje (v študijskem letu 2001/02) spet povezalo s področjem agrarne ekonomike in razvoja podeželja v Katedro za agrarno ekonomiko, ruralno sociologijo in razvoj podeželja. Ob reorganizaciji/združevanju kateder Oddelka za agronomijo Biotehniške fakultete po prehodu na bolonjski študij pa se je oblikovala programsko pestra Katedra za agrometeorologijo, urejanje kmetijskega prostora ter ekonomiko in razvoj podeželja.

Tako podatki o uvajanju in izvajanju (ruralno) sociooloških predmetov v okviru študijskih programov Biotehniške fakultete Univerze v Ljubljani kot podatki o vključevanju ruralne sociologije v ustrezno katedro/inštitut ter podatki o spreminjanju poimenovanja katedre/inštituta opozarjajo vsaj na dvoje: prvič, ruralni sociologiji se je v okviru študijskih programov in organizacijskih enot Biotehniške fakultete uspelo uveljaviti kot matično raziskovalno in izobraževalno področje, in drugič, obseg in status (obvezen/izbirni predmet) ruralno-sociooloških predmetov v okviru študijskih programov posameznih študijskih usmeritev znotraj oddelkov Biotehniške fakultete sta bila v treh desetletjih v veliki meri odvisna od (ne)formalne moči vodstva fakultete, posameznih oddelkov, in/ali strokovnih skupin znotraj njih. Ruralno-sociološki predmeti bodo ustrezen obseg in status v okviru študija kmetijstva na Univerzi v Mariboru in Univerzi v Ljubljani dobili šele takrat, ko bo uvrščanje posameznih strokovnih področij in študijskih predmetov znotraj njih določeno z opredelitvijo želenih znanj diplomantov/diplomantk posameznih študijskih usmeritev.

Interes študentov Biotehniške fakultete za ruralno-sociološke vsebine poleg njihovega aktivnega sodelovanja v pripravi in predstavitvi seminarjev ter izvajanju študentskih raziskovalnih nalog, ki so občasno nadomestile seminarske izdelke, potrjuje tudi število diplomskih nalog, izdelanih pod mentorstvom vsakokratne nosilke predmeta Ruralna sociologija. Tako je bila A. Barbič v obdobju 1984-2001 mentorica šestindvajsetim diplomantom/diplomantkam na dodiplomskem študiju, od katerih je ena prejela Prešernovo nagrado Biotehniške fakultete, ena pa Prešernovo nagrado Univerze v Ljubljani. Hkrati sta bila pod njenim mentorstvom dosežena dva magisterija znanosti in v somentorstvu dva doktorata, od katerih je bil eden opravljen na Univerzi v

Wageningnu na Nizozemskem. Pod mentorstvom M. Černič Istenič pa je bilo v obdobju 2004-2012 izdelanih osemnajst diplomskih nalog na univerzitetnem študiju, tri diplomska dela na visokem strokovnem študiju ter eno magistrsko delo. M. Černič Istenič pa je bila v tem obdobju tudi mentorica eni diplomantki na Filozofski fakulteti Univerze v Mariboru in somentorica dvema doktorandkama na Fakulteti za družbene vede Univerze v Ljubljani.

Na Višji agronomski šoli Maribor se je ruralna sociologija začela predavati v šolskem letu 1970/1971. Kot prvi predavatelj predmeta Sociologija vasi je Zdravko Mlinar pripravil tudi prvo študijsko gradivo za študente pod naslovom Uvod v ruralno sociologijo (Mlinar, 1971c). Kot predavatelj mu je sledil Franci Pivec. Predmet se je predaval v prvem semestru v obsegu 60 ur vse do šolskega leta 1985/86, ko je bil njegov obseg prepolovljen. Od takrat do šolskega leta 1994/1995 je predmet predavala višja predavateljica Majda Japelj, diplomirana sociologinja. Na magistrskem študiju pred bolonjskim programom je bil v študijskem modulu Pravno-institucionalne oblike upravljanja v kmetijstvu v 2. letnik vključen predmet Kmetijska politika in ruralna sociologija, ki sta ga poučevala Emil Erjavec z Biotehnične fakultete Univerze v Ljubljani in Stefan Vogel s Kmetijske univerze na Dunaju (Universitäet für Bodenkultur Wien). Slednjega je kot predavateljica v študijskem letu 2010/2011 zamenjala Andreja Borec, redno zaposlena na Fakulteti za kmetijstvo in biosistemske vede Univerze v Mariboru (tako se je leta 2008 poimenovala Fakulteta za kmetijstvo Univerze v Mariboru, ki je med letoma 1992 in 1994 nosila ime Visoka kmetijska šola Univerze v Mariboru, pred tem pa Višja agronomska šola Maribor). Od šolskega leta 2006/2007 (bolonjski program) se predava predmet Sociologija podeželja in sicer na prvi stopnji strokovnega študija v obsegu 30 ur predavanj in vaj ter 35 ur individualnega dela študenta, ter na 2. stopnji (magistrski študij) v okviru Podiplomskega študijskega programa Agrarna ekonomika v obsegu 60 ur predavanj in vaj ter 60 ur individualnega dela študenta. Nosilka in izvajalka obeh programov je Đurđica Žutinić z Agronomske fakultete Univerze v Zagrebu. V programih univerzitetnega bolonjskega študija ni nobenega ruralno-sociološkega predmeta.

4 CELOVIT PRISTOP K NAČRTOVANJU VZDRŽNEGA RAZVOJA PODEŽELSKIH SKUPNOSTI IN NEKAJ PREDLOGOV ZA NJEGOVO UVELJAVITEV[2]

Želja po nenehnem izboljševanju kakovosti življenja je osnovni motiv človekovega delovanja tako na individualni kot na družbeni ravni. Pri tem kakovosti življenja sodobne razvite in demokratične družbe ne opredeljujejo zgolj s količinskim večanjem dobrin, temveč v vse večji meri z njihovo dostopnostjo, zdravstveno neoporečnostjo, varnostjo, daljšim trajanjem in vzdržno rabo, pa tudi s kakovostnimi odnosi med ljudmi (sodelovanje, medsebojno spoštovanje, medsebojna pomoč) in družbenimi skupinami (demokratičnost, enakopravnost, miroljubnost). V prizadevanjih za višjo kakovost življenja so prebivalcem podeželja in njihovim lokalnim skupnostim lahko v veliko pomoč ugotovitve in predlogi znanstvenikov različnih strok, ki se ukvarjajo s preučevanjem podeželja. V nadaljevanju omenjam zgolj nekaj znanih, pomeni, že večkrat opisanih in predstavljenih dejstev in predlogov, ki pa so bili pogosto spregledani ali vsaj ne dovolj upoštevani v načrtovanju in izvajanju konkretnih razvojnih projektov posameznih podeželskih skupnosti ali širših podeželskih območij.

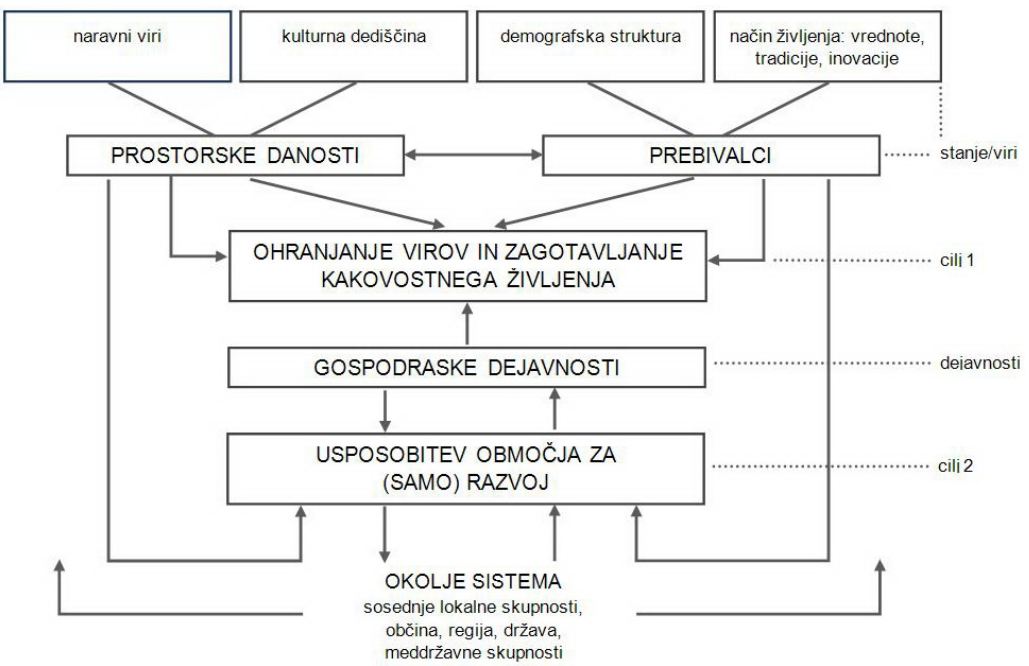
Celovito načrtovanje na vseh ravneh družbene organiziranosti (lokalna skupnost, regija/ pokrajina, država, meddržavne skupnosti) terja usklajevanje varovanja okolja in ohranjanja naravne in kulturne dediščine ter gospodarskega in socialnega razvoja (slika 1).

2 Manjši del tega besedila je povzet in predelan po že objavljenem prispevku (Barbič, A. (2010b).

Slika 1: Usklajevanje okoljskega/prostorskega, socialnega in gospodarskega razvoja (Barbič, 2005:19)

Vsak od treh podsistemov (okolje/prostor, družba, gospodarstvo) deluje sicer po lastnih zakonitostih, hkrati pa so soodvisni. Torej razvoj posameznega podsistema ne sme ogrožati razvoja drugih dveh, temveč ju mora podpirati in prispevati k njuni vzdržnosti.

Usklajevanje prostorskih, okoljskih in naravnih danosti ter potreb in želja ljudi (posameznikov, lokalnih skupnosti, države) na eni strani zagotavlja optimalno reševanje težav in konfliktov, na drugi strani pa spodbuja oblikovanje takšnih gospodarskih proizvodov, ko bodo zagotavljali vzdržno rabo razpoložljivih virov in kakovostno življenje ljudem.



Slika 2: Model celovitega pristopa k razvojnemu projektu (Barbič, 1997:24)

Razpoložljiv prostor, tako njegov obseg kot viri, je omejena danost, ki je podlaga za bivanje in delovanja prebivalcev konkretnega območja. Zato je pri vsakem načrtovanju nujno izhajati iz prostorskih razmer (naravnih virov) in potencialov prebivalstva (človeških virov) za vzdržen gospodarski razvoj, ki bo zagotavljal tako ohranjanje virov kot kakovostno življenje ljudi (slika 2).

Slovensko podeželje, vsaj njegov pretežni del, je razmeroma dobro opremljeno s prometno in komunalno infrastrukturo ter s storitvenimi dejavnostmi, nakupovalna središča pa so večini prebivalcev dostopna v krajih zaposlitve. Zaradi številnih dnevnih delovnih migracij s podeželja v mesta se zmanjšujejo razlike v življenjskem slogu in sistemu vrednot med podeželskim in kmečkim prebivalstvom, hkrati pa se kljub lokalnim prizadevanjem izgublja identiteta slovenskega podeželja. Poleg nenadzorovane pozidave kmetijskih zemljišč (Prosen, 2008; 2009; Matjašec, 2013) sta ogroženi naravna in kulturna dediščina in s tem lokalni razvojni potenciali, vključno z zmanjševanjem obsega kmetijskih zemljišč in števila prebivalcev, ki so se pripravljeni ukvarjati s pridelavo živeža za trg.

Prebivalci slovenskega podeželja, tako kmetje kot prebivalci drugih poklicev ali statusov (upokojenci, šolajoča se mladina, brezposelni, osebe s posebnimi potrebami) ne potrebujejo (samo)pomilovanja, ki izvira predvsem iz primerjav glede dostopnosti delovnih mest in storitev v mestu in na podeželju, ter že tradicionalno vzvišenega odnosa meščanov do podeželanov, ki se kaže v slabšalnem pomenu besed kmet, kmečki, vaški, ruralni. V zadnjem času se je beseda ruralno pojavila celo v slabšalnem opredeljevanju slovenske prestolnice kot ruralnega mesta.

Za spodbudo razvoja (slovenskega) kmetijstva in podeželskih skupnosti na osnovi domačih in mednarodnih izkušenj naj izpostavim naslednje predloge:

Tekmovanja z mesti nadomestiti s sodelovanjem. Kmetje zagotavljajo meščanom vsaj delno oskrbo s kakovostnim živežem in najkrajšo pot od njive do mize. Hkrati omogočajo mestnemu prebivalstvu kakovostno preživljanje prostega časa, kot so izleti v naravo, pohodništvo, tek, kolesarjenje in nabiralništvo, zagotavljajo gostinske in počitniške storitve (kmetije odprtih vrat, kmečki turizem, gostinski lokali) ter omogočajo spoznavanje/doživljanje naravne in kulturne dediščine slovenskega podeželja (turistične, etnološke, kulturne prireditve na podeželju). Hkrati pa obiskovalci iz mest zagotavljajo prebivalcem podeželja vsaj del potrebnega dohodka od prodaje živeža, gostinskih in turističnih storitev ter športnih, rekreacijskih in kulturnih prireditev. Seveda pa je temelj za uspešno sodelovanje meščanov in prebivalcev podeželja medsebojno spoštovanje. To pomeni, da meščani spoštujejo delo kmečkega prebivalstva (se držijo uhojenih poti, ne pobirajo pridelkov in plodov v zasebni lasti) ter naravno in kulturno dediščino podeželja, lastniki zemljišč pa ne omejujejo ali celo s postavljanjem preprek ne zapirajo uhojenih sprehajalnih in pohodniških poti, saj se zavedajo, da so jim zemljišča dana zgolj v rabo in upravljanje, ne pa v absolutno lastnino, hkrati pa se zavedajo, da mestni obiskovalci njim in drugim prebivalcem podeželja zagotavljajo proizvode in storitve, nepogrešljive za njihovo kakovostno življenje.

Ne čakati na pomoč od zunaj temveč se opreti na lastne moči in lokalne vire. Že v 16. stoletju je v Franciji pri opisu kmetov veljalo, da so kmetje »prekanjeni« in da je »v vsakem kmetu nekaj trgovca« (Zenon Davis, 2009:209). Politika kmetijskih nadomestil EU in držav članic pa je s tem, da finančnih podpor kmetijstvu ni vezala na pridelavo, temveč zgolj na obdelavo kmetijskih površin, ustvarjalnost kmečkega prebivalstva v zagotavljanju primernega dohodka precej zatrla. Očitno poskuša EU s ponovno navezavo finančnih podpor kmetijstvu na pridelke to »napako« popraviti, saj se zaveda, da bo prehod od pasivnega sprejemanja nadomestil k aktivnemu ustvarjanju dohodka potreboval nekaj časa. Upati je le, da ustvarjalno razmišljanje kmečkega in podeželskega prebivalstva drugih poklicev v Sloveniji še ni povsem zamrlo in ga bo mogoče ob podpori sodobnih informacijsko-komunikacijskih tehnologij, ki so razširjene tudi na slovenskem podeželju (Mlinar, 2008b), ter ob primerni državni politiki in ustrezni finančni podpori hitro obnoviti.

Ustvarjanje novih proizvodov v kmetijstvu in na podeželju ter odpiranje novih delovnih mest. Ustvarjalno razmišljanje kmečkega/podeželskega prebivalstva v kmetijski pridelavi in predelavi, razširitev pestrosti organske/ekološke pridelave ter obogatitev turistično-rekreacijske ponudbe na podeželju bo ponudilo nova delovna mesta ali priložnost za dopolnilno delo kmečkim/podeželskim prebivalcem, predvsem tistim, ki so ali še bodo izgubili delo zaradi gospodarske krize. Pričakovati pa je, da bo ustvarjalni duh pritegnil na podeželje tudi

strokovnjake sodobnih informacijsko-komunikacijskih tehnologij kot optimistično napoveduje Mlinar (2008b). Kot na področju novih tehnologij (Tyrangiel, 2012) je tudi za razvoj kmetijstva in podeželja bistvenega pomena inovativnost ne le v oblikovanju povsem novih gospodarskih proizvodov, temveč tudi, morda celo predvsem v (po)iskanju najboljših rešitev konkretnih težav. Pri tem gre pomembna vloga povezovanju predvsem s sosednjimi državami in EU kot povezovalki evropskih držav in različnih družbenih skupin. Takšna usmeritev bo prispevala k odpiranju navzven, izpostavljanje mednarodni konkurenci pa neprestano izboljševanje ponudbe. Ker pa je vsako oblikovanje in uvajanje nove ponudbe povezano z večjim ali manjšim tveganjem, je hkrati z oblikovanjem novega gospodarskega proizvoda ali že pred tem treba vsaj okvirno spoznati interes zanj v smislu povpraševanja/ prodaje. Hkrati pa kaže upoštevati že mnogokrat potrjeno ugotovitev, da lahko dobra ponudba samodejno ustvarja povpraševanje. Tako neuspeh kot uspeh uvajanja novega gospodarskega proizvoda pa utrjuje spoznanje, da brez tveganja ni razvoja.

Povezovanje pridelovalcev in sodelovanje s potrošniki. Povezovanje kmetov v okviru kmetijskih zadrug ima v Sloveniji več kot 130-letno tradicijo (Ocepek, 1983). Zadruge so se v času spreminjale tako v organizaciji kot vsebini dela, temeljna naloga zadružništva kot podpornika kmetov kot pridelovalcev živeža (oskrba s sredstvi za pridelavo) in odkupovalcev njihovih proizvodov pa se je ohranila. Medtem ko sodobni večji pridelovalci v večini primerov sami tržijo svoje pridelke, ostajajo kmetijske zadruge življenjskega pomena za manjša kmetijska gospodarstva, čeprav je tudi med njimi vedno več takih, zlasti med pridelovalci organskih vrtnin, ki formalno povezovanje v kmetijski zadrugi dopolnjujejo/nadomeščajo z neposrednimi stiki s potrošniki (tedensko dostavljanje zabojčkov svojih pridelkov, neposredna prodaja na tržnici ali na domačem dvorišču). Seveda pa sodobne zadruge, ki jih Božič (2013) imenuje kar zadruge za 21. stoletje, lahko igrajo pomembno vlogo tudi pri posredovanju kmetijskih pridelkov in proizvodov med manjšimi pridelovalci in večjimi porabniki kot so vrtci, šole in bolnišnice.

Spodbuditi obveščanje, izobraževanje in ozaveščanje o ciljih, strategijah in vsebinah vzdržnega razvoja. Obveščenost in znanje sta osnovni pogoj za ustvarjalno sodelovanje državljanov v procesih odločanja o vzdržni rabi prostora ter ustreznih programih/projektih gospodarskega in socialnega razvoja podeželskih skupnosti. Na področjih obveščanja in ozaveščanja se je v Sloveniji v zadnjem času pojavilo kar nekaj poljudno-strokovnih prispevkov (na primer: Prosen, 2008, 2009; Plut, 2010; Ravbar, 2010) in glasil okoljevarstvenih društev (na primer: Glasilo Društva Pangea, 2007). Pojavljajo pa se tudi obsežnejša poljudno-strokovna besedila, namenjena širitvi znanja na ustreznem področju (na primer: Simoneti, Zavodnik Lamovšek, 2009), in posveti, na katerih strokovnjaki poljudno opozarjajo na razvojne priložnosti konkretnega območja ob hkratnem zagotavljanju trajnosti virov in dediščine (Ohranitev kraške krajine…, 2008). Medtem ko je obveščanje predvsem domena medijev, pa morata biti izobraževanje in ozaveščanje za ustvarjalno sodelovanje državljanov v procesih odločanja in v vsakdanjem delovanju sestavina rednega izobraževanja (učni programi) kot tudi izobraževanja odraslih (seminarji, tečaji). Na področju obveščanja in spodbujanja sodelovanja ter medsebojne pomoči med posameznimi organizacijami, ki sodelujejo pri razvoju podeželja, kaže omeniti slovensko Mrežo za podeželje (www.mko.gov.si; 21.04.2013), ki deluje v okviru Ministrstva za kmetijstvo in okolje, ter na Društvo za razvoj slovenskega podeželja LAS (lokalne akcijske skupine) (www.prc.si; 21.04.2013). Tako mreža kot društvo izpostavljata spodbujanje sodelovanja in medsebojno pomoč med posamezniki in organizacijami na podeželju preko dopolnjujočih se ciljev svojega delovanja.

5 SKLEP

Povezovanje družboslovnih in tehnoloških znanj je nujno tako na področju prenosa kmetijskih tehnologij v kmetijsko pridelavo kot na področju načrtovanja in izvajanja projektov razvoja podeželskih skupnosti. Sociologija, predvsem ruralna in prostorska, namreč v obeh primerih

izpostavlja človeka kot pridelovalca živeža in kot prebivalca podeželske skupnosti, ter nujnost njegovega upoštevanja kot deležnika in aktivnega sodelavca v razvojnem načrtovanju.

Interdisciplinarnost oziroma interdisciplinarno sodelovanje v Sloveniji sicer ni neznanka, le v praksi je vse preveč primerov, ko so bodisi kmetijski strokovnjaki (agronomi, zootehniki) bodisi prostorski načrtovalci (urbanisti, arhitekti, krajinski arhitekti) prepričani, da poznajo znanja, potrebe in želje ljudi, ki kmetujejo, se ukvarjajo s kakšno drugo gospodarsko dejavnostjo na podeželju ali tam zgolj živijo. Toda samo »zdrava pamet« posameznih skupin strokovnjakov, čeprav z osnovnim poznavanjem drugih področij, je za strokovno optimalno načrtovanje premalo. Zadošča le za to, da se strokovnjaki določene stroke pri načrtovanju razvoja na svojem področju zavedajo, katere stroke oz. strokovnjake morajo povabiti k sodelovanju v načrtovanju optimalne rabe prostora tako na področju kmetijstva kot drugih posegov v prostor s ciljem celovitega in vzdržnega razvoja podeželskih skupnosti.

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CONTENT ANALYSIS OF THE PAPERS IN THE ACTA AGRICULTURAE SLOVENICA

VSEBINSKA OBDELAVA PRISPEVKOV V ACTA AGRICULTURAE SLOVENICA let. 103 št. 2

Tomaž BARTOL[a], Karmen STOPAR[b],

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VSEBINSKO KAZALO PO SKUPINAH ZNANJA (PREDMETNIH KATEGORIJAH)

NAVODILA AVTORJEM

(letniki z liho številko - rastlinska proizvodnja)

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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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