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NaCl salinity and Zn foliar application influence essential oil composition of basil (Ocimum basilicum L.)

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ABSTRACT

Essential oils composition of hydroponically grown *Ocimum basilicum* L. plant was evaluated in response to salinity (control and 50 mM NaCl) and Zn foliar application (control, 100 and 200 mg l^{-1}). Essential oil constituents were quantified and identified by GC/EI-MS. In total, fifty seven components were identified in the six treatment combinations. Methyl chavicol (43.9–61.2 %) and linalool (11.4-16%) were the major components of all treatments. Salinity had deteriorative effect on methyl chavicol biosynthesis and accumulation. In contrast, integrated levels of salinity and 200 mgl^{-1} Zn had increment effects on linalool content. Germacrene D (2.2-3.9 %), 1,8-cineole (2.4-3.8 %), (Z)-α-bergamotene (0.1-2.6 %), (E)-β-farnesene (1.4-2.6 %), α-bulnesene (0.9-2.4 %), camphor (0.7-1.3 %) and (E)-β-ocimene (0.2-1.3 %) were the other main common constituents of oil. Considering the constant levels of zinc foliar application, salinity had raising effects on the contents of most above mentioned constituents. In conclusion, it seems that moderate salinity stress along with balanced levels of Zn foliar application changed the primary metabolites pathways in favor of major volatile oil components biosynthesis and that basil plant has the production potential under prevalent semi-saline conditions.

Key words: *Ocimum basilicum* L., essential oil, GC/MS, salinity, Zn foliar application, methyl chavicol, linalool

IZVLEČEK

KONCENTRACIJA NaCl V HIDROPONSKI RAZTOPINI IN FOLIARNI NANOS RAZTOPINE CINKA VPLIVAJO NA SESTAVO ETERIČNIH OLJ PRI BAZILIKI (*Ocimum basilicum* L.)

Raziskana je bila sestava eteričnih olj hidroponsko gojene bazilike (*Ocimum basilicum* L.), glede na vpliv slanosti (kontrola in 50 mM NaCl) ter foliarnega nanašanja cinka (Zn) (kontrola ter 100 oziroma 200 mg l^{-1}). Sestava eteričnih olj je bila ugotovljena z GC/EI-MS tehniko. Pri šestih kombinacija tretiranja je bilo ugotovljenih 57 sestavin. Metil kavikol (43,9–61,2 %) in linalool (11,4-16%) sta bili glavni sestavini pri vseh tretiranjih. Sol je negativno vplivala na sintezo in akumulacijo kavikola. Nasprotno, skupen vpliv solne raztopine in višje koncentracije cinka je povzročil povečanje vsebnosti linaloola. Germakren D (2,2-3,9 %), 1,8-kineol (2,4-3,8 %), (Z)-α-bergamoten (0,1-2,6 %), (E)-β-farnesen (1,4-2,6 %), α-bulnesen (0,9-2,4 %), kafra (0,7-1,3 %) in (E)-β-ocimen (0,2-1,3 %) so tudi bile glavne sestavine olja. Pri nespremenjeni koncentraciji cinka pri foliarni aplikaciji je sol vplivala na povečano koncetracijo omenjenih metabolitov. Zmerna slanost skupaj s foliarnim tretiranjem z raztopino cinka vpliva na spremenjeno sestavo metabolitov s tem da poveča biosintezo hlapnih sestavin olj; tako je ugotovljena prednost pridelovanja bazilike v razmerah zmerne slanosti.

Ključne besede: *Ocimum basilicum* L., bazilika, eterična olja, GC/MS, sol, Zn foliarno nanašanje, metil kavikol, linalool

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1 INTRODUCTION

The increasing importance of essential oils in various domains of human activities including pharmacy, perfumery, cosmetics, aromatherapy, food and drinks industry has opened a new era for exploitation of different intrinsic and extrinsic factors influencing their biosynthesis, accumulation and biological properties (Sonwa, 2000). Essential oils are very complex mixture of volatile compounds and their chemical composition and concentrations of individual components are strongly influenced by several integrated factors such as genetical and biochemical criteria (subspecies, natural hybridization and chemovariety) climatological and geographical conditions (light quality and quantity, soil characteristics, water availability and temperature), growing conditions (wild habitats, greenhouse production and different soilless culture systems) as well as agronomic parameters (fertilization, salinity level, macro and micronutrients availability and irrigation regime) (Hassanpouraghdam *et al*., 2009 & 2010).

High saline sodic condition has become a major restrain adversely affects plants physiological processes and limits field and horticultural crops performance particularly in arid and semi-arid regions of the world. High salt occurrence in the soil solution is linked to create the high osmotic pressure in the rhizosphere and ultimately reduced availability of water and nutrients to plant. Salinity conditions are known to affect plants physiological and biochemical potential, which in turn affect crops primary and secondary metabolism (Hebbara *et al*., 2003; Hendawy and Khalid, 2005).

Micronutrients greatly influence plants growth and development (Grejtovsky *et al.*, 2006; El-Tohamy *et al*., 2009; Akhtar *et al*., 2009). Among micronutrients, zinc is an essential one that plays role either as metal component of various enzymes or as a functional, structural or regulatory cofactor and is thus linked with photosynthesis and carbohydrate metabolism (Farahat *et al*., 2007). Zn has critical action on activity of carbon metabolism enzymes such as carbonic anhydrase, ribulose 1,5-bisphosphate carboxylase/oxygenase and fructose 1,6-bisphosphate (Misra *et al*., 2005). Misra *et al*. (2005) reported that essential oil biosynthesis in geranium was strongly influenced by Zn acquisition or deficiency. Overall, Zn is involved in carbon assimilation, saccharids accumulation, reactive oxygen radicals scavenging and finally carbon utilization in volatile oil biosynthesis (Misra *et al*., 2005; Nahed and Balbaa, 2007). Furthermore, Zn stimulate IAA production, starch formation and chlorophyll biosynthesis (Nahed and Balbaa, 2007; El-Wahab and Mohamad., 2008) and is necessary for DNA, RNA and protein synthesis (Nahed and Balbaa, 2007; El-Wahab and Mohamad., 2008).

Common basil (*Ocimum basilicum* L.) is a cosmopolitan herb and aromatic plant commonly growing in all parts of the world. Basil is a multipurpose plant with great applications in pharmaceutical, food and fragrance industries. Medicinally, this plant and its essential oil have long been used to treat nausea, dysentery, mental fatigue colds and rhinitis. The main volatile components of basil oil have been characterized as terpenoids and phenylpropane derivatives (Hassanpouraghdam *et al*., 2009 & 2010). In a series of studies conducted on basil we identified menthone (33.1 %) / estragol (21.5 %) and methyl chavicol (37.2-56.7 %) / linalool (13.1-21.1 %) as major essential oil components of field and hydroponically grown basil plants from Iran respectively (Hassanpouraghdam *et al*., 2009 & 2010).
The present experiment was conducted under the auspices of our previous works on the hydroponic management of high-value added crops production in order to finding an alternative production system for field grown *O. basilicum* plants encountered with progressive salinity conditions. Therefore, an attempt was made to evaluate the effects of salinity conditions and zinc foliar application on the essential oil composition of hydroponically grown *O. basilicum* L.

2 MATERIAL AND METHODS

This experiment was carried out at the Research Greenhouse of Department of Horticultural Sciences, University of Tabriz, Iran during spring-summer of 2009.

2.1 Plant material, growing conditions and treatments

Seeds of a native *O. basilicum* L. plants were directly sowed in 5L pots filled with medium sized perlite. During germination period and two first weeks of plantlets growth, they were irrigated with tap water. The quarter–strength modified Hoagland's nutrient solution was used for regular irrigation of plants for upcoming two weeks. Thenafter treatments were imposed. Treatments were factorial combinations of salinity [control (without salinity) and 50 mM NaCl] and Zn foliar applications [control (sprayed with distilled water) and 100 and 200 mg l^{-1} $ZnSO_4$, $7H_2O$] as randomized complete block design with 3 replications. For salinity study, 50 mM NaCl added to the half–strength solution was employed for the nourishment of related treatments.

$ZnSO_4$, $7H_2O$ solution was twice sprayed on plants. Once during early growth stage *i.e.* one month after sowing date

followed by second application about 2 weeks later. The plants leaves were thoroughly wetted until solution drops.

pH and EC of Hoagland's nutrient solutions were adjusted at 6–6.5 and 2 dSm^{-1} by H_2SO_4 or KOH and water respectively. The experiment was carried out in an one–layer polyethylene covered greenhouse under natural sunlight. Temperature, humidity and PAR of greenhouse were 15-30 ºC, 40-50 % and 500 $\mu Molm^{-2}s^{-1}$ during growing period respectively. No pesticide or herbicide was applied on the plants. The aerial parts of plants were harvested at the full flowering stage, dried at room temperature and subjected to essential oil extraction.

2.2 Volatile oil extraction

Fifty grams of air dried powdered plant materials (aerial parts) were extracted by the hydrodistillation method during 3 hours in an all–glass Clevenger type apparatus. The extracted essential oils were dried over anhydrous Na_2SO_4 and stored in sealed glass vials, covered with aluminum foil to protect the contents from photo-conversion and kept under refrigeration until analysis. Pooled essential oil samples from three replicates were evaluated for its components by GC/EI-MS.

2.3 Instrumentation

A GC/MS instrument (Agilent 6890N GC and Agilent 5973 mass selective detector operating in the EI mode, USA) was employed for the compositional analysis of volatile oil. Ultra pure helium (99.99%, Air Products, UK) passed through a molecular sieve trap and oxygen trap (Chromatography Research Supplies, USA) was used as the carrier gas at a constant velocity of 1 ml/min. The injection port was held at 300ºC and used in the split mode; Split ratio 1:100, Volume injected: 5µl of the pure volatile oil. Detector temperature was 200ºC. Separation was carried out on an apolar HP5MS (5%-phenyl methyl poly siloxane; 30m × 0.25 mm i.d. and 0.25µm film thickness) capillary column (Hewllet-Packard, USA). The oven temperature was programmed as follows: 50ºC (held 2 min) raised to 110ºC at a rate of 10ºC/min, then heated to 200ºC at the 10ºC/min rate and finally increased to 280ºC at 20ºC/min, isothermal at the temperature for 2 min. The mass operating parameters were as follows: ionization potential: 70eV, interface temperature: 200ºC and acquisition mass range: 50-800.

2.4 Identification and quantification of volatile oil components

Relative percentage amounts of the volatile oil constituents were evaluated from the total peak area (TIC) by apparatus software. The components of the essential oil were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from the database (NIST and WILEY library) as well as by comparing their Kovats gas chromatographic retention indices with those of the literature.

3 RESULTS AND DISSCUSION

The results obtained from the essential oil compositional analysis of *Ocimum basilicum* L. plants subjected to salinity and Zn foliar applications are presented in table 1. Salinity and Zn application combinations had appreciable quantitative but slight qualitative effects on essential oil constituents of basil. In total, fifty seven components were identified in the essential oil of six treatment combinations. Methyl chavicol – a phenylpropane derivative– was the most abundant components of all treatments with $NaCl_0$ Zn_{100} (61.2 %) and $NaCl_{50}$ Zn_{200} (43.9 %) treatment combinations had the highest and the least amount for this component respectively. It seems that salinity had negative effects on the biosynthesis and accumulation of this high–valued compound. Meanwhile, increasing Zn levels from 100 to 200 mg l^{-1} had no influential potential to compensate the deteriorative effects of salinity depression on this compound. Linalool – a fragrant oxygenated monoterpene – was the second major volatile oil component which $NaCl_{50}$ Zn_{200} (16 %) had the greatest amount for this compound. $NaCl_{50}$ Zn_0 (12 %) had the lowest sum for linalool. It is likely that salinity had promotive effects on this compound and the increasing levels of Zn foliar application had raising influence as well. Coolong *et al*. (2004) suggested that Zn fertility can influence changes in glucosinolates (GS) that may affect related plants flavor or medicinal attributes. In their study Zn linearly decreased gluconapin and sinalbin content of *Brassica rapa* plants. Gerjtovsky *et al*. (2006) reported that soil based Zn application only slightly affected the essential oil constituents, *i.e*. chamazulene and (E)-*β*-farnesene content of chamomile. Furthermore, an increased supply of Zn did not affect the content of flavone apigenin and coumarin herniarin in aforementioned study. Regarding the main components of essential oil it seems that there is some discrepancy and/or similarity between present study and reports of other scientists from elsewhere (cited in Hassanpouraghdam *et al*., 2009 & 2010). Germacrene D (2.2-3.9 %), 1,8-cineol (2.4-3.8 %), (Z)-*α*-bergamotene (0.1-2.6 %), (E)-*β*-farnesene (1.4–2.6%), *α*-bulnesene (0.9–2.4%), (E)-caryophyllene (1–2.1%), bicyclogermacrene (0.8–1.5 %), camphor (0.7–1.3 %) and (E)-*β*-ocimene (0.2–1.3 %) were the other common components of treatment combinations with amounts greater than one percent. Taking into account the constant levels of Zn foliar application, salinity had the elevating effects on the contents of 1,8-cineol, (Z)-*α*-bergamotene, (E)-caryophyllene, (E)-*β*-farnesene, germacrene D, bicyclogermacrene and *α*-bulnesene. It seems that salinity stress resulted growth reduction along with appropriate amounts of nutrient elements especially micronutrients changes the primary photosynthetic metabolic pool (sugars, amino acids and organic acid) in favor of secondary metabolites biosynthesis and accumulation. Like our results

Table1. Effects of salinity and zinc foliar application on essential oil composition of hydroponically grown *Ocimum basilicum* L.

No.	Compound	RI	%					
			$NaCl_0Zn_o$	$NaCl_0Zn_{100}$	$NaCl_0Zn_{200}$	$NaCl_{50}Zn_0$	$NaCl_{50}Zn_{100}$	$NaCl_{50}Zn_{200}$
1	*α*-Pinene	0939	0.2	0.1	0.1	-	0.1	0.2
2	Camphene	0954	0.1	0.1	0.1	0.1	-	
3	Sabinene	0975	0.2	0.1	0.1	0.1	0.1	0.1
4	*β*-Pinene	0979	0.6	0.5	0.4	0.7	0.5	0.6
5	Myrcene	0991	0.7	0.4	0.4	0.5	0.3	0.5
6	*α*-Terpinene	1017	0.1	0.1	tr	-	-	0.1
7	(P)-Cymene	1025	-	-	tr	-	-	-
8	Limonene	1029	-	0.2	0.3	-	-	0.2
9	*β* –Phellandrene	1030	-	-	0.1	-	-	-
10	**1,8-Cineole**	**1031**	**3.5**	**2.4**	**2.3**	**3.8**	**3.1**	**2.8**
11	(Z)-*β*-Ocimene	1037	0.1	0.1	0.1	-	0.1	**1.5**
12	(E)-*β*-Ocimene	1050	**1.2**	**1.2**	**1**	**1.1**	**1.3**	0.2
13	*γ*-Terpinene	1060	0.1	0.1	0.1	0.1	0.1	0.1
14	(Z)-Sabinene hydrate	1070	0.1	-	-	-	-	-
15	Fenchone	1087	0.8	0.6	0.8	0.6	0.6	0.4
16	Terpinolene	1089	-	-	-	tr	tr	-
17	**Linalool**	**1097**	**13.1**	**13**	**15.4**	**11.4**	**12**	**16**
18	Camphor	1146	**1.1**	**1**	**1.3**	**1**	0.7	0.8
19	Borneol	1169	0.2	0.1	0.2	0.5	0.2	0.2
20	Terpinene-4-ol	1177	0.1	0.1	0.1	0.1	0.1	0.1
21	*α*-Terpineol	1189	-	0.3	-	-	-	0.3
22	**Methyl chavicol**	**1196**	**54.7**	**61.2**	**58.4**	**57.5**	**51.5**	**43.9**
23	Geraniol	1253	-	0.1	0.1	-	tr	-
24	*α*-Cubebene	1351	0.8	0.5	0.2	0.6	0.2	0.9
25	*α*-Longipinene	1353	-	-	-	-	0.2	-
26	Eugenol	1359	**1**	-	0.1	-	0.1	0.1
27	*α*-Ylangene	1375	-	-	-	0.1	-	-
28	*α*-Copaene	1377	0.2	0.2	0.6	0.4	0.4	0.4
29	*β*-Bourbonene	1388	0.1	0.1	0.1	0.1	0.2	0.2
30	*β*-Cubebene	1388	0.5	0.4	0.4	-	0.6	0.2
31	*β*-Elemene	1391	0.7	0.6	0.7	0.9	**1.1**	**1.8**
32	Methyl eugenol	1404	0.6	0.2	0.4	0.3	0.8	**1.5**
33	*α*-Cedrene	1412	-	0.1	-	-	-	0.2
34	***α*-(Z)-ergamotene**	**1413**	**2.6**	**0.1**	**1.8**	**1.8**	**-**	**2.6**
35	**(E)-Caryophyllene**	**1419**	**1**	**1.3**	**1.3**	**1.3**	**1.1**	**2.1**
36	*α*-Guaiene	1440	0.4	0.5	0.5	0.7	0.8	**1.2**
37	Aromadendrene	1441	0.1	-	0.1	0.2	-	0.2
38	**(E)-*β*-Farnesene**	**1457**	**1.6**	**2.4**	**1.4**	**2**	**2.8**	**2.6**
39	**Germacrene D**	**1485**	**2.7**	**2.2**	**2.5**	**3.3**	**3.9**	**3.9**
40	***α*-Amorphene**	**1485**	**-**	**-**	**1.6**	**1.7**	**-**	**-**
41	*α*-Zingiberene	1494	-	**1.9**	0.1	0.1	**2.2**	-
42	Bicyclogermacrene	1500	**1.3**	**1**	0.8	**1.1**	**1.3**	**1.5**
43	(E)-*α*-Farnesene	1506	-	-	-	0.4	-	-
44	*α*-Bulnesene	1510	**1.1**	0.9	**1.2**	**1.5**	**1.9**	**2.4**
45	*γ*-Cadinene	1514	**2**	**1.4**	-	0.1	**2.6**	**2.4**
46	*Δ*-Cadinene	1523	0.1	0.4	**2.2**	**2.8**	0.1	0.6
47	(Z)-Nerolidol	1533	0.1	-	0.1	0.1	0.1	0.2
48	*α*-Cadinene	1539	0.1	-	tr	0.1	0.1	-
49	(Z)-Calamenene	1540	0.4	-	0.3	-	0.5	-
50	Spathulenol	1578	-	-	-	0.3	0.3	0.5
51	Caryophyllene oxide	1583	0.1	0.1	0.1	0.2	0.3	0.4
52	Alloaromadendrene	1641	0.1	-	-	-	0.3	-
53	*β*-Eudesmol	1651	0.1	0.1	0.1	0.1	0.2	-
54	***α*-Cadinol**	**1654**	**3**	**2**	**-**	**-**	**3.7**	**4.1**
55	*α*-Bisabolol	1686	0.1	-	0.1	0.1	0.2	-
56	*β*-Sinensal	1700	-	-	-	0.1	0.1	-
57	Phytol	1943	0.1	-	tr	0.1	0.1	-
	Total		97.8	98.1	98	98	96.9	98

Compounds are reported according to their elution order on non-polar column

Srivastava *et al.* (1997) noted that increased sugar content of leaves under Zn stress conditions positively influenced essential oil accumulation of peppermint plants. (Z)-*β*-Ocimene (1.5 %), *β*-elemene (1.8 %), methyl eugenol (1.5 %) and *α*-guaiene (1.2 %) were the constituents with their maximum amounts possessed by $NaCl_{50}$ Zn_{200}. Seemingly, moderate levels of salinity combined with raising levels of Zn foliar application had synergistic effects on production of some compounds. Misra and Sharma (1991) mentioned that Zn application stimulated the menthol concentration in Japanese mint. Furthermore, Chand *et al.* (2007) reported that integrated supply of vermicompost and zinc–enriched compost improved the percentage amount of geranium oil components such as cis-rose oxide, isomenthone and linalool. *α*-amorphone (1.6–1.7 %) and *Δ*-cadinene (0.1–2.8 %) were the other components with a scatter distribution between treatment combinations. *α*-Zingiberene (0.1–2.2 %) was a constituent with its greatest amounts under 100 mgl^{-1} Zn foliar application and only with minor impacts of salinity level. Srivastava *et al.* (2006) in their work on turmeric stated that there is a strong relationship between primary metabolic pathways and biosynthesis/accumulation of secondary metabolites. Those authors declared that proper correlation of carbon assimilation pathways and accumulation of secondary metabolic compound needs association of several intrinsic and extrinsic factors particularly optimum levels of micronutrients. *α*-Cadinol (2–4.1 %) and *γ*-cadinene (0.1–2.6 %) were two major sesquiterpene hydrocarbon constituents of studied oils with their maximum quantites belonged to $NaCl_{50}$ Zn_{200}. This means that combined controlled application of salinity and Zn had complement effects on major fifteen carbons sesquiterpenoidal components of essential oil. Similar to our results Hendawy and Khalid (2005) reported that zinc application under salinity levels had diverse increasing and/or decreasing effects on the volatile components of sage plants.

In general it appears that moderate salinity levels had promising effects on volatile oil profile of basil, and integrated foliar application of zinc ameliorated the presumable unhealthy effects of salinity in support of major volatile oil components. In summary, it seems that regarding volatile oil constituents, basil plant has the production potential under semi saline conditions. This production needs optimum growing conditions and balanced nutrient availability especially appropriate levels of micronutrients. However this claim needs detailed studies with other nutrient elements.

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Agrovoc descriptors: monilinia, fungi, pathogens, infection, symptoms, fungal diseases, crop losses,damage, morphology, geographical distribution, hosts, disease transmission, identification

Agris category code: h2

Monilinia pathogens of cultivated and native *Vaccinium* species in Slovenia

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ABSTRACT

The fungal genus *Monilinia* comprises several aggressive and economically important plant pathogens. The aim of this study was to examine *Monilinia* species that belong to the Disjunctoriae group and are specifically pathogenic to *Vaccinium* hosts. 23 samples of *Vaccinium* hosts showing symptoms of infection with *Monilinia sp.* were collected in the years 2004 – 2009. *Monilinia* species were isolated and identified using morphological and molecular methods. Three species from the Disjunctoriae group were identified: *M. baccarum*, *M. urnula* and *M. vaccinii-corymbosi*. Morphology of the encountered species is briefly described. Their distribution and host range are presented and the diseases they cause on their respective hosts are described.

Key words: Monilinia baccarum, Monilinia urnula, Monilinia vaccinii-corymbosi, Disjunctoriae, Vaccinium, Slovenia

IZVLEČEK

PATOGENE GLIVE IZ RODU *MONILINIA* NA GOJENIH IN SAMONIKLIH VRSTAH RODU *Vaccinium* V SLOVENIJI

Rod *Monilinia* združuje številne agresivne in gospodarsko pomembne patogene glive. Predmet raziskave so bile vrste rodu *Monilinia*, ki sodijo v skupino Disjunctoriae in so specializirane na gostitelje iz rodu *Vaccinium*. V letih 2004 - 2009 smo zbrali 23 vzorcev rastlin iz rodu *Vaccinium*, pri katerih smo ugotovili znamenja okužbe s temi glivami. S standardnimi mikroskopsko morfološkimi in molekularnimi tehnikami smo identificirali tri glive iz skupine Disjunctoriae: *M. urnula*, *M. baccarum* in *M. vaccinii-corymbosi*. V prispevku so opisane glavne morfološke značilnosti ugotovljenih vrst, njihova razširjenost in gostitelji v Sloveniji ter bolezni, ki jih posamezne vrste povzročajo pri svojih specifičnih gostiteljih.

Ključne besede: Monilinia baccarum, Monilinia urnula, Monilinia vaccinii-corymbosi, Disjunctoriae, Vaccinium, Slovenija

1 INTRODUCTION

The fungal genus *Monilinia* Honey (*Ascomycota*, *Leotiomycetes*, *Helotiales*, *Sclerotiniaceae*) comprises several aggressive and economically important plant pathogens (Batra, 1991). They affect several fruit tress and shrubs and cause blights, cankers and fruit rot. Their hosts mainly belong to the families *Rosaceae* and *Ericaceae*.

Altogether 31 *Monilinia* species have been described. Honey (1936) established two informal sections within the genus *Monilinia*, the *Junctoriae* and the *Disjunctoriae.* This division was based on morphological characters, life histories and host preferences of included species and was also supported by phylogenetics analyses (Holst-Jensen et al., 1997). All major pathogens of fruit trees and shrubs belong to the Junctoriae group. The best known representatives of this group in Europe are *M. laxa* and *M. fructigena*. They are polytrophic and affect a wide range of rosaceous hosts. Another Junctoriae species, *M. fructicola*, is native to North and South America, Australia and New Zealand and has been introduced to

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Europe only recently (EPPO, 2002). All three species induce similar symptoms: blossom blight, dieback of twigs, canker formation and fruit rot known as *Monilinia* brown rot. They consequently cause conspicuous economic loss in fruit growing regions worldwide.

The vast majority of *Monilinia* species belong to the Disjunctoriae group; 27 species are currently included in this section (Batra, 1991). They have a common morphological characteristic, the disjunctors i.e. spindle like appendages intercalated between conidia in the conidial chain. This characteristic is reflected in the name of the group. Disjunctoriae species inhabit a variety of wild and cultivated *Rosaceae* and *Ericaceae*. Host specialization is more pronounced in the Disjunctoriae group than in the Junctoriae group. While some species specifically infect *Rosaceae* from genera *Sorbus*, *Crategus*, *Amelanchier* and *Prunus*, the majority of Disjunctoriae species specialize to *Ericaceae*. They affect different genera, those with fleshy fruits (*Vaccinium*, *Gaylussacia*, *Oxycoccus*) and those with capsular fruits (*Rhododendron*, *Azalea*, *Ledum*). They cause blight of flower clusters, dieback of shoots and mummification of fruits of their respective hosts. Only two species from this group cause economically significant damage, *M. vaccinii-corymbosi* and *M. oxycocci*, the causative agents of the mummy berry disease and the cotton boll disease of blueberries and cranberries, respectively (Batra, 1983).

Monilinia species from the Disjunctoriae group have a complex life cycle and regularly form a sexual and an asexual reproductive stage (Batra, 1983). They produce two types of spores that have different dispersal mechanisms and infect specific host tissues. Each spore type is produced only once in a growing season and has very limited opportunity to cause infection. In spring, apothecia develop on overwintered mummified fruits (termed pseudosclerotia) and release ascospores that initiate primary infection of newly emerging tissues. Asexual spores (macroconidia) are produced on blighted plant parts and subsequently spread infection to flowers. In contrast, the Junctoriae species rely mainly on asexual reproduction and produce several crops of macroconidia that spread infection to flowers and fruits.

The aim of this study was to examine the occurrence, ecology and diseases caused by *Monilinia* species from the Disjunctoriae group in Slovenia. Species that are specifically pathogenic to *Vaccinium* hosts were of our primary interest.

2 MATERIALS AND METHODS

2.1 Field sampling

In the years 2004 – 2009 we collected 23 samples of ericaceous hosts showing symptoms of infection with *Monilinia sp*. Several samples were collected from high-bush blueberry (*Vaccinium corymbosum*) growing in the plantations situated in the Ljubljana Wetland. Other samples were collected from native *Vaccinium species* growing in forests situated in the lowland and mountainous regions of Gorenjska and Štajerska (Table1).

Monilinia species were isolated from blighted twigs, leaves and mummified fruits as well as from mature apothecia. Isolation was performed by culturing pieces of necrotic host tissue on potato dextrose agar (PDA) or by spreading ascospore suspension on PDA plates and isolating individual germinating ascospores. The PDA medium was amended with a mixture of streptomycin sulphate and penicillin to prevent bacterial contamination during the isolation. Isolates were kept on PDA slants at 4 ^{0}C for long term storage. Apothecia and pseudosclerotia were dried and saved as herbarium specimens.

2.2 Identification of isolates

Monilinia isolates were identified by colony characteristics, morphology of macroconidia, microconidia and disjunctors as well as by characteristics of pseudosclerotia and apothecia.

Morphological characteristics were studied by using standard microscopic techniques. Free hand sections of apothecia and pseudosclerotia were used for morphological studies. Observations and measurements were done in lactophenol with cotton blue. 25 to 50 asci, ascospores, conidia and disjunctors were measured. Cultural characteristics were observed after growing ten replicates of isolates for three weeks in the dark at 20 and 25 ^{0}C on PDA and YEMEA (malt agar with yeast extract).

Morphological identification of isolates was confirmed by molecular tools. DNA was extracted from pure cultures using the DNeasy Plant Mini Kit (Qiagene, Germany). Amplification of the ITS rDNA region, comprised of the 3' end of the 18S rRNA gene, the internal transcribed spacer (ITS) 1, the 5.8S rRNA gene, ITS2, and the 5' end of the 26S rRNA gene, was performed with the primer pair ITS1 and ITS4 (White et al., 1990). The PCR cycling parameters were: one cycle of 94 ^{0}C for 3 min; 35 cycles of 94 ^{0}C for 40 sec, 54 ^{0}C for 50 sec, 72 ^{0}C for 2 min; and a final cycle of 72 ^{0}C for 10 min. The PCR products were cleaned using Jetquick (Genomed, Germany). Sequencing was done at a sequencing facility (Macrogen, Korea) using the same primers as for the PCR reactions. Sequence data were analyzed using BioEdit Alignment Editor. Sequences were analyzed against the GenBank "nr/nt" database using BLASTN.

Table 1: Isolates of *Monilinia* species examined in the study

Isolate code *	Species of *Monilinia*	Locality	Host	Source of isolates
M311	*M. vaccinii-corymbosi*	Drenov Grič, UTM 33TVL59	*V. corymbosum*	conidia
M422	*M. vaccinii-corymbosi*	Valburga, UTM 33TVM51	*V. corymbosum*	blighted twig
M423	*M. vaccinii-corymbosi*	Valburga, UTM 33TVM51	*V. corymbosum*	conidia
M424, CBS 120174**	*M. vaccinii-corymbosi*	Borovnica, UTM 33TVL58	*V. corymbosum*	conidia
M425	*M. vaccinii-corymbosi*	Borovnica, UTM 33TVL58	*V. corymbosum*	blighted twig
M426	*M. vaccinii-corymbosi*	Smrekovec, UTM 33TVM94	*V. corymbosum*	conidia
M427	*M. vaccinii-corymbosi*	Drenov Grič, UTM 33TVL59	*V. corymbosum*	blighted blossoms
M430	*M. vaccinii-corymbosi*	Borovnica, UTM 33TVL58	*V. corymbosum*	apothecium
M060824.1	*M. urnula*	Pohorje, Pesek , UTM 33TWM24	*V. vitis-idaea*	mummified fruit
M060824.2	*M. urnula*	Pohorje, Rogla, UTM 33TWM24	*V. vitis-idaea*	mummified fruit
M060824.3	*M. urnula*	Ribnica na Pohorju, UTM 33TWM25	*V. vitis-idaea*	mummified fruit
M060824.4	*M. urnula*	Pohorje, Lovrenško barje, UTM 33TWM24	*V. vitis-idaea*	mummified fruit
M060824.5	*M. urnula*	Pohorje, Osankarica, UTM 33TWM34	*V. vitis-idaea*	mummified fruit
M419	*M. urnula*	Sp. Palovče nad Kamnikom, UTM 33TVM71	*V. vitis-idaea*	mummified fruit
M420	*M. urnula*	Pokljuka, Šijec, UTM 33TVM23	*V. vitis-idaea*	mummified fruit
M421/K	*M. urnula*	Pokljuka, Šijec, UTM 33TVM23	*V. vitis-idaea*	conidia
M060824.7	*M. baccarum*	Pohorje, Pesek , UTM 33TWM24	*V. myrtillus*	mummified fruit
M060824.8	*M. baccarum*	Pohorje, Klopni vrh, UTM 33TWM34	*V. myrtillus*	mummified fruit
M060824.9	*M. baccarum*	Pohorje, Lovrenško barje, UTM 33TWM24	*V. myrtillus*	mummified fruit
M313	*M. baccarum*	Pokljuka, pod pl. Lipanco, UTM 33TVM13	*V. myrtillus*	mummified fruit
M429	*M. baccarum*	Sp. Palovče nad Kamnikom, UTM 33TVM71	*V. myrtillus*	mummified fruit
M431	*M. baccarum*	Krvavica nad Taborom, UTM 33TVM91	*V. myrtillus*	mummified fruit
M432	*M. baccarum*	Pokljuka, Mrzli Studenec, UTM 33TVM23	*V. myrtillus*	apothecium

* all isolates are kept in the culture collection at the Agricultural Institute of Slovenia
** isolate was deposited at Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands

3 RESULTS AND DISCUSSION

Three species of the genus *Monilinia* were detected on ericaceous hosts with fleshy berries: *M. baccarum*, *M. urnula* and *M. vaccinii-corymbosi*. Brief morphological description and notes on the ecology of each species are given below. Only representatives of the genus *Vaccinium* were found to be infected with *Monilinia* species. No signs of infection were observed on *Oxycoccus* and *Arctostaphylos* species although they were regularly examined for the occurrence of twig blight, mummified fruits and apothecia.

3.1 *Monilinia urnula* (Weinmann) Whetzel

Anamorph: *Monilia urnula* Batra

Morphology of the fungus

Pseudosclerotia were reddish brown, hollow, open above and below. They retained the shape of a mature fruit and incorporated some remains of host tissue

(mesocarp, ovules, aborted seed). In cross section an outer and inner rind and a thick medulla were clearly visible. Mature pseudosclerotia were collected in late August and September.

Apothecia occurred in late spring (from the end of May until the beginning of June, depending on locality and weather conditions). Apothecia were rare and difficult to find. They arose from fallen mummified fruits (pseudosclerotia) that overwintered in moist patches. We often found them among *Sphagnum* moss. Usually only one apothecium developed per pseudosclerotium. Apothecia were nut brown, cupulate when young, later discoid with slightly everted margin, disc 5 -8 mm in diameter. Stipe of apothecia was up to 50 mm long, dark brown. Fascicles of thick walled hyphae, the so-called rhizoidal tufts, were clearly visible at the base of stipes. Hymenium was light brown, asci cylindrical, eight spored and measured 152.8 – 166.3 µm (mean162.1 µm) x 9.7 – 10.9 µm (mean 10.3 µm). Ascospores were hyaline, ellipsoid and measured 11.4 - 15 µm (mean 13 µm) x 5 – 6.5 µm (mean 5.8 µm). Paraphyses were filiform and septate at the base.

Macroconidia of the anamorphic state *Monilia urnula* were observed in early summer (from June until the beginning of July). They appeared as white covering on the convex side of young shoots and on the under surface of leaves, particularly along midribs. Macroconidia were limoniform, hyaline and measured 25.6 – 41.4 µm (mean 31.13 µm) x 15.5 – 27.5 µm (mean 20.7 µm). Mature macroconidia were separated by spindle like structures - disjunctors. The disjunctors are involved in the dissemination of macroconidia. They were first described by Woronin (1888) in *M. urnula* and later found to be present in all species belonging to the Disjunctoriae group. The disjunctors of *M. urnula* are conspicuous and robust. They measure 4.4 x 2 µm when intercalated between macroconidia and 9.4 x 2 µm when detached.

The colonies on PDA and YEMEA were slow growing; at 25^0 C they overgrew a 90 mm petri dish in 21 days. The mycelium was white and beige on reverse, loose but compact in places. Black stroma, buried deep in agar, was observed in aging colonies. Macroconidia did not develop, but microconidia were abundant, particularly in mature colonies growing in darkness. They were globose, hyaline, 2 - 3 µm wide. Microconidia are characteristic for many *Monilinia* species. They form on macroconidia, ascospores and vegetative hyphae either directly or on flask shaped phialides (Batra, 1991).

Habitat, distribution and diseases caused

M. urnula is a monotrophic species that inhabits only *Vaccinium vitis-idaea.* There are several records of this species in Europe, mainly from Scandinavia, Austria and United Kingdom (Gjaerum, 1969; Woronin, 1888; Dennis, 1968). It was reported also from Japan (Kobayashi, 2007).

M. urnula infections result in the blight of young shoots and leaves and the development of brown, hard berries (mummy berries). Infection of shoots and leaves in early summer is performed by ascospores while mummification of berries results from conidial infection of flowers.

M. urnula was thoroughly studied by the Russian mycologist Woronin (1888) in his monographic study of *Sclerotiniaceae* on *Vaccinium* species with fleshy berries. He investigated the life cycle of the fungus and established the anamorph / teleomorph relationship. Woronin (1888) described entry of conidia through the stigma, growth along the stylar canal and development of white cottony mass of mycelium around ovules and placenta. The infected berries appear normal until the fungus invades the mesocarp and forms pseudosclerotium. Then they turn brown and fall to the ground.

3.2 *Monilinia baccarum* (Schröter) Whetzel
Anamorph: *Monilia baccarum* Migula

Morphology of the fungus

Pseudosclerotia were light grey, sometimes grayish pink and could be found in the litter below host plants. Mature pseudosclerotia were hollow and open at the distal end. Remains of seeds and ovules were present in the young ones. An outer and an inner rind were visible on cross section. Mature pseudosclerotia were collected from July to September.

In spring, one or two apothecia developed from the overwintered pseudosclerotia. Apothecia developed by the end of May at higher elevations in the mountainous region. They probably occur earlier in the lowland. They were dark brown, cupulate with upward margins and measured 6 mm in diameter. Stipe was dark brown, 40 - 50 mm long, not ornamented with rhizoidal tufts. Hymenium was brown, asci cylindrical, eight spored and measured 140 - 180 µm (mean 170 µm) x 10 - 14 µm (mean 13 µm). Ascospores were hyaline, ellipsoid and measured 14.5 – 20.8 µm (mean 17.3 µm) x 5.9 – 7.3 µm (mean 6.6 µm). Paraphyses were filiform, septate at the base and unbranched.

Macroconidia of the anamorphic state *Monilia baccarum* were observed from late spring to early summer, depending on the locality. They appeared as grayish white mantle on the concave side of new shoots and leaves. Macroconidia were limoniform, hyaline and

measured 19 – 28 µm (mean 23.5 µm) x 14 - 21 µm (mean 17 µm). They were separated by 3 - 4 µm long disjunctors.

The colonies on PDA and YEMEA were slow growing and reached the diameter of 50 mm in 21 days. The mycelium was white, grayish brown on reverse, compact. Black superficial stroma developed in older colonies. Macroconidia were not observed in culture. Microconidia were present on hyphae, they were globose, hyaline, 2 - 3 µm wide.

Habitat, distribution and diseases caused

M. baccarum is a monotrophic species restricted to *Vaccinium myrtillus.* It is known from Scandinavia, Austria, Belgium, Germany and United Kingdom (Batra, 1991; Gjaerum, 1969; Rehm, 1885; Woronin, 1888; Dennis, 1968; Palmer, 1988).

M. baccarum causes blight of newly emerging shoots; they turn brown and droop. The infected bilberries turn pale, dry, shrivel, mummify and fall to the ground. They are called white berries due to the fine whitish layer of host cells (including epidermis) that cover the berry (Batra, 1991).

3.3 *Monilinia vaccinii-corymbosi* (Reade) Honey
Anamorph: *Monilia vaccinii-corymbosi* Reade

Morphology of the fungus

Pseudosclerotia were dark brown, robust, hollow, distinctively ribbed, flattened and opened at both poles. In cross section an outer and an inner black rind were visible. Mature pseudosclerotia fell to the ground. They were found in masses under infected host plants by the end of summer. They persisted until next spring.

At the end of March until the end of April three to four apothecia developed from the overwintered pseudosclerotia. Apothecia were reddish brown to umber, cupulate with recurved margin when young, later discoid with slightly crenate margin, disc 10 -12 mm in diameter. Stipe was dark brown, 40 - 50 mm long, rhizoidal tufts were not observed. Hymenium was brown, asci cylindrical, eight spored and measured 177 - 230 µm (mean 181.6 µm) x 8 – 14.7 µm (mean 11.2 µm). Ascospores were hyaline, ellipsoid and measured 15 – 19.2 µm (mean 16.7 µm) x 8 – 10.7 µm (mean 9.6 µm). Paraphyses were filiform and unbranched.

Macroconidia of the anamorphic state *Monilia vaccinii-corymbosi* were observed in spring (early May), just before the onset of flowering. They appeared as dense grayish covering on the convex side of bent current year twigs, on petioles and along midribs of blighted leaves. Macroconidia were limoniform, hyaline and measured 21.9 – 30.1 µm (mean 25.7 µm) x 11.7 – 15.3 µm (mean 13.8 µm). They were borne in long, branched moniloid chains. Individual macroconidia were intercalated with 3 – 5 µm long, fusiform disjunctors.

The colonies on YEMEA were slow growing and reached 7 - 8 cm in diameter after 21 days at 20 ^{0}C in the dark. The mycelium was white to beige and compact. Brown stroma developed in older cultures. The reverse of plates was brown, with yellow or honey pigmentation in some cultures. Hyphae were broad and often assembled in fascicles. Production of macroconidia was scarce. Microconidia were abundant on hyphae, on germinating ascospores and macroconidia. They were hyaline, globose, 3 - 4 µm in diameter.

Habitat, distribution and diseases caused

M. vaccinii-corymbosi is endemic to North America. It is oligotrophic there and affects several species of the genus *Vaccinium* (Batra, 1983). In our study we found it on *Vaccinium corymbosum* only.

M. vaccinii-corymbosi is a major problem of commercial and wild blueberries throughout North America. It causes the mummy berry disease of high-bush blueberry (*V. corymbosum*), low-bush blueberry (*V. angustifolium*), rabbiteye blueberry (*V. ashei*) and several other North American *Vaccinium* species (Batra, 1991). The pathogen induces two kinds of damage: wilting and browning of young shoots, leaves and flower clusters in early spring and mummification of maturing fruits in summer. Blighting of flower clusters and mummification of fruits significantly reduce the current year yield while blighting of shoots can have a long term effect on blueberry production in the following years. Blighting is caused by ascospore infection of buds in early spring. The development and release of ascospores are well correlated with the bud brake of host plants (Batra 1983; Lehman and Oudemans 2000). Macroconidia later develop on blighted plant parts. Insect pollinators and wind transfer them to stigmas of open flowers where they germinate, subsequently enter the fruit locules and finally invade the fruit mesocarp (Batra and Batra, 1985; Ngugi et al., 2002). Young fruits show no signs of infection and do not differ from healthy ones until maturity. Then they become wrinkled, light pink to brown, hard and drop to the orchard ground.

M. vaccinii-corymbosi was first documented in Europe in 2003 in Austria (Gosch 2003). A year later (in summer 2004) high-bush blueberry growers in Slovenia also found the first mummified fruits in their plantations. Already in spring 2005 severe infection of new shoots and flowers occurred. The extent of damage reached up to 50 % in certain high-bush blueberry varieties.

4 CONCLUSIONS

The primary goal of our work was to study *Monilinia* species that inhabit *Vaccinium* hosts and belong to the Disjunctoriae group. Species from the Disjunctoriae group have been much less thoroughly studied than those from the Junctoriae group. There are several reasons for this. Most Disjunctoriae species are rare or restricted in their distribution. Their fruit bodies are small and difficult to find in the field. Besides, they mostly cause economically less significant damage comparing to the Junctoriae and therefore receive less attention from phytopathologists.

Three Disjunctoriae species were encountered in Slovenia: *M. baccarum*, *M. urnula* and *M. vaccinii-corymbosi*. *M. baccarum* and *M. urnula* were pathogenic to *V. myrtillus* and *V. vitis-idaea*, respectively. These two hosts are sympatric in our region and frequently inhabit the same site. However, each host was strictly colonized only by its respective *Monilinia* pathogen and no cross infections were observed. In contrast to the monotrophic species *M. urnula* and *M. baccarum*, *M. vaccinii-corymbosi* exhibits a polytrophic character and infects several North American blueberry species. It is listed among pathogens that have the greatest economical impact on blueberry production in North America. It was presumably introduced to our country with the infected planting material of high-bush blueberry. It remained restricted to this North American host. In the last years it became well established and widespread in our high-bush blueberry plantations where it significantly affects the production of high-bush blueberries.

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Agris category code: h20, f30

Total RNA extraction method and *Prunus* species influence the detection of *Plum pox potyvirus* by real-time RT-PCR

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ABSTRACT

Successful total RNA (totRNA) extraction is a prerequisite for a successful real-time PCR. In the present work we compared one manual and one automated totRNA extraction method for detection of *Plum pox potyvirus* (PPV) in leaves of different *Prunus* sp. using developed real-time RT-PCR assay. Advantages and disadvantages of compared methods are described in the view of sensitivity, reproducibility and in terms of laboratory use. The results suggest that the column based totRNA extraction method is more effective for apricot, plum and peach. In the case of damson the differences in real-time PCR results between both extraction methods were negligible. In case of negative results obtained with automated method, manual column based extraction method should be used additionally.

Key words: 18S rRNA, MGB, PCR inhibition, PPV

IZVLEČEK

RASTLINSKA VRSTA IN METODA IZOLACIJE CELOKUPNE RNA VPLIVATA NA USPEŠNOST DETEKCIJE VIRUSA ŠARKE S PCR V REALNEM ČASU

Uspešnost izolacije RNA je osnova za uspešno izvedbo reakcije PCR v realnem času (qPCR). V svojem delu smo primerjali ročno in avtomatsko metodo izolacije celokupne RNA (totRNA) iz listov različnih rastlinskih vrst iz rodu *Prunus*. Uspešnost izolacije totRNA smo preverjali z uvedeno metodo qPCR za detekcijo virusa šarke (*Plum pox potyvirus* – PPV). Prednosti in slabosti uporabljenih metod smo ugotavljali s primerjavo občutljivosti detekcije, ponovljivosti in intenzivnosti laboratorijskega dela. Rezultati raziskave so pokazali, da je ročna metoda izolacije totRNA učinkovitejša pri marelicah, breskvah in slivah, medtem ko so bile razlike med obema metodama pri ciborah zanemarljive. Pri uporabi preizkušene avtomatske metode izolacije totRNA je priporočljivo, da v primeru negativnih rezultatov le-te še dodatno preverimo z uporabo ročne metode izolacije.

Ključne besede: 18S rRNA, MGB, inhibicija PCR, PPV

1 INTRODUCTION

Sharka caused by the *Plum pox virus* (PPV) is the most devastating viral disease of stone fruits, predominately peaches, nectarines, apricots, plums and prunes. In susceptible varieties it can cause high yield losses and tree decline. The fruit quality is also affected. Estimated cost associated with sharka management worldwide in the last 30 years exceeded 10 000 million euros. Costs of sanitary controls, surveys and eradication programs, but not of indirect trade losses, are included in the estimation (Cambra et al., 2006).

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Sharka was first observed in Slovenia in 1987. In the following years the presence of PPV was confirmed by Double Antibody Sandwich Enzyme-linked Immunosorbent Assay (DAS-ELISA) in samples originating from orchards, individual trees and propagation material of stone fruits. Due to the high incidence of PPV infection a systematic survey of PPV was initiated in 1998 to prevent and control the spread of sharka. Since 2000 the survey has focused mainly on nurseries, mother trees and stool-beds (Viršček Marn et al., 2004).

Due to the possibility of low virus concentration in plants and its irregular distribution, a great sensitivity and accuracy is required in PPV diagnostics.

Real-time PCR and RT-PCR is extensively used for pathogen detection and quantification; it is a method of choice for gene quantification and it is more and more used also in plant virology. Its use also eliminates or reduces the use of hazardous chemicals (e.g. ethidium bromide) in the laboratories.

Real-time PCR is a method of choice in the view of sensitivity and accuracy but the choice of target tissue and RNA extraction methods can also greatly influence the detectability of viruses in plant material. Plants are known to contain a lot of possible PCR inhibitors such as polysaccharides, polyphenols, proteins and other plant secondary metabolites (Gambino et al., 2008; Demeke and Adams, 1992; Osman and Rowhani, 2006). Their content differs between different plant organs and changes during the growing period. Previous experiences show the importance of validating RNA extraction procedure for different sample matrixes and the ability of the extraction method to provide a suitable nucleic acid free of PCR inhibitors from each sample matrix. Numerous RNA extraction methods have been used in the preparation of total RNA (totRNA) from woody plants with a more or less sufficient quantity of isolated totRNA and reduction of inhibitors, but most of them are time consuming and technically demanding (Gambino et al., 2008; Singh et al., 2002; Maliyakal, 1992).

In the present work we have compared one manual and one automated method for totRNA extraction from leaves of different *Prunus* sp. The quality and quantity of extracted totRNA was evaluated using developed real-time RT-PCR assay for the detection of PPV. Advantages and disadvantages of compared methods are discussed in the view of sensitivity, reproducibility and in terms of laboratory activities.

2 MATERIALS AND METHODS

2.1 Plant material

10 samples of each of 4 different *Prunus* species (*P. armeniaca* (apricot), *P. domestica* ssp. *domestica* (plum), *P. domestica* ssp. *insititia* (damson) and *P. persica* (peach)) were included in the study. Leaves were collected from PPV infected trees previously tested by DAS-ELISA. For each plant species a negative tree was also included.

2.2 Total RNA extraction

TotRNA was extracted from leaf tissue of different *Prunus* species by two extraction methods. Extracts were prepared by homogenizing 100 mg of fresh leaf tissue in 1100 µL of RLT extraction buffer (RNeasy Plant Mini kit (Qiagene, Valencia, USA)). The homogenisation was done using TissueLyser (Retsch, Haan, Germany) shaker for 3 min at 30 Hz in the presence of one stainless steel bead of 5 mm in diameter. Extracts were centrifuged and supernatant was used for further totRNA extraction.

In Method 1 (manual method), 450 µL of each extract were used for totRNA extraction using RNeasy Plant Mini kit (Qiagene, Valencia, USA) according to the manufacturer's instructions. The totRNA elution volume was 50 µL.

In Method 2 (automatic method), the other 450 µL of each extract were used for totRNA extraction using RNeasy Tissue Kit (Qiagene, Valencia, USA) with KingFisher mL instrument (Thermo Electron, Vantaa, Finland), and according to the manufacturer's instructions. The totRNA elution volume was 100 µL.

5 µL of isolated totRNA were treated with DNase I for further analysis with real-time PCR method. The reaction conditions were according to the manufacturer's instructions (Invitrogen Corporation, Carlsbad, USA), using 1 U of DNase I. The final volume of the reaction was 50 µL. Aliquots of the resulting RNA were analyzed by electrophoresis on 1 % agarose gel with ethidium bromide staining to check its quantity and integrity (data not shown).

2.3 Reverse transcription

Equalized amounts of DNase treated totRNA (according to agarose gel analysis) was reverse transcribed using a cDNA Archive kit (Applied Biosystems, Foster City, USA) according to manufacturer's instructions with minor modifications. 25 U of RNase inhibitor (Applied Biosystems, Foster City, USA) were added to the final volume of 25 µL. Reactions were incubated at 25°C for 10 min followed by 37°C for two hours.

2.4 Real-time PCR primers and probe design

A high homology 103 bp region of the PPV genome was selected as a target for PCR amplification by aligning 61 sequences published in GenBank database. Primer Express software v2.0 (Applied Biosystems, Foster City, USA) was used to design primers and sequence specific hydrolyzing TaqMan MGB probe (Table 1). The probe was labeled at the 5'-end with 6-carboxyfluorescein (FAM) dye. A GenBank

BLAST search for short nearly exact matches revealed no significant database alignments of either primer or the probe with other than the region of interest in the PPV genome.

Table 1: Primers and probe used for PPV detection

Primer name	Orientation	Sequence (5'→3')
PPV-f	forward	GGA GAC ACA AGT GGA GTA TCC AAT AAA
PPV-r	reverse	AAT GTA CGC TTC AGC CAC GTT A
PPV-probe	probe	FAM-CAC TTT TAG ACA AAT TAT GGC A-MGB

Assay specific for eukaryotic 18S rRNA (PDARS, Applied Biosystems, Foster City, USA) and TaqMan RNaseP Detection Reagents (Applied Biosystems, Foster City, USA) were used for confirmation of successful totRNA extraction and for detection of inhibitors in real-time PCR.

2.5 Real-time PCR (TaqMan) assay

The cDNA of each sample was used in separate real-time PCR reactions for detection of PPV and 18S rRNA.

The 20 µL real-time PCR reactions for PPV were performed in 1X TaqMan Universal Master Mix (Applied Biosystems, Foster City, USA), with 900 nM of each primer and 200 nM probe (Table 1) and 1/10 diluted cDNA as a template.

The 20 µL real-time PCR reactions for 18S rRNA were carried out according to the manufacturer's instructions with 1/10 diluted cDNA as a template.

Real-time PCR reactions were run in duplicates for each undiluted or diluted cDNA on ABI PRISM 7500 (Applied Biosystems, Foster City, USA) using universal cycling conditions (50ºC for 2 min, 95ºC for 10 min, 45 cycles of 95ºC for 15 s and 60ºC for 1 min). Data were acquired and analyzed using the ABI PRISM 7500 Real-Time PCR System Sequence Detection System Software v1.3.

cDNA from a non-infected plant of each *Prunus* species was used as a negative control. A PCR non-template control (NTC) was set up with molecular grade water instead of template.

10-fold serial dilutions of cDNA of each *Prunus* species (samples: apricot 2, damson 10, peach 2, plum 3) were used to obtain standard calibration curve for PPV. For the assessment of inter-run variability a standard calibration curve of three 10-fold serial dilutions of the same sample (apricot 2) was prepared on each of four different real-time PCR plates.

The 18S rRNA assay was used for confirmation of successful totRNA extraction. Additionally, assay for *RNase P* gene was used for detection of inhibitors in real-time PCR step. 10 ng/µL of control human DNA from TaqMan RNaseP Detection Reagents (Applied Biosystems, Foster City, USA) was added to each real-time PCR reaction as external standard of inhibition control.

2.6 Performance of real-time PCR

The ABI Prism 7500 Real-Time PCR System Sequence Detection Software v1.3 generated fluorescence data. For the comparability of the results, identical settings for threshold and baseline were used in all experiments for PPV detection. The baseline was established automatically and the threshold was set at 0.2. The dynamic range of PPV real-time PCR reactions was determined by performing 10-fold serial dilutions of each *Prunus* species template cDNA in water. Standard calibration curves were obtained by plotting cycle threshold (Ct) values against the log of the sample cDNA dilutions.

The real-time PCR efficiency was calculated by the following equation: PCR efficiency [E] = $10^{(1/-S)}$ (Pfaffl, 2001; Rasmussen, 2001).

3 RESULTS

3.1 Total RNA extraction

The quantity of extracted totRNA differed greatly between plant species. The quantity of totRNA from peach samples with Method 1 was high and uniform while the quantity with Method 2 was much lower and there was a big variation between samples (data not shown).

With other three *Prunus* species the used totRNA extraction methods were comparable but the quantity of extracted totRNA was low.

3.2 Real-time PCR (TaqMan) assay

All samples were tested for the presence of eukaryotic 18S rRNA and the results confirmed successful totRNA extraction.

Real-time PCR parameters were determined by amplifying PPV RNA from different *Prunus* species (Table 2). A comparison was made between Method 1 and Method 2. The average coefficients of determination (R^2) were between 0.987 and 0.999 indicating a good correlation between the amount of template and Ct values. Efficiencies and dynamic range of detections of the real-time PCR were assessed (Table

2). No significant differences were observed in efficiency between both isolation methods and between the tested species. Differences were observed in the dynamic range between both methods for plum and peach. The dynamic range with Method 2 was broader for plum but narrower for peach.

The amplification of 10-fold serial dilutions of selected samples of each species (apricot 2, damson 10, peach 2, plum 3) was performed. The Ct values of undiluted samples of peach, apricot and damson ranged between 31 and 39 while the Ct values of samples diluted 1/10 ranged between 18 and 23, what indicates a strong inhibition in undiluted samples using both methods. The strong inhibition of amplification was observed also for plum with Method 2 while the inhibition with Method 1 was weak.

The amplification of *RNase P* gene as external standard of inhibition control (control human DNA) was performed for all tested samples. The results indicate elimination of inhibition in 1/10 diluted samples of all species. Standard deviation (SD) between the different samples was constant and similar to positive control (control human DNA diluted in ddH2O) (Method 1: the average Ct is 27.33 ± 0.14 and Method 2: the average Ct is 27.07 ± 0.13).

Table 2: Comparison of dynamic ranges, efficiencies (E) and coefficients of determination (R^2) between both totRNA extraction methods and plant species

	Method 1			Method 2		
	Dynamic range	E	R^2	Dynamic range	E	R^2
plum	$5x10^{-1} – 10^{-4}$	2.08	0.987	$5x10^{-1} – 10^{-6}$	2.01	0.996
peach	$5x10^{-1} – 10^{-6}$	1.99	0.995	$5x10^{-1} – 10^{-4}$	2.06	0.994
apricot	$5x10^{-1} – 10^{-6}$	2.04	0.999	$5x10^{-1} – 10^{-6}$	1.95	0.995
damson	$5x10^{-1} – 10^{-6}$	1.97	0.999	$5x10^{-1} – 10^{-6}$	2.02	0.997

Most of tested samples were in linear dynamic range of specific species independently of the amount of totRNA used for reverse transcription reactions. All other samples were treated as negative (Fig. 1). Only four apricot samples were found negative with Method 2 and one of them also with Method 1.

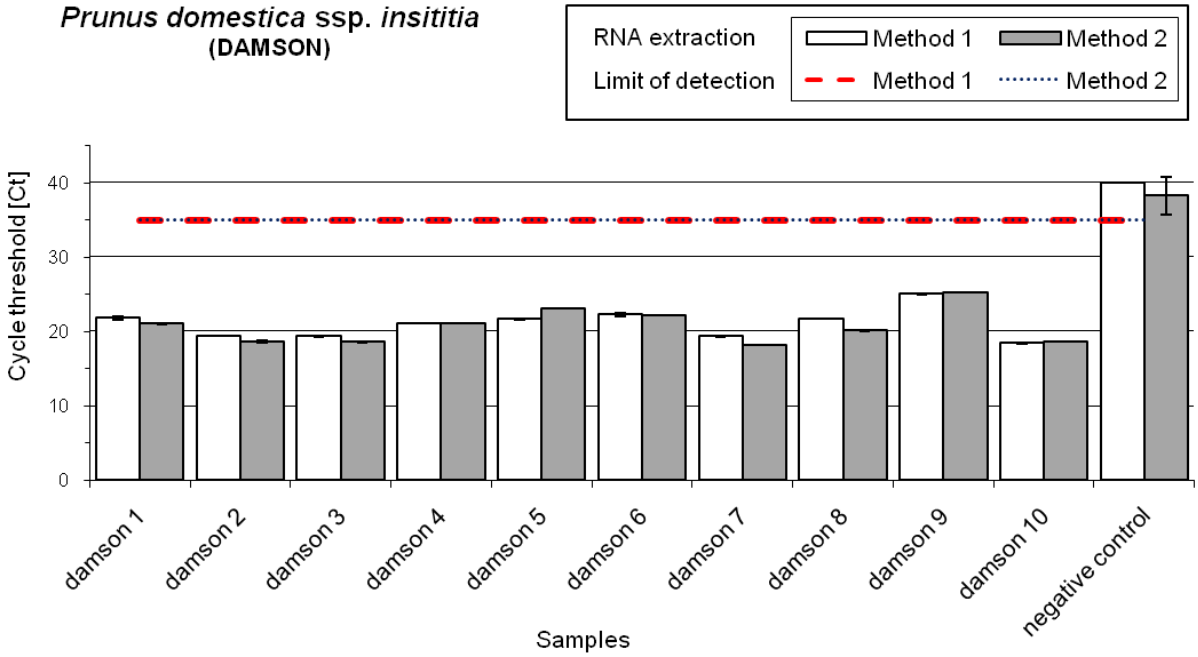
According to average Ct of tested samples, the totRNA extraction from plum with Method 1 was much more effective than the extraction with Method 2. The average difference in Ct between Method 1 and Method 2 for all 10 samples was 6.65 ± 3.79. The Method 1 performed better.

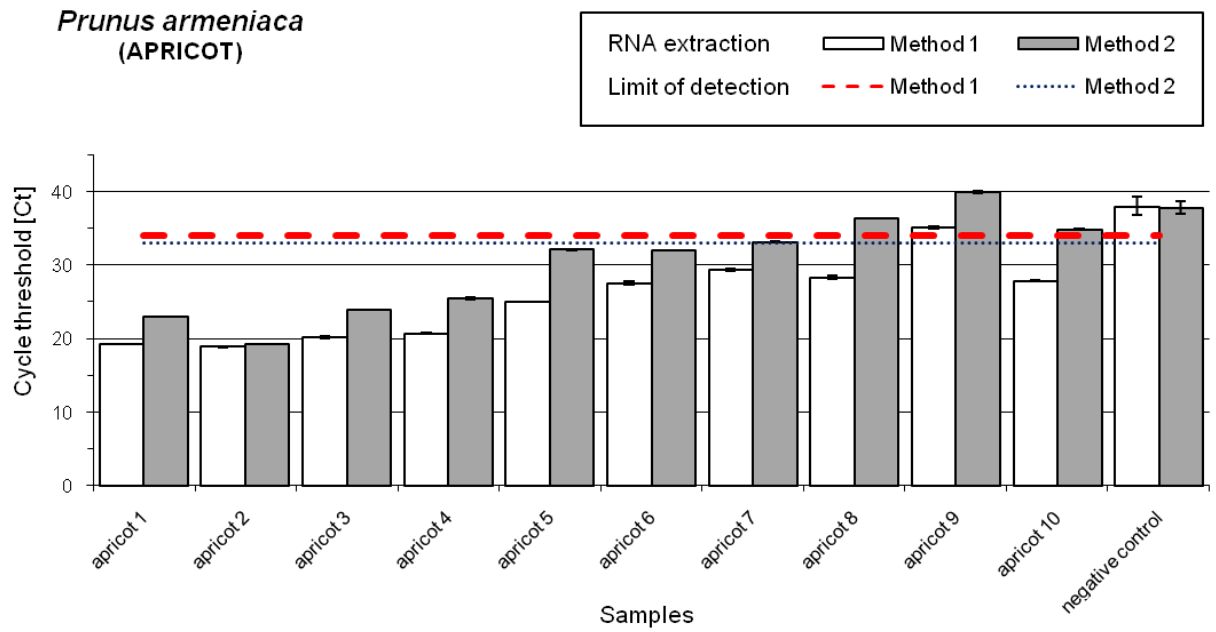
For damson we observed lower variability in average Ct compared to plum. The average difference in Ct between Method 1 and Method 2 for all damson samples was -0.34 ± 0.85. Both methods were comparable.

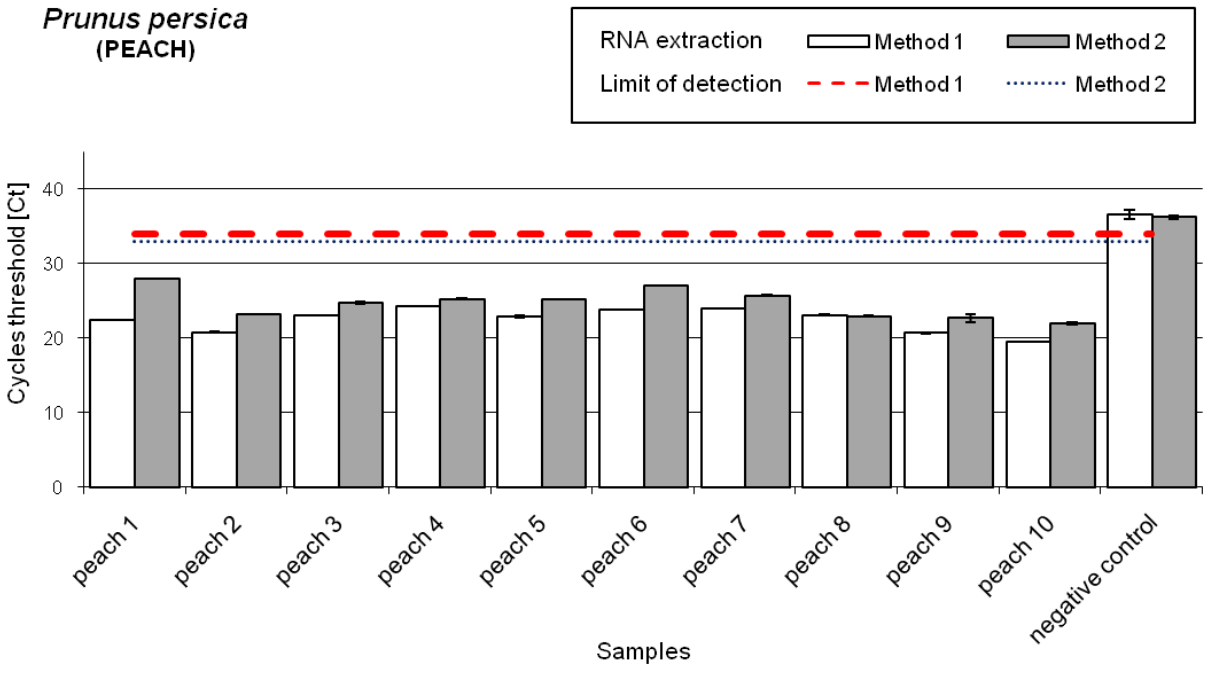
The average difference in Ct between Method 1 and Method 2 for all 10 samples of peach was 2.22 ± 1.48. The Method 1 performed better.

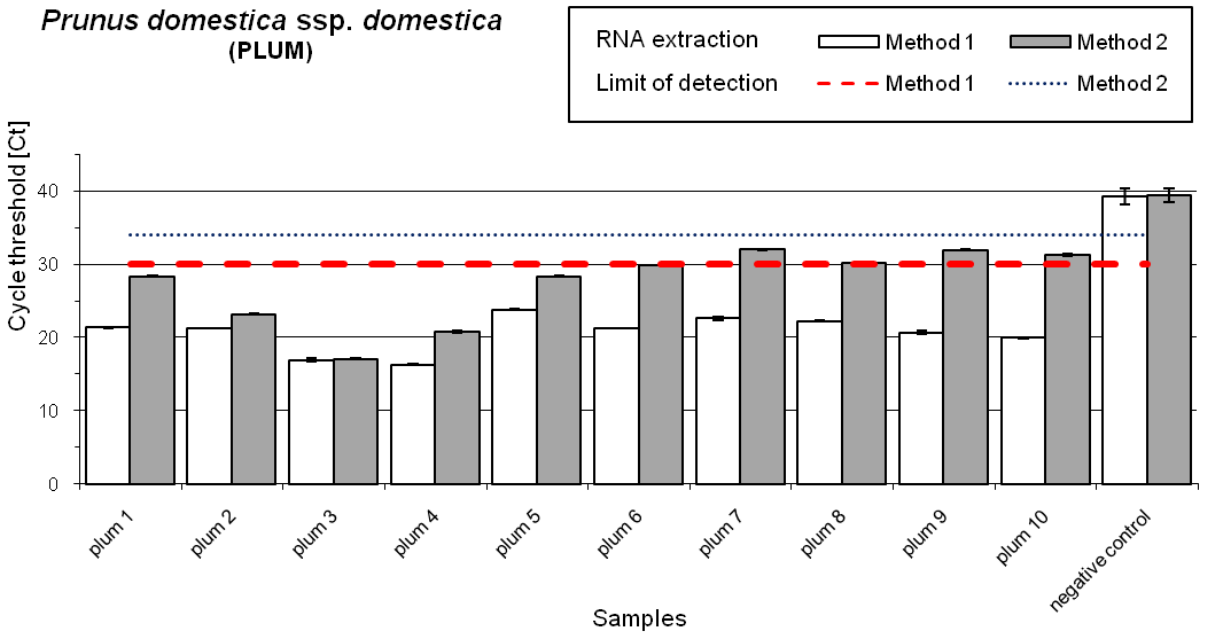
The average difference in Ct between Method 1 and Method 2 for positive apricot samples was 4.04 ± 2.20. The Method 1 performed better.

Figure 1: Real-time PCR cycle threshold (Ct) values for detection of PPV in samples of four different *Prunus* species extracted with two different totRNA extraction methods.









Samples with C_t values of less than marked Ct, indicating the detection limit for each totRNA extraction method and tested *Prunus* species, were considered as positive.

3.3 Inter-run variability

Inter-run variability was assessed by preparing the standard curves with three 10-fold serial dilutions of the same sample in four different plates. The quantification was linear over a range of all three log units. The average slope of all compared standard curves was – 3.355 ± 0.028, the efficiency was 1.986 ± 0.011 and the average R^2 was 0.997 ± 0.003 (data not shown). These results show that the conditions of amplification on all plates were stable and the comparison of the results from different plates was possible.

4 DISCUSSION

PCR based methods, including real-time PCR are widely used for the detection of plant viruses. The choice of nucleic acid extraction procedure can greatly influence the reliability of detection and quantification of target sample. It is important to validate the extraction procedure for different sample matrixes and the ability of the extraction method to provide suitable nucleic acid from each matrix. It is known that different matrixes can contain different substances that could affect the efficiency and reliability of real-time PCR (Cankar et al., 2006). Components that inhibit the amplification of nucleic acids by PCR based methods are present with the target nucleic acids from many sources and inhibitory effects may have important implications for clinical investigations, investigations in food and environmental screening (Wilson, 1997). The mechanism of inhibition is considered to be chelation of the Mg^{2+} cofactor which is important for Taq polymerase activity, or by binding to target DNA/RNA/Taq polymerase or by inhibition of Taq polymerase (Wilson, 1997; Mayr et al., 2005). Inhibitors can generate inaccurate quantitative results and high degree of inhibition can create false-negative results (Nolan et al., 2006).

The significant reduction in the sensitivity and kinetics of real-time PCR caused by inhibitory components frequently found in biological samples is well known (Rådström et al., 2004; Jiang et al., 2005).

Different *Prunus* species may have different concentration of secondary metabolites, similar to other plants like citrus, strawberry and blackberry (Borah et al., 2008; Wei et al., 2008) which can have inhibitory effects. In plant tissue we usually find polysaccharides and polyphenols as components with inhibitory effect on PCR (Demeke and Adams, 1992). Numerous extraction methods have been used in the preparation of totRNA from plant samples with a more or less sufficient quantity of isolated totRNA and reduction of inhibitors (Gambino et al., 2008; Singh et al., 2002; Maliyakal, 1992; Koonjul et al., 1999; Thompson et al., 2003). These approaches are often time-consuming but they are essential for reliable diagnostic work.

In our study one manual and one automated totRNA extraction method were compared in terms of elimination of potential PCR inhibitors. The column based method for totRNA extraction (e.g. RNeasy Plant Mini Kit) is easily adapted for high throughput processing, and can yield amounts of up to 10 µg of totRNA from small amount of starting material, which is sufficient for real-time PCR applications. Samples isolated by the column based method contain fewer impurities such as phenol compounds which are a particular problem with plant samples and have a significant influence on the efficiency of PCR amplification (Singh and Singh, 1996; Singh, 1998; Myslik and Nassuth, 2001). The use of RNeasy columns manually is time consuming but a higher quantity of totRNA is isolated. An automated alternative for manual extraction with RNeasy columns could be advanced technology, instrument called QIAcube (Qiagen) which we were not able to use during our experiments. Sample preparation on the QIAcube use RNeasy spin columns follows the same steps as the manual procedure and no change in purification chemistry is required.

The KingFisher instrument uses patented technology in which magnetic rod moves particles through purification process. The technology used is based on silica particles which bind totRNA in the presence of chaotropic salt. The use of KingFisher instrument is automated and fast, it saves time and money. The financial disadvantage is the initial investment into the instrument but it can be used for many different applications (for DNA, RNA and protein extraction) (Lassailly et al., 2007; Zhao et al., 2008; Josefsen et al., 2007).

For comparison of manual and automated totRNA extraction we developed a real-time PCR assay that can be used for detection of PPV in different *Prunus* species.

With both totRNA extraction methods real-time PCR assay for PPV detection was shown to be reproducible and linear amplification was achieved over a range of 4 or 5 orders of magnitude. The range depends on the extraction method and the *Prunus* species used.

The results of this study suggest that the column based totRNA extraction method is more effective for apricot, plum and peach. It is a manual method and more time is

needed for processing of the same amount of samples compared to automated method using KingFisher instrument. The differences between both methods were very small when analyzing damson. For the processing high number of samples in large scale detection of PPV the automated method such as KingFisher instrument is more appropriate. It saves time even if the amount of extracted totRNA is not as high as with manual method. According to our results we would recommend to use cDNA diluted 1/10 in real-time PCR of *Prunus* sp. In case of negative results obtained with automated method, manual column based extraction method should be used additionally.

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Economic growth and trend changes in wildlife hunting

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ABSTRACT

Petty-Clark's law suggests that as the economy of a country develops, its proportion of primary industries declines while those of its secondary and tertiary industries increase. Traditionally, hunting has played a crucial role in a country's food supply; however, currently, it is increasingly viewed as a leisure activity. This paper empirically examines whether Petty-Clark's law holds in case of hunting in European countries. The results reveal that the proportion of hunters across countries increases when the per capita GDP is between 5,000 and 15,000 USD. Once the per capita GDP crosses the 15,000 USD mark, two major trends are detected: the number of hunters continues to increase in some countries but decreases in some other countries. Finally, the number of hunters in a country stabilizes when its per capita GDP reaches around 25,000 to 30,000 USD.

Key words: hunting, economic growth. Petty-Clark's law

IZVLEČEK

GOSPODARSKA RAST IN SPREMEMBE PRI LOVU DIVJADI

Po Petty-Clarkovem zakonu se z razvojem gospodarstva manjša relativni delež primarne gospodarske dejavnosti, medtem ko se veča sekundarni in terciarni. V zgodovini je imel lov odločilno vlogo pri zagotavljanju prehrane prebivalstva, toda v novejšem času gledamo na lov čedalje bolj kot na aktivnost v prostem času. V tem članku je empirična raziskava če Petty-Clarkov zakon velja v primeru lova v evropskih državah. Ugotovljeno je, da se število lovcev veča v povezavi z večanjem bruto domačega proizvoda (BDP) na prebivalca v državi, v razponu 5.000 do 15.000 USD. Ko pa BDP na prebivalca preseže vrednost 15.000 USD, sta odkrita dva glavna trenda. V nekaterih državah se število lovcev še nadalje povečuje, v nekaterih drugih pa se zmanjšuje. Število lovcev postane stabilno ko BDP doseže okoli 25.000 do 30.000 USD.

Ključne besede: lov, ekonomska rast, Petty-Clarkov zakon

1 INTRODUCTION

Man has been hunting wild animals since prehistoric times. Since hunting began, human beings have both exploited and co-existed with wild animals, and this holds true even in the present day. Therefore, a decline in the hunting of wild animals by humans will naturally change the relationship between the two—the wild animal population may drastically increase and cause various serious imbalances in the ecosystem. The impact of an increase in the wild animal population on the ecosystem can be especially problematic when there is a decrease in the hunting of ungulates whose predators are now extinct. This is because the growth rate of ungulates is generally high, and at times, they graze so extensively on the vegetation in their region that it leads to a drastic decline in the same.

Although recent times have witnessed a decline in the hunting of wild animals in some countries, it remains prevalent in others. The reasons behind this prevalence could be one or more of the following: the tradition and culture of hunting that exist in these countries and which have been maintained over time, the continuing

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use of game meats in the traditional cuisine in these countries, or a shift in the role of hunting from a means of procuring food to a leisure activity. The hunting of wild animals may have declined in the countries where the above reasons do not hold good. If the movement against hunting gets stronger, there may be a decline in hunting activities (for example, Bambi syndrome is one such examples wherein people, especially those living in the city, advocate not to kill bambi, without considering the importance of deer population control; Banta, 2002; Takatsuki, 2006). In addition, we should note that the same change may bring about different results depending on the methods of hunting, tastes and/or difference in attitude of the inhabitants of the regions or countries. For example, an income increase in a certain region may result in a decline in the region's hunting activities because of the increased availability of staple foods. On the other hand, in another region, an income increase may result in an increase in hunting because it will enable the region's inhabitants to purchase advanced and expensive hunting gear—a factor that contributes to hunting effectiveness.

As mentioned above, the rise and decline in hunting depends on various factors, which suggests that Petty-Clark's law will not necessarily hold in the case of hunting. Petty-Clark's law is one of the most well-known empirical rules to suggest that as an economy develops, its proportion of primary industries declines while those of its secondary and tertiary industries increase. However, although hunting is considered to be a primary industry, it may not decline as an economy develops. The purpose of this paper is to analyse the current situation in Europe with regard to animal hunting. Data pertaining to Europe's GDP, hunting activities, and forests will be used in the analysis.

The motivations behind this investigation are as follows. First, it will enable us to ascertain whether Petty-Clark's law is applicable to hunting. Second, a rise or decline in hunting activity within a region may substantially influence the population size of game animals in the region, which, in turn, has a significant impact on the region. An examination of the reasons underlying rises and declines in hunting activity may enable us to determine a mechanism for influencing demand for game hunting, which could be helpful for game management.

The structure of this paper is as follows. In section 1, we provide a brief review of related existing researches; we also present a theory-based argument that Petty-Clark's law does not necessarily hold in the case of hunting. In section 2, to empirically show that Petty-Clark's law does not necessarily hold in the case of hunting, we use empirical data to graphically examine the relationships between per capita GDP and the number of hunters and between per capita GDP and the proportion of hunters across countries. In section 3, we discuss the reasons why Petty-Clark's law holds true in some cases but not in others. On the basis of our findings in sections 1 and 2, we expect the following result: the income elasticity of demand for the hunting of wild animals is greater than 1 in northern and eastern European countries, while it is less than 1 in middle, western, and southern European countries. The reasons for the difference in the income elasticity of demand can be attributed to the following points, all of which are related to differences in latitude: (1) differences in the body size of game animals, (2) differences in hygienic conditions, and (3) differences in forest areas and the abundance of game populations. In section 3, we examine these three points in detail and apply the ordinary least squares method and correlation analysis to statistically investigate the third point. The last section concludes the paper.

2 BACKGROUND

2.1. Previous researches

In this paper, we examine the relationship between hunting and economic development. The relationships between industry and economic development have been examined in several fields of economics. Some of the most well-known relationships are as follows: (1) the relationship between economic development and inequality in income distribution, known as the Kuznets curve; (2) the relationship between economic development and environmental quality, known as the environmental Kuznets curve; and (3) the relationship between economic development and a change in industrial structure, known as Petty-Clark's law. The idea behind Petty-Clark's law had first appeared in *Political Arithmetick* which was written by W. Petty in 1690. This fact was revealed in *The Conditions of Economic Progress* by C. Clark (1940) and confirmed by S.S. Kuznets in *Modern Economic Growth* (1966). The Kuznets curve was developed by Kuznets (1955), and it states that inequality of income distribution tends to rise in the early stages of economic development but then diminishes gradually; this relationship is often referred to as an inverted U-curve. Research on the environmental Kuznets curve was undertaken after the 1990s. It states that the quality of the environment

worsens in the early stages of economic development but then begins to improve gradually.

This paper examines the proposition that although hunting is a primary industry, Petty-Clark's law does not necessarily hold in the case of hunting. In general, the production of goods in the primary industry tends to show an inverted U-curve; however, we empirically demonstrate that this is not true in the case of hunting. We also show that this result is theoretically sound. To the best of our knowledge, there is no existing study that has examined this topic.

One of the reasons that Petty-Clark's law does not always hold in the case of hunting is that there are several types of hunting, and the type of hunting prevalent in a region depends on its level of economic development. Chardonnet et al. (2002) and Loveridge et al. (2006) classify hunting as subsistence hunting, commercial hunting, and sport hunting. Among these, only subsistence hunting is classifiable as a primary industry. As the level of economic development increases, subsistence hunting might decline and/or commercial hunting and sport hunting might increase. These changes may be influenced by regional differences in the body size of game animals, hygienic conditions, forest areas, or strength of protest campaigns. We will discuss these issues later.

2.2. Theoretical background

In this section, we theoretically examine the situations in which hunting activity declines as the economy develops. We trace this phenomenon in the context of hunting based on Egaitsu (2008). The following equation—obtained by modifying an equation of income elasticity of demand—holds true in the abovementioned context.

ratio of change in demand = income elasticity of demand × ratio of change in income (1)

Let us rewrite this relationship in the context of hunting. Suppose the ratio of change in the demand for wild animals, income elasticity of demand for wild animals, and GDP growth rate are denoted by WD_R, η_W, and GDP_R, respectively. Here, we substitute the ratio of change in income with GDP growth rate. Thus, we have the following equation.

$$WD_R = \eta_W \times GDP_R \tag{2}$$

Next, we consider an equation that describes the ratio of wild animal-related products in the GDP. Suppose the wild animal-related production value, ratio of wild animal-related products in the GDP, and GDP are denoted by WP, WP_R, and GDP, respectively. We then have the following equation.

$$WP = WP_R \times GDP \tag{3}$$

Let us denote the variations in WP, WP_R, and GDP by wp, wp_R, and gdp, respectively. Then, the ratio of change in Eq. (3) can be denoted as follows.

$$\frac{wp_R}{WP_R} = \frac{wp}{WP} - \frac{gdp}{GDP} - \frac{wp_R \cdot gdp}{WP_R \cdot GDP} \tag{4}$$

We substitute $\frac{gdp}{GDP}$ with GDP_R because $\frac{gdp}{GDP}$ is the ratio of change in the GDP. We ignore $\frac{wp_R \cdot gdp}{WP_R \cdot GDP}$ because of its small value. We further substitute the ratio of change in the wild animal-related production value $\frac{wp}{WP}$ with the ratio of change in demand for wild animals WD_R. Then, by substituting Eq. (2) into Eq. (4), we obtain the following result.

$$\varpi = [\eta_W - 1] \times GDP_R \tag{5}$$

where $\varpi = \frac{wp_R}{WP_R}$. It follows from Eq. (5) that if the income elasticity of demand for wild animals η_W is less than 1, the ratio of the wild animal-related product value in the GDP will decrease. This is the application of the Petty-Clark law for wild animal products based on Egaitsu (2008).

Several inter-related factors are indicative of the demand for wild animals, and we can select one or more of these factors to examine the said demand. Two major factors are (1) the hunting of wild animals as a primary source of food and (2) hunting for game meat or trophy hunting as a leisure activity. As stated in the introduction section of this paper, an increase of income in a particular region might reduce the demand for game meat in that region. In this case, since $\eta_W < 1$, ϖ will decrease. On the other hand, in another region, an increase in income might promote hunting as a leisure activity and lead to an increase in the hunting of wild animals. In the above case, since $\eta_W > 1$, ϖ will increase.

As seen above, we cannot predict increases or decreases in ϖ on the basis of theoretical analysis; rather, an empirical examination is required to make such a prediction. Moreover, empirical results are influenced by factors such as the purpose of hunting and the use of products derived from the hunted animals. In the following sections, we examine these topics in detail, particularly from an economic perspective.

In our analysis, the ratio of the change in WP_R (the left-hand side of eq. (5)) is substituted with the 'number of hunters' and the 'hunters' proportion in the population' in sections 1.1 and 1.2, respectively. This is because it is difficult to use the ratio of the change in WP_R because different (sub)species of game animals are hunted in different countries and valued at different standards. In addition, poaching activities degrade the accuracy of these data. The 'number of hunters' and the 'hunters' proportion in the population' may reflect hunting (including poaching) statistics more realistically, and can also be used as standardized data.

3 RELATIONSHIP BETWEEN PER CAPITA GDP AND HUNTING

3.1. Increases or decreases in the number of hunters

In this subsection, we examine the influence of increases in the per capita GDP of various countries on changes in the number of hunters in the countries. For this purpose, we use per capita GDP data for 2007 obtained from the 'World Economic Outlook' (WEO), which is an annual report published by the International Monetary Fund (Table 1). For data pertaining to changes in the number of hunters in various countries, we utilized Table III of Chardonnet et al. (2002, p. 27) and categorized the data by assigning the following values: increased (2), stable or slightly increased (1), stable (0), slightly decreased (–1), decreased (–2), and drastically decreased (–3). Chardonnet et al. (2002) provides data for 20 countries.

The results are illustrated in Figure 1. Chardonnet et al. (2002) states that the number of hunters decreased in the Latin countries of southern Europe but increased in the Scandinavian countries. We further have the following results. In the western and southern European countries, the population trend of hunters averages to less than –1, which suggests a decrease in the number of hunters. In the eastern and northern European countries, the average is greater than 1, which suggests an increase in the number of hunters. In the middle European countries, it is between –1 and 1, which suggests that the number of hunters has stabilized. In addition, the middle European countries generally tend to have high incomes as compared to the other European countries. This suggests that as the income of a country increases, the fluctuations in the number of hunters in the country reduces, and the number tends to stabilize.

3.2. Hunters' proportion of the population

In this subsection, we examine the influence of increases in per capita GDP on the proportion of hunters across countries. For the data of per capita GDP, we used 2007 WEO data. For data on the proportion of hunters across countries, we utilized the values of their *proportion of the population* in various countries given in Table III of Chardonnet et al. (2002, p. 27).

The results of the above analysis are shown in Figure 2. The proportion of hunters in a nation is low when the nation's per capita GDP is low. When the per capita GDP reaches a certain level, the proportion of hunters varies from nation to nation. To clarify this, we have

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used arrows in Figure 2 to indicate the changing direction in the number of hunters. Figure 2 shows that the proportion of hunters across nations demonstrates an upward trend when the per capita GDP is between 5,000 and 15,000 USD. However, once the per capita GDP crosses 15,000 USD, the countries exhibit two main trends: the number of hunters continues to increase in some countries but decreases in some other countries. Finally, the number of hunters appears to stabilize when the per capita GDP reaches around 25,000 to 30,000 USD.

Table 1. Data used in the analysis

Country	Per capita GDP of 2002	The proportion of hunters across countries	Number of hunters	Forest area	Latitude
Austria	25,801	0.0139	0	47.0%	47.52
Belgium	24,397	0.0029	0		50.50
Denmark	32,493	0.0345	1		56.26
Finland	26,145	0.0588	2	72.0%	61.92
France	24,449	0.0286	–2	27.9%	46.23
Germany	24,523	0.0040	1	30.7%	51.17
Greece	15,486	0.0286	–3	27.9%	39.07
Hungary	6,548	0.0049	2	19.9%	47.16
Ireland	31,394	0.0333	0		53.41
Italia	21,318	0.0167	–2	34.0%	41.87
Luxembourg	50,970	0.0063	0		49.82
Netherlands	27,207	0.0022	0		52.13
Norway	42,526	0.0400	0	28.9%	60.47
Poland	5,185	0.0026	2	29.7%	51.92
Portugal	12,349	0.0250	0		39.40
Slovenia	12,079	0.0119	0	55.0%	46.15
Spain	16,693	0.0256	–2		40.46
Sweden	27,326	0.0370	2	65.9%	60.13
Switzerland	38,659	0.0043	0	30.3%	46.82
United Kingdom	26,719	0.0172	0		55.38
Source	[1]	[2]	[3]	[4]	[5]

[1] IMF (2007) World Economic Outlook.
[2] 'Proportion in the population' in Table III (p. 27) of Chardonnet et al. (2002).
[3] The 'trends' in Table III (p. 27) in Chardonnet et al. (2002) are transformed to the following values: increased (2), stable or slightly increased (1), stable (0), slightly decreased (–1), decreased (–2), and drastically decreased (–3).
[4] Values in the 'Forest and Woodland' section of von Arx et al. (2004).
[5] Based on search results (country names were used for search) at the following website: http://www.benricho.org/chimei/get_LatLon/.

In the northern and eastern European countries, as an overall trend, the proportion of hunters across countries continues to increase and thereafter stabilizes at high GDP levels. In the middle, southern, and western European countries, the proportion of hunters across countries increases for a certain amount of time, but subsequently diminishes and stabilizes at low GDP levels.

4 DISCUSSION ON THE REASONS FOR OUR RESULTS

The theoretical and empirical results presented in sections 1 and 2 suggest that in the northern and eastern European countries, the income elasticity of demand for wild animals η_W is greater than 1 and the ratio of change in wild animal-related production ϖ is increasing while in the middle, western, and southern European countries, $\eta_W < 1$ and ϖ is decreasing. The reasons for these results may be as follows. (1) The northern and eastern European countries are located in relatively cool areas, and the wild animals in their forests have relatively larger body sizes than those in other European countries. (2) The post-hunt treatment of game animals is relatively easy with respect to the maintenance of hygienic safety. (3) The forest area in these countries is large such that there are abundant populations of game animals in the forests. In the following subsections, we examine each of these three points.

3.1. Differences in the body size of game animals

First, Bergmann's law—which states that the body sizes of homeothermic animals of the same species tend to be larger as latitude increases— supports the presence of relatively large animals in cool areas. Further, the purpose of hunting in a country has changed depending on its economic conditions, among other factors (Chardonnet et al., 2004; Loveridge et al., 2006). Irrespective of the purpose of hunting, hunters tend to seek larger game animals. Once the purpose of hunting changes from the acquisition of food to the pursuit of a leisure activity, those who live in areas where there is an abundance of larger game animals can easily begin considering hunting as a leisure activity, and such hunters will abound in these areas. On the other hand, hunters who live in areas where only small game animals are present have no choice but to hunt the small animals when they have limited economic means; however, when economic development takes place in these areas, some of the hunters might begin travelling to other areas where they can hunt larger game animals. However, the decline in the number of hunters may be greater in the areas with small game animals as compared to that in areas with large game animals. From this economic perspective, these phenomena can be explained as follows. The decrease in the number of hunters is greater in these areas because they obtain smaller benefits from hunting (e.g. because of the small size of the game animals). The hunters living in the areas with small game animals may travel to other areas; however, this translates into additional travel and accommodation costs and reduces the net benefits that accrue from hunting, resulting in an overall reduction in the number of hunters in the area.

3.2. Differences in hygienic conditions

It is a matter of common sense that in comparison with areas with a hot climate, areas with a cool climate are more conducive to the hygienic and safe treatment of the carcasses of game animals. The cool climate also helps the carcasses remain fresh for a longer period of time. The hunting of ungulates is often scheduled for the winter months, and in countries whose general climate is cool, the winters are cooler than those of warmer countries. A cooler winter climate leads to the maintenance of a low body temperature in carcasses, which results in a higher quality of game meat. In the warmer countries, on the other hand, it is necessary to carry the carcasses to a slaughterhouse as soon as possible in order to increase hygienic safety and maintain a quality of meat; otherwise, the meat may become rotten or unsafe to eat. However, hunters might not always find a slaughterhouse near the hunting site. Thus, it is clear that from the perspective of the efficient use of hunted game animals, warmer countries have certain disadvantages as compared to the cooler ones.

3.3. Difference in forest and woodland cover

Given the natural abundance of game animals in forests, it is expected that countries with greater forest areas will have more abundant populations of game animals. However, the forest area of a country may decrease in direct proportion to its economic development. In this subsection, we examine these relationships using the *Forest and Woodland Cover* data provided in von Arx et al. (2004) and data from the studies already mentioned.

A correlation analysis revealed that there was a statistically significant correlation at the 5% level—with r = 0.58 (n = 12, two-tailed test)—between the proportion of hunters across countries and the forest areas in the countries. On the other hand, we could not detect a statistically significant correlation even at the 10% level (r = 0.24, n = 11, two-tailed test) between per capita GDP and forest area. We also employed the ordinary least squares method with the proportion of hunters across countries as the explained variable and forest area and per capita GDP as the explanatory variables. The regression equation is as follows (t-values in parentheses)

$$\underset{}{Y} = \underset{(-0.92)}{-0.012} + \underset{(0.91)}{3.800 \times 10^{-7}}\, GDP + \underset{(2.36)}{0.067}\, FOREST$$

We found that forest area was statistically significant at the 5% level but the per capita GDP was not significant even at the 10% level with the adjusted $R^2 = 0.37$.

The lack of a statistically significant relationship between forest area and per capita GDP in our results might be explained by the small sample size and the fact that the forest area of a country is dependant on various other factors. In addition, the historical background of the use of the forests may also have been one of the factors for the above finding. On the other hand, we found that there was a statistically significant relationship between the proportion of hunters across countries and forest area. This finding supports our inference that the population size of the wild animals and hunters in a region is directly proportion to the forest area in the region.

Countries located at a high latitudes have larger forest areas. The correlation analysis shows that this hypothesis is statistically significant at the 10% level (r = 0.54, n = 12, two-tailed test). From the above-mentioned results, we can infer that as the latitude of a country increases, the forest area also increases, resulting in higher game animal populations and a higher proportion of hunters in the country as compared to other countries. However, as stated above, we could not detect any significant relationship between per capita GDP and forest area from our data.

5 CONCLUSIONS

It is well known that as the economy of a country develops, the proportion of its primary industries declines while those of its secondary and tertiary industries increase. This paper focuses on hunting, which is a primary industry, and empirically examines whether Petty-Clark's law holds in case of hunting in European countries. The results reveal that the proportion of hunters across countries increases when the per capita GDP is between 5,000 and 15,000 USD. Once the per capita GDP crosses the 15,000 USD mark, two major trends are detected: the number of hunters continues to increase in some countries but decreases in some other countries. Finally, the number of hunters in a country stabilizes when its per capita GDP reaches around 25,000 to 30,000 USD. However, the proportion of hunters in the population may differ across countries in which the number of hunters has stabilized, as illustrated in Fig. 2. In the northern and eastern European countries, as an overall trend, the proportion of hunters across countries stabilizes at high GDP levels. In the middle, southern, and western European countries, the proportion of hunters across countries stabilizes at low GDP levels.

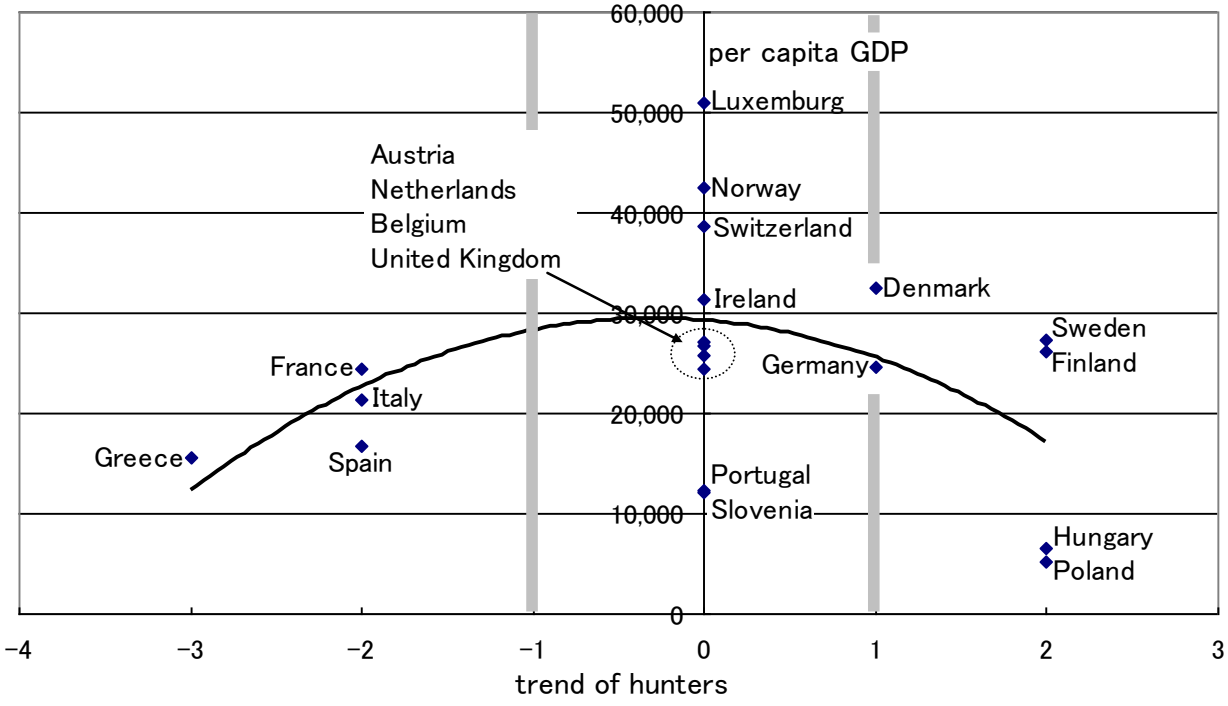

Fig. 1. Relationship between GDP and hunting trends

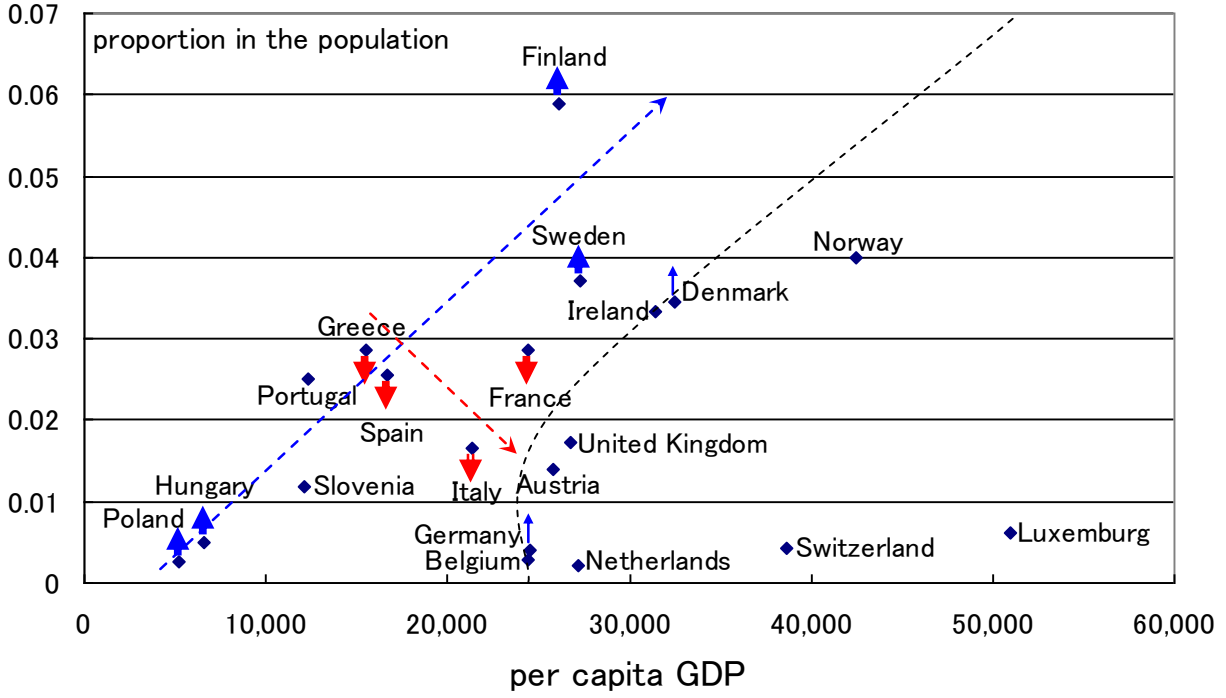

Fig. 2. Relationship between GDP and hunters

The reason for these results is that apart from being part of the primary industry of an economy, hunting also involves the aspect of leisure. Hunting as a leisure activity is more frequently observed in the following areas: areas where large-sized game animals are present, areas where the population sizes are large, and areas where the climate is sufficient cool to allow for relatively easy and hygienic treatment of carcasses. Our data show that the proportion of hunters is higher in the areas satisfying the above conditions.

It is natural that hunting activities decline in proportion to the development of industries. However, if the population of ungulates drastically increases in an area where hunting has declined, there is a significant possibility that the increase will lead to serious damage of the regional vegetation and/or agriculture, which would pose a substantive problem to the human population. Our results imply that the proportion of hunters in a country begins to stabilize when the per capita GDP reaches 25,000–35,000 USD.

An issue that has not been examined in this paper is whether—in countries where the proportion of hunters has stabilized at these per capita levels—the proportion of hunters is sufficient to manage the ungulate population and prevent damage to regional vegetation and/or agriculture. In addition, in the presence of strong movements against hunting activities, the number of hunters will decrease and stabilize at a low level, which may worsen the condition of the ungulates and their habitat. Future studies on these issues are required to understand them better.

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Agris category code: q04, f60

Inhibicija amilolitične aktivnosti mok z neoptimalnim padajočim številom

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IZVLEČEK

Študija proučuje vpliv dodatka ekstrakta nekaterih stročnic, ki naj bi vsebovale inhibitorje amilaz, na amilolitično aktivnost mok z nizkim padajočim številom (FN). Aktivnost amilaz je bila spremljana posredno z metodo določanja padajočega števila. Ugotovljen je bil optimalni čas vodne ekstrakcije. Raziskava je pokazala pozitivno povezavo dodatka moke stročnic z dvigom FN pšeničnih mok. Prikazane so povezave med različnimi koncentracijami ekstrakta in spremembo FN. Največji učinek je bil ugotovljen pri sorti fižolaTop Crop. Opravljena je bila peka kruha z dodatki različnih koncentracij moke fižola z največjim vplivom na padajoče število. Učinek dodatka moke fižola se je izrazil v manjšem volumnu pečenega kruha. Spremembe organoleptičnih lastnosti ni bilo zaznati pri 5 % dodatka, pri kruhih z dodatkom nad 2,5 odstotka je bilo zaznati daljšo svežino sredice.

Ključne besede: inhibitorji amilaz, fižol, padajoče število

ABSTRACT

AMYLOLYTIC ACTIVITY INHIBITION OF FLOURS WITH UNOPTIMAL FALLING NUMBER

The aim of this research was to discover the influence of supplementing pulses extract containing amylase inhibitors, on amylolytic activity of flour with low falling number. The amylase activity in flour was indirectly scaned with the falling number (FN) method. The optimal time of aqueous extraction was established. The research showed a positive correlation between pulses flour supplement and the rise of FN of wheat flour. Correlations between different concentrations of extract and change of FN are presented. The biggest effect on the FN was established with Top Crop beans. Bread-baking tests with different concentrations of bean flour that had the most pronunced effect of FN were made. The result of adding bean flour was a smaller volume of the bread. When adding 5% or less of bean flour, organoleptic characteristics did not change. The freshness of interior of the bread proved to be longer with breads containing more than 2.5% of the supplement.

Key words: amylase inhibitors, bean, falling number

1 UVOD

Na kvaliteto pšeničnega zrnja in posledično moke odločilno vplivajo vremenske razmere v sklepnem obdobju dozorevanja. Padavine z vmesnimi vročimi obdobji lahko povzročijo pričetek biokemijskih procesov, značilnih za kalitev zrnja. Spremembe je mogoče zaznati v sestavi in sicer v ključnih kvalitetnih parametrih, pomembnih za peko kruha. Pecilne lastnosti takih mok se lahko drastično poslabšajo. Razlog je v encimatski aktivnosti, zlasti beta-amilazni, ki ima za posledico razgrajevanje za peko pomembnih makromolekul škroba. Na aktivnost amilaz bi lahko vplivala tudi morebitna kontaminacija in rast gliv v deževnem obdobju (Kovač, 2010). Amilazno encimsko aktivnost ugotavljamo s pomočjo analize padajočega števila, ekvivalenten angleški izraz je falling number (FN) (Apling, 1993). Vrednost FN je obratno sorazmerna z aktivnostjo amilaz. Optimalne pecilne lastnosti lahko pričakujemo pri mokah, ki izkazujejo zmerno amilolitično aktivnost in se vrednost padajočega števila giblje med 220 in 250 enotami (Edwards, 2007).

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Moke z nizkimi vrednostmi padajočega števila, to je med 180 in 220 enot, so encimsko zelo aktivne in imajo preveč prostih sladkorjev. Zaradi povečane proteolitične aktivnosti, so lahko delno razgrajene tudi beljakovine. V kalečih žitih se namreč poleg amilolitične poveča tudi proteolitična aktivnost (Goesaert in sod., 2005). Kruh pečen iz take moke ima zaradi razgrajenih beljakovin lepka slabši volumen in packavo sredico. Pecilna vrednost moke z izrazito nizkim padajočim številom je lahko vprašljiva tudi zaradi preveč razgrajenih beljakovin. Visoka amilolitična aktivnost je torej lahko tudi indikator za razgrajene beljakovine (Lai in Lin, 2006). Pšenice in moke z vrednostjo padajočega števila, ki je manjša od 180, so v pekarstvu neuporabne. Take pšenice so primerne za animalno prehrano.

Pšenica ima lahko tudi prenizko amilolitično aktivnost, ki se kaže v visokem padajočem številu. Pecilne vrednosti takih mok lahko izboljšamo z dodatkom encimov amilaz v obliki sladne moke. Uporabljajo se tudi industrijsko pripravljeni dodatki, ki vsebujejo amilaze plesni in bakterij (Kovač in Raspor, 1994, Kovač, 2008). Mlinska industrija se zlasti ob slabih letinah sprašuje, kako bi moke z neoptimalnim padajočim številom, a še sprejemljivimi reološkimi lastnostmi uporabila. Pogoj je kvantitativno in kvalitativno ugodna vrednost lepka, torej nerazgrajenost beljakovin (Giorilli in Lauri, 2000). Neoptimalne moke bi bilo mogoče uporabiti v pekarstvu, če bi uspeli aktivnost amilaz zmanjšati z inhibitorji, literaturnih podatkov, kako to doseči ni na voljo. Nasprotno, veliko virov navaja in raziskuje inhibitorje α-amilaz v povezavi z inhibiranjem amilaz sline in trebušne slinavke s ciljem manjše izkoristljivosti škroba (Harry, 2009).

Semena in posledično moke stročnic vsebujejo inhibitorje amilaz (Grant in sod., 1995), ki vplivajo na zmanjšano delovanje le-teh. Inhibitorji amilaz so relativno termostabilne beljakovine, ki imajo inhibitorni učinek na amilaze trebušne slinavke in jih najdemo v vodnih ekstraktih stročnic, pšenice in rži. Zaradi visoke termostabilnosti je inhibitorno aktivnost mogoče zaznati tudi v pripravljenih živilih. Inhibitor amilaz postane aktiven šele po predhodni inkubaciji z encimom. Inhibitorji amilaz delujejo inhibitorno na amilaze sline in trebušne slinavke *in vitro* in *in vivo*. Povprečne količine inhibitorjev, ki jih zaužijemo s hrano so v primerjavi z izločenimi amilazami v prebavnem traktu premajhne za evidenten učinek. V sodobni farmaciji in prehrani se že uporabljajo tudi kot sredstva za uravnavanje prekomerne telesne teže in v zdravilih za bolnike s sladkorno boleznijo, iščejo se novi naravni inhibitorji različnih izvorov (Subramanian in sod., 2008). Prisotni so v različnih vrstah stročnic, v kidney fižolu jih najdemo v znatnih količinah, v grahu in leči jih je manj (Dilis in Trichopoulou, 2009). Inhibitorje amilaz najdemo tudi v ajdi (Ikeda in sod., 2004).

Na amilolitično aktivnost mok bi lahko vplivali z dodajanjem različnih kovinskih ionov (Umar Dahota in sod., 2004). Problematike mok z nizkim padajočim številom se loteva tudi Rudolf, (2009); v praktičnem delu diplomske naloge ugotavlja vpliv inhibitorjev amilaz nekaterih stročnic, zelenega boba, zelenega stročjega fižola, boba, fižola češnjevca, belega fižola in rdečega »kidney« fižola ter graha na moko z nizkim padajočim številom. V poskusih ugotovi, da ima med testiranimi vzorci največji inhibitorni učinek na delovanje amilaz rdeč fižol. Rezultati so pokazali, da je koncentracija inhibitorjev višja v vzorcih zrelih semen stročnic kot pa v njihovih nezrelih plodovih. Natančnega podatka o uporabljenih sortah stročnic v omenjenem delu ni. Dejstvo, da je bila najmočnejša inhibicija dosežena s fižolom, je izhodišče za to študijo.

Namen raziskave je ugotoviti učinek petih sort fižola na vrednost padajočega števila moke, ki je glede na amilazno aktivnost neoptimalna za peko. Ugotavljane so bile spremembe vrednosti FN kot posledica dodatka vodnega ekstrakt stročnice. V nadaljevanju raziskave so bili testirani vplivi dodatka na kakovost kruha, pripravljenega iz mok z nižjim FN od optimalnega. Spremljan je bil učinek na organoleptične lastnosti in volumen pečenega kruha.

2 MATERIALI IN METODE

Iz kakovostno neoptimalnih pšenic letine 2010, po poreklu iz Slovenije in Madžarske, so bile na laboratorijskem mlinu zmlete polnozrnate moke. Z mešanjem mok so bili pripravljeni vzorci s padajočim številom 160, 180, 206, 225 in 235. Določanje amilolitične aktivnosti in inhibitornega učinka dodatka vodnega ekstrakta testiranih stročnic je potekalo z napravo »Falling Number« po standizirani metodi ICC 107/1 (AACC 56-81B, ISO/DIS 3093). Metoda je zasnovana na hitri zaklejitvi škroba iz suspenzije moke ter merjenju razgradnje škroba z amilazami. Ta se odraža v zmanjšanju viskoznosti suspenzije, ki je v korelaciji s časom padanja mešala viskozimetra med dvema točkama.

V testih so bile uporabljene naslednje sorte fižola:

- -Starozagorski, Semenarna Ljubljana, lot 1A0016, 15. 10. 2012,
- Anellino verde, Semenarna Ljubljana, lot N24/850-ML, 15. 10. 2011,
- Bergold, Semenarna Ljubljana, lot D:02298/112-OC, 15. 10. 2012,

- Top Crop, Semenarn, Ljubljana, lot 0608/150-OC, 15. 10. 2012,
- Češnjevec, Hrib d.o.o., lot 180810, 16. 08. 2011.

Vzorci so bili zmleti na laboratorijskem mlinu Perten Laboratory Mill 3100. Ekstrakti so bili pridobljeni s suspendiranjem mletih vzorcev v destilirani vodi 60 minut z mešanjem na magnetnem mešalu pri temperaturi 30 stopinj C. Postopek priprave kruha je potekal na aparatu za hitro pripravo kruha po naslednjem hodogramu: mesenje testa pri počasni hitrosti 5 min, mesenje testa pri hitri hitrosti 15 min, počivanje testa10 min, mesenje 2 minuti, vzhajanje 45 minut in peka pri avtomatski nastavljeni temperaturi. Receptura testa za pšenični kruh je bila naslednja: 500 g moke s testiranim padajočim številom, proizvajalec Mlinotest d.d. lot 15. 3. 2012, 270 ml vode, 7 g soli, 15 g kvasa, proizvajalec Kvasac d.d. Zagreb, lot 30. 4. 2011. Beli pšenični kruh z 1, 2.5, 5, in 10% fižolove moke, je imel za dodani odstotek fižola zmanjšano količino pšenične moke.

Volumen je bil določen šest ur po peki kruha. Določanje volumna je bilo izvedeno z merjenjem volumna izpodrinjene tekočine po potopitvi. Kruh se je organoleptično ocenilo 24 in 48 ur ter 7 dni po peki. Kruh je bil hranjen v PE/PP vrečki za pakiranje kruha s perforacijo 100 luknjic/kvadratni decimeter.

3 REZULTATI

V prvem testu je bila proučevana sprememba padajočega števila preiskovanega vzorca polnozrnate moke s padajočim številom 206 ob dodatku vodnega ekstrakta različnih stročnic. Koncentracija fižola v vodnem ekstraktu je bila uravnana tako, da je bil dodatek med 1-15 % moke fižola/na testirano količino moke. Rezultati so podani v preglednici 1.

Preglednica 1: Odvisnost spremembe FN od koncentracije dodatka ekstrakta različnih mok stročnic.

Table 1: Correlation between variation of FN and concentration of the extract of different types of pulses flour supplement.

vrsta fižola/ bean types	padajoče število/Falling Number					
	% dodatka fižola /bean supplement					
	0	1	2,5	5	10	15
Starozagorski	206	206	210	115	220	220
Anellino verde	206	202	210	217	223	225
Bergold	206	204	208	210	230	227
Top Crop	206	204	212	216	235	235
Češnjevec	206	205	208	213	210	215

Iz preglednice je razvidno, da so vsi vzorci izkazovali dvig FN testirane moke z začetno vrednostjo 206 enot. Pri nekaterih meritvah je bilo pri 1% dodatku zaznati rahel padec FN, razlog bi lahko bil v interakciji med sestavinami filtrata, ki je vseboval ekstrakt fižola s sestavinami moke. Porast FN se stabilizira pri povprečno 10 % dodatka. Največja inhibicija amilaz, torej porast FN, je zaznati pri sorti Top Crop, ekstrakt te sorte je bil uporabljen v nadaljnih poskusih. Testiran je bil učinek 10% dodatka na inhibicijo, če je začetni FN moke variabilen. Rezultati so prikazani v Preglednici 2.

Preglednica 2: Odvisnost spremembe FN ob 10 % dodatku ekstrakta sorte Top Crop glede na začetno vrednost FN.

Table 2: Correlation between variation of FN when adding 10% Top Crop extract and initial FN value of the tested flour.

Padajoče število/Falling number		
začetna vrednost /initial value	z dodatkom/ with supplement	sprememba/change
160	190	30
180	205	25
206	236	30
225	259	34
233	258	35

Iz rezultatov je razvidno, da je pri mokah z višjo začetno vrednostjo ob dodatku 10% ekstrakta vrste Top Crop sprememba padajočega števila večja. Za optimalne lastnosti pečenega kruha vrednost padajočega števila naj ne bi bila manjša od 235. Glede na rezultate bi lahko sklepali, da padajoče število najslabše moke, ki bi lahko še izboljšali z dodatkom, ne sme znašati manj kot 200 enot. Moko s tako vrednostjo smo tudi pripravili in spekli kruh v modelih v obliki štručk. Pečenim izdelkom je bil določen volumen, višina štručke, ocenjeni so bili morebitni priokusi po stročnicah. Rezultati meritev volumna in višine kruhov z vsebnostjo moke fižola Top Crop so podani v preglednici 3. Kruhi iz moke s FN 206 so bili spečeni z 1, 2,5, 5 in 10% dodatkom fižolove moke. Kontrolni kruh je bil pečen samo iz pšenične moke. Pri zamesu testa z 5 in 10% fižolove moke je bilo potrebno korigirati dodatek vode za 20 in 40ml vode zaradi izrazito suhega testa.

Preglednica 3: Volumen in višina kruhov iz moke s FN vrednostjo 206 v odvisnosti od dodatka fižolove moke.

Table 3: Volume and height of bread made of flour with 206 FN value in correlation with bean flour supplement.

dodatek/supplement (%)	volumen (ml)/volume (ml)	višina/height (cm)
0	2500	11,7
1	2340	10,1
2,5	2270	11
5	2200	10,3
10	2105	10,2

Z namenom ugotoviti morebitne stranske učinke dodatkov fižolove moke smo spekli tudi kruh iz moke z optimalno vrednostjo padajočega števila. Rezultati so podani v preglednici 4.

Preglednica 4: Volumen in višina kruhov iz moke s FN vrednostjo 250 v odvisnosti od dodatka fižolove moke.

Table 4: Volume and height of bread made of flour with 250 FN value in correlation with bean flour supplement.

dodatek/supplement (%)	Volumen (ml)/ volume (ml)	višina/height (cm)
0	2700	12,4
1	2640	12,0
2,5	2502	11,5
5	2340	10,7
10	2300	10,5

Iz rezultatov v preglednicah 3 in 4 ugotovimo, da ima dodatek fižola negativen vpliv na volumen kruha. Podoben učinek zasledimo pri obeh testiranih mokah, volumen je slabši pri moki z nižjim padajočim številom. Zmanjšanje volumna je v korelaciji z količino dodatka fižolove moke. Vzrokov za nižji volumen bi lahko bilo več. Delno je za padec volumna krivo dejstvo, da je v kruhih z deležem fižolove moke manj pšenične moke in s tem lepka, ki omogoča večji volumen. Delno bi lahko pripomoglo k zmanjšanju volumna več vezane vode pri zamesu testa. Zmanjšanje volumna je preveliko, da bi ga lahko pripisali samo slednjima dejstvoma. Mogoča je razlaga, da komponente fižolove moke lahko negativno vplivajo na kvasovke in zavirajo njihovo delovanje. Pričakovan rezultat je bil namreč drugačen, dvig FN z dodatkom moke fižola naj bi tudi vplival na povečanje volumna kruhov.

Kruhi iz moke s FN 206 z dodatkom fižola niso imeli vidnih napak. Pri kontrolnem kruhu, pripravljenemu iz same pšenične moke pa je bilo na dotik čutiti, da je sredica malenkost lepljiva. To je potrdil tudi organoleptični test, predvsem teksture in občutka v ustih. Razlika je bila očitna ob vzporednem poskušanju kruha iz optimalne moke. Lepljivost je pričakovana napaka kruha, ki je spečen iz moke s prenizkim padajočim številom. Kruhi s fižolovo moko pa so bili za razliko od zgoraj omenjenega tudi po teksturi brez napak.

Kruhi iz moke s FN 250 niso imeli vidnih napak, njihova sredica pa prav tako ni bila packasta ne na otip in na občutek v ustih. Kruhi so bili brez napak verjetno tudi zato, ker je bil FN moke optimalne vrednosti za peko. Kruhi z dodatkom fižolove moke so bili bolj sveži, potreba po večjem dodatku vode se je pokazala že

pri zamesu testa. Večji vezavi vode gre pripisati tudi boljšo svežino kruha.

Poleg packavosti, ki je služila kot test za preverjanje prenizke vrednosti FN, je bilo pri vseh kruhih, v katerih je bil delež moke fižola, zanimivo to, da so bili celo po 5 dneh hrambe v perforiranih plastičnih vrečkah na sobni temperaturi skoraj povsem sveži. Medtem pa je bil kruh, pečen samo iz pšenične moke skoraj povsem izsušen in trd. Vsekakor dodatek fižolove moke prispeva k svežini kruha, saj je fižol sposoben vezati več vode kot pa sama pšenična moka. V majhni koncentraciji dodatka, to je do 5 %, ni bilo zaznati priokusa fižola.

Glede na rezultate raziskave lahko zaključimo, da so v testiranih sortah prisotni inhibitorji amilaz, to dokazujejo porasti FN moke ob dodatku vodnega ekstrakta fižola. Rešitev izboljšanja FN slabših mok z dodatkom moke fižola je vsekakor zanimiva, je pa res, da je odprtih še nekaj vprašanj, da bi lahko ta način prenesli v prakso. Ob dvigu padajočega števila moke s pomočjo inhibitorja amilaz ne smemo pozabiti na to, da ima moka z nizkim FN tudi druge slabše lastnosti, ki prav tako pomembno vplivajo na končen rezultat in z njim na izdelek. Pri peki kruha z dodano moko fižola je bila ugotovljena negativna korelacija z volumnom kruha. Razlog za manjši volumen bi lahko bil v inhibiciji kvasovk, kar bi pa vsekakor morali še potrditi. Kompleksnost problema ne onemogoča enostavne uporabe izsledkov raziskave v praksi. Volumen kruha je vsekakor za potrošnika zelo pomembna lastnost. Kljub vsemu pa so rezultati raziskave kot taki lahko tudi tržno zanimivi, kruhi z dodatki fižola so imeli v primerjavi z običanimi kruhi izrazito daljšo svežino.

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Pregled potvorb živil skozi zgodovino – dejanja slučaja, nuje ali namere?

Vida ŠKRABANJA[1)]

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IZVLEČEK

Sodobni potrošnik se želi prehranjevati čim bolj zdravo in kakovostno, vendar zaradi intenzivnega tempa življenja živil skoraj ne prideluje več sam, temveč izbira tista, ki mu jih nudi trg. Odgovornost za zagotavljanje zdrave in varne hrane je s tem v veliki meri prenesena na živilsko industrijo, ki pa do potrošnika ni vedno lojalna. Okoriščanje zaradi potvorb hrane ni nov pojav ali značilnost sodobne družbe, je neustavljiva skušnjava, ki sega daleč v preteklost. V članku so izpostavljeni primeri potvarjanja živil skozi zgodovinska obdobja kot tudi vzroki, obseg in posledice zlorab potrošnikove odvisnosti od tržnega prehranskega sistema.

Ključne besede: hrana, potvorbe, živila

ABSTRACT

HISTORICAL OVERVIEW OF FOOD ADULTERATION – ACT OF ACCIDENTS, NEEDFULNESS OR INTENTION?

Availability of healthy, nutritious and safe foods should be considered as fundamental and contemporary consumers would accept this as their granted right. The intensive lifestyle enables them to use own food sources very rarely. Instead, they are forced to choose products being available on the market. Responsibility for ensuring healthy and safe food is thus largely transferred to the food industry, but its offer to the consumer is not always fair and honest. Benefits stemming from the supply of adulterated foods are not a new phenomenon or a characteristic of modern society; it is irresistible temptation that reaches far into the past. The article highlights examples of food adulterations through the history as well as the causes, extent and consequences of abuse of consumer's dependence on food marketing system.

Key words: food, adulteration, food products

1 UVOD

Hrana je osnovni predpogoj za preživetje človeka, zagotavlja mu socialno in ekonomsko blaginjo ter omogoča njegov razvoj. Zaradi njenega ključnega pomena sta skrb in strah v zvezi s hrano ves čas prisotna, le da se vzrok, obseg in vpliv po regijah in obdobjih močno razlikujejo (Scholliers, 2008).

Dobiček, pridobljen protizakonito s potvorbami, ponaredki živil in napačnimi predstavitvami le-teh, je neustavljiva skušnjava, ki sega daleč v preteklost in jo zasledimo v vseh časih, pri vseh družbah. Niso vsi strupi v hrani goljufija: nekateri so prisotni po povsem nesrečnem naključju. Pri sami goljufiji (namerni nedovoljeni spremembi kakovosti živila) je od samega kvara živila hujša namera, s katero je hrana potvorjena (Wilson, 2008).

Barva živil je bistvena lastnost, ki določa, kako hrano okušamo in sprejemamo. Arheologi trdijo, da so se živilska barvila pojavila že leta 1500 pr.n.št.. Žafran je kot barvilo omenjen v Homerjevi Iliadi, Plinij starejši omenja umetno barvanje vina 400 let pr.n.št. Uporabljene so bile le tiste barve, ki so jih ljudje brez težav pridobili v naravi: iz že omenjenega žafrana,

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sipinega črnila, paprike, kurkume, pese ali cvetnih listov različnih cvetov (Burrows, 2009).

V Indiji je 300 let pr.n.št. zakon že prepovedal prevare pri žitih ter potvorbe jedilnih maščob, dišav in zdravil (Ottaway, 2003). V Egiptu so s predpisi hoteli zaščititi meso pred kvarom, Atene so imele javnega inšpektorja za vina, razne primesi v hrani in potvorbe pa so značilne tudi pri Rimljanih (Tsimidou in Boskou, 2003).

Iz pisem stare zaveze razberemo prepoved uživanja mesa živali, ki so umrle drugačne smrti, kot je posledica zakola. Predpisane so bile teže in mere živil in ostalega blaga. Pravila in predpisi so prav tako znani iz kitajskih, hindujskih, grških in rimskih zapisov. Rimljani so imeli zagotovljen nadzor nad dobavo živil in -po dostopnih virih - tudi zaščito potrošnika pred slabo kakovostjo in potvorbami. Pravila v zvezi s prodajo živil so bila detajlno razdelana. Primeri iz 1. stol. opisujejo ponarejanje oljčnega olja s produkti, predelanimi iz lesa, listov in plodov dreves, ter vina s snovmi različnih drugih rastlin. Ta oblika nadzora nad živili naj bi se v Rimu obdržala do konca 7. stoletja.

V preteklosti je prevladovala predvsem skrb v zvezi s pomanjkanjem hrane, z revnejšo hranilno vrednostjo, okuženostjo, pa tudi že z goljufijami, kot so odstopanja v teži ali zamenjave sestavin hrane, lahko tudi s strupi. Čeprav je večina prodajalcev ravnala pošteno, se je zgodaj pokazala potreba po zakonih za zaščito potrošnikov kot tudi poštenih prodajalcev pred tistimi, ki niso sledili sprejetim predpisom dobre prakse. Čim težje je bilo priti do hrane ali do ostalega blaga, več je bilo goljufij v zvezi z živili, kot so kruh, ribe, mleko, vino, pivo in ostalo.

V srednjem veku so se v nekaterih družbah Evrope (Anglija, Francija) oblikovali trgovski cehi, ki so imeli močan vpliv na zakonodajo blagovnega prometa. Namen le-teh je bil zagotavljanje kontrole in splošnega pregleda nad poštenostjo oziroma popolnostjo njihovih članov ter nad kvaliteto njihovih izdelkov. Med živili z najbolj natančno definiranimi postopki izdelave je bil kruh. Znan je anglo-saksonski zakon, razglašen v Angliji v 13. stol., ki predpisuje hude kazni za peke, ki izdelujejo slabši ali lažji kruh od predpisanih meril.

Kruh, ki so ga peki prodajali pred industrijsko revolucijo, je bil relativno neoporečen, pripravljen iz moke, dodatka kvasa ali kislega testa za fermentacijo, soli in vode. Zaslediti je bilo kvečjemu prisotnost pepela in peska kot tudi primes mletega starega kruha k svežemu testu. Pomanjkanje hrane in lakota v preteklosti pa sta povečevala delež nadomestnih surovin in dodatkov za kruh, ki so lahko povzročali tudi neželene učinke, škodljive za zdravje (primer: dodatek omotne ljulke je povzročal podobne učinke kot opiati). V Rusiji so bili uporabljeni naslednji dodatki, kot so: slama, lubje breze in bresta, luske ajde, metlika/ščir (amarant), želod, slad, otrobi, krompir, krompirjevi listi, leča, lipovo listje, gozdna krebuljica (Smith in Christian, 1984).

Naravna barvila so bila težko dostopna, draga in namenjena le za višji sloj. V renesančni Evropi so barvo živil povezovali s prehransko vrednostjo in pripadajočo zdravilno močjo. Prestiž belega kruha je dolgo znan in s tega vidika zelo primeren za potvarjanje (Burrows, 2009). Da bi peki popravili barvo in poroznost kruha, so moki dodajali galun: kalijev (ali natrijev) aluminijev sulfat, ki so ga od nekdaj uporabljali za strojilo kož. V letih 1757-1758, ko je bila letina britanske pšenice še posebej slaba, izbruhne eden večjih škandalov v zgodovini pekarstva. Količina dodanega galuna je takrat presegla vse meje in zdravniki so pri vladi ostro nastopili proti lakomnim pekom, ki (po njihovem mnenju) povzročajo več škode kot najhujše naravne katastrofe (Wilson, 2008). Leta 1857 je Snow objavil hipotezo o pojavnosti rahitisa pri otrocih zaradi uživanja kruha z dodatkom galuna (Snow, 1857 in odzivi na članek: Hardy, 2003; Dunnigan, 2003; Paneth, 2003). Analitske metode, če bi jih takrat izvajali, bi to hipotezo lahko potrdile ali ovrgle, prav tako tudi dodajanje nekaterih drugih strupenih dodatkov za povečanje teže kruha (kreda, apno, mlete kosti), za katere so krivili peke.

Zgodnejše razglase, ki so prepovedovali barvanje masla (1396) in peciva, s katerim so simulirali dodatek jajc (1574), zasledimo tudi v Franciji. Goljufi, ki so potvarjali živila, so kljub posledicam svojih dejanj podobne odredbe zaradi velikega zaslužka redko upoštevali (Burrows, 2009). Kazni so bile v času Ludvika XI. (1461-1483) še posebej ostre: za redčenje mleka so kršitelju v grlo vstavili lij in vanj vlivali razredčeno mleko toliko časa, dokler po zdravnikovem ali padarjevem mnenju požiranje ni bilo več možno brez nevarnosti (Lásztity s sod., 2004; Wilson, 2008).
Nemški kemik Frederick Accum, ki se je izpopolnjeval v prestižnih angleških ustanovah in v Londonu tudi deloval, je pomembno prispeval k osveščanju angleške in širše javnosti glede pristnosti živil. Leta 1820 je izdal knjigo *A treatise on adulteration of food, and culinary poisons, exhibiting the fraudulent sophistications of bread, beer, wine, spirituous liquors, tea, oil, pickles, and other articles employed in domestic economy. And methods of detecting them* (Accum, 1820) in resno opozoril na problem potvarjanja živil, po drugi strani pa si je nabral veliko sovražnikov, tako, da sam potrditve o prepoznavnosti in pomembnosti svojega dela ni dobil (Collins, 2007; Wilson, 2008).

Accum (1820) je opisal številne primere takratne nedovoljene predelave živil iz koristoljubja prodajalcev

ali posrednikov. Poleg beljenja kruha avtor opozarja na barvanje sladkorčkov, ki so jih na ulicah prodajali v raznih oblikah, z živosrebrovim sulfidom (rdeč pigment), s primesjo rdečega svinca ali z bakrom. Za intenzivno zeleno obarvano vloženo zelenjavo so ljudje uporabljali postopke kuhanja v bakrenih posodah (Accum, 1820). V tem primeru niti ne moremo govoriti o sleparstvu, saj so bila navodila za ohranjanje zelene barve zapisana celo v tedanjih kuharskih knjigah (Tsimidou in Boskou, 2003). Okus po oreščkih je namesto z dražjimi mandlji možno nadomestiti z lovorikovcem (*Prunus laurocerasus*), ki so ga dodajali v mleko, jajčne kreme, pudinge, smetano. Sir so barvali z anato barvilom, na katerega so nekateri ljudje sicer alergični, vendar ta ni smrtonosen, kot je lahko nadomestni rdeči svinec. Iznajdljivi izdelovalci likerjev so uporabljali ekstrakt anamirte (indijanske jagode; *Anamirta paniculata*) in ga dodajali tudi v pivo, da so pri pivcih dosegli močnejšo opitost. Popru so pogosto dodajali cenejše primesi – med drugimi tudi prah, potvorjena je bila čokolada, oljčnemu olju je bilo včasih primešano cenejše olje makovih semen, kavi cikorija, h kislemu okusu limonade je namesto limone pripomogla vinska kislina, h gostoti smetane pa namesto mlečne maščobe dodatek riževe moke. Premeteni trgovci so z raznimi triki obdelovali rastlinske liste (npr. trnulje ali gloga) toliko časa, da so dosegli podobnost s kitajskim zelenim čajem, pri čemer ni šlo samo za namerno zamenjavo listov, temveč tudi za povzročeno dodatno toksičnost končnega izdelka z barvanjem listov z bakrovim acetatom (Accum, 1820).

Kvalitetno vino je rezultat kompleksnega sožitja med človekom in okoljem. Napake in posledično popravljanje okusa vina sega daleč v preteklost (Charters, 2006; Wilson, 2008). Nekateri dodatki, kot so med, timijan ali rožmarin, cvetni listi, cimet, ki so jih uporabljali Grki, so bili neškodljivi. V teh primerih je težko ločiti, ali gre za ponarejanje vina ali za kulinarično inventivnost. Drugače je bilo z dodajanjem zdravju škodljivih konzervansov, ki so jih zaradi hitrega kvara vina pridelovalci dodajali vinu. Poleg morske vode je bil najpogosteje uporabljen svinec, strup, ki povzroča glavobol, utrujenost, bolečine v želodcu, v večjih količinah pa tudi gluhost, slepoto, izgubo govora, paralizo, piktonsko koliko, lahko tudi smrt (Eisinger, 1982). Nekateri zgodovinarji trdijo, da je bilo zaradi zaužitih količin svinca v vinu veliko število Rimljanov sterilnih (Gilfillan, 1965; Lessler, 1988). V 19. stol. so vinarji iz Sicilije vinu pogosto dodajali kredo, tako vino je bilo tako običajno, da so bili tuji potrošniki pripravljeni plačati več za nepotvorjeno vino, torej višjo ceno za nekaj, kar bi moralo biti standard (Loubère, 1978).

V ruralnem okolju so bile potvorbe živil tvegano dejanje, v večjih mestih pa je pot živila od pridelovalca do potrošnika dolga in večinoma nesledljiva. Posledice industrijske revolucije so bile med drugimi tudi, da prebivalci mest niso mogli več pridelovati lastne hrane, temveč so bili odvisni od drugih (Lásztity s sod., 2004). Možnosti in priložnosti za ponarejanje hrane so bile v tem okolju veliko večje. Pomanjkanje in večje potrebe po hrani so bile vzrok, da je razsežnost ponarejanja hrane v prvi polovici 19. stoletja dosegla višek, kar je pritegnilo širšo pozornost in sprožilo vrsto ukrepov kot npr. opuščanje starih predpisanih metod kontrole živil, intenzivnejšo tekmovalnost v tedanji trgovini z živili ter povečano skrb na področju varnosti in zdravja ljudi (Kassim, 2001).

Čeprav so strokovnjaki s področja kemije in medicine na problem potvarjanja živil opozarjali že zgodaj, se je resen poseg v reševanje le-tega na državnih ravneh pričel šele po letu 1850. Tega leta je urednik revije Lancet Thomas Wakley ustanovil Analytical Sanitary Commission, komisijo za analizo zdravstva in javne higiene (Charnley, 2008), ki jo je vodil Arthur Hill Hassal, prvi, ki je za raziskave potvorjenih živil uporabil mikroskop. Poročila o raziskavah, ki so bila objavljena v reviji Lancet, so izzvala sestavo britanskega parlamentarnega odbora, ki je leta 1860 sprejela prvi zakon o živilih (Food and Drink Act). Zakon je opredelil ponarejanje oz. potvorbe hrane in pijače kot kaznivo dejanje ter poimenoval analitike, ki so bili javno pooblaščeni za nadzor nad živili v celi državi. Zaradi kritik, da zakon bolj ali manj le potrjuje potvorbe, so sledile številne dopolnitve, ki so leta 1875 vpeljale tudi ustrezne sankcije (Burrow, 2009). Prav tako so bili v novem zakonu bolje opredeljeni pristnost (čistost) živil ter profil in vloga analitikov za vzpostavitev primernih zakonskih podlag za pregon ponarejanja živil (Kassim, 2001). Podobni začetki so zabeleženi v Novi Zelandiji (1866) in Kanadi (1874) (Tsimidou in Boskou, 2003).

Vzporedno s pojavljanjem potvorjenih živil je ves čas potekal tudi razvoj metod za odkrivanje le-teh. Sprva so temeljile zgolj na senzorični detekciji, v 18. in 19. stol. so sledile preproste kemijske reakcije in prej omenjena uporaba mikroskopa za določanje posameznih sestavin v živilih (Wilson, 2008). Velik korak v razvoju predstavlja Pasteurjevo odkritje mikroorganizmov in znanje o fermentacijskih procesih v živilih. Pomembnost in moč živilske kemije je naraščala, ustanavljale so se kmetijske eksperimentalne postaje, znanstvene ustanove, laboratoriji za kontrolo živil, znanstvene revije pa so objavljale članke o živilski kemiji in izobraževanju specialistov v živilski industriji (Deadly Adulteration and Slow Poisoning, 1839?; Foods and Food Adulterants, 1887; Bruce, 1917; Lásztity s sod., 2004; Lásztity, 2006).

Regulativa na področju živil se je v ZDA oblikovala v zgodnjem 20. stol. Leta 1906 je zakon o neoporečnih živilih in zdravilih definiral potvorbe le-teh in označil izdelovanje in prodajo potvorjenih živil in zdravil za nelegalno dejanje (Tsimidou in Boskou, 2003; Ottaway, 2003). Ameriški vladni urad za prehrano in zdravila – Food and Drug Administration (FDA), zadolžen za kontrolo živil z vidika potvorb in napačnega označevanja, je bil ustanovljen leta 1931.

Z namenom oblikovanja programa mednarodnih standardov za živila je bila leta 1963 s strani FAO (Food and Agriculture Organization) in WHO (World Health Organization) ustanovljena komisija za Codex Alimentarius. Gre za zbirko standardov za živila oziroma referenčni dokument, ki ga uporabljajo tako potrošniki kot proizvajalci, predelovalci, trgovci in državni organi.
Komisija Codex Alimentarius predpisuje standarde, dobro prakso, navodila in priporočila za olajšano in harmonizirano mednarodno trgovanje, zagotavlja pošteno prakso v trgovini z živili ter potrošnika ščiti pred potvorbami in zagotavlja varno in kakovostno hrano, ki mora biti ustrezno označena. Standardi so v predpisani obliki za posamezno živilo ali skupino živil ter standardi o aditivih, kontaminantih, ostankih pesticidov in veterinarskih zdravilih (Direktorat za varno hrano pri Ministrstvu za gospodarstvo, kmetijstvo in prehrano, RS).

2 RAZSEŽNOSTI DANAŠNJIH POTVORB IN ŠKANDALOV NA PODROČJU OSKRBE S HRANO

Razvoj na analitskem področju in izpopolnjevanje zakonov, predpisov in standardov so ilegalno in neetično prakso do neke mere zamejili, vendar do sedaj primerov potvarjanja živil s toksikološkimi posledicami niso popolnoma izkoreninili.

V nadaljevanju so navedeni nekateri zadnji masovni primeri zlorab oziroma škandalov v živilski industriji ter njihove posledice. V Španiji so leta 1981 pri približno 20000 ljudeh zabeležili simptome dihalnih motenj, slabosti, vročine, bruhanja, glavobolov, bolečin v mišicah, trebušnih bolečin in kožnih izpuščajev, več kot 400 ljudi pa je umrlo. Španski sindrom je bil posledica uporabe denaturiranega repičnega olja za jedilne namene. Toksikologi še danes niso enotni v razlagi glede glavnega povzročitelja oz. komponente iz uporabljenega olja izmed naslednjih spojin: anilin, fenilalamin ali N-fenil-amino propandiol estri maščobnih estrov, ki nastajajo nastajajo med postopkom dehidracije (Tsimidou in Boskou, 2003).

K nekaterim avstrijskim, nemškim in italijanskim vinom so 1985 nelegalno dodajali dietilen glikol. Ta naj bi oblikoval telo vina, povečal sladkost in poudaril njegovo polnost. Škandalu je sledilo za 30 % nižje povpraševanje po vinu v Avstriji. Dve leti kasneje so isti dodatek zasledili tudi v japonskih vinih (Charters, 2006).

Leta 1986 se je začela serija škandalov z BSE krizo (Bovine Spongiform Encephalopathy) oziroma z boleznijo norih krav – najprej v Veliki Britaniji, Belgiji (1993), Nizozemski (1997), Danski, Franciji, Nemčiji, Portugalski, Švici in Španiji (2000), Italiji (2001), Kanadi (2003) (Bánáti, 2011). V Sloveniji je bil prvi primer okužbe z BSE potrjen leta 2001 (VURS, 2009). Zaradi pomankljive ocene tveganja in nasprotujočih si sporočil medijev je bila zmeda med potrošniki zelo velika, posebej zaradi možne povezave med BSE in Creutzfeldt-Jacobsovo boleznijo (Berg, 2004). Poraba govejega mesa je v Nemčiji v obdobju enega leta padla za 40 %. BSE kriza je posledično doprinesla k izboljšavam na področju sledljivosti v celotni (živalski in rastlinski) živilski industriji.

V Belgiji so v letu 1999 z dioksinom kontaminirano živalsko maščobo v različnih odstotkih primešali v krmo za kokoši, rejo perutnine in prašičev. Zaradi posledic je v državi upadla poraba piščančjega in svinjskega mesa za 69 % oziroma za 93 %. Krizo, ki je izbruhnila leta 1999, mnogi označujejo kot gospodarsko, politično in medijsko hkrati. Embargu na vso belgijsko hrano živalskega izvora (nekatere države so zavračale celo belgijske čokoladne izdelke) je sledil program sledenja in uvedba harmoniziranih evropskih predpisov za poliklorirane dibenzodioksine in furane v živalski krmi in živilih živalskega izvora, celokupni stroški krize so bili ogromni (Covaci, 2008).

Kljub vsem ukrepom je 2008 na Irskem dobavitelj s kontaminirano živalsko krmo povzročil nov škandal, vsebnost dioksinov in dioksinom podobnih polikloriranih polifenilov v svinjskem mesu je 80-200 krat presegala priporočene EU meje (Bánáti, 2011).

Zaradi ptičje gripe (H5N1), ki se je 2003 pojavila v vzhodni in južni Aziji, je po podatkih WHO do 1.4.2011 umrlo 318 ljudi v 12 državah, dve tretjini teh v Indoneziji in Vietnamu (WHO, 2011).

Nov strah pred globalno epidemijo, podobno španski gripi iz leta 1918, se je 2009 začel širiti iz Mehike. Virus prašičje gripe (H1N1) je dosegel 208 držav (podatek iz decembra 2009), histerijo pa so dodatno

podpihovali mediji, delno oblasti, ki so opozarjale na potrebo po uporabi in koristnosti novih cepiv. Zaradi predvidevanj, da so lahko tudi prašiči okuženi z novo gripo (in s tem meso nevarno za uživanje), so le-to v začetku poimenovali »prašičja gripa«. Da potrošnikov ne bi zavajali, so ustrezni mednarodni organi začeli z uporabo pojma »nova gripa« (Bánáti, 2011).

V septembru 2008 je kitajska oblast odpoklicala mleko v prahu zaradi vsebnosti melamina, kemikalije, ki se običajno uporablja v plastiki. Primer melamina se razlikuje od zgoraj navedenih novodobnih afer v tem, da ni izbruhnil po nesreči, slučajno. Melamin, ki je bogat z dušikom, je bil namerno dodan razredčenemu mleku. S tem je bila navidezna vsebnost beljakovin višja, saj rutinsko določanje le-teh poteka preko analize vsebnosti dušika. Odkrili so, da je melamin pri dojenčkih povzročal nastanek ledvičnih kamnov, kar brez zdravljenja lahko vodi do ledvičnega popuščanja, možna je tudi smrt. Za posledicami kontaminacije z melaminom so umirali dojenčki, otroci, številni so oboleli. Škandal je šokiral svetovno javnost, neposredno vpleteni so bili obsojeni na smrt, močno je padel ugled kitajskim podjetjem in izdelkom, vendar vsa statistika ne more vključiti številnih čustvenih travm, s katerimi živijo starši otrok, za katere je vselej možnost pojava kasnejših zdravstvenih težav (Xiu in Klein, 2010).

3 ZAKLJUČEK

Pred potvorbami živil se lahko zavarujemo predvsem tako, da skušamo razumeti njihov vzrok.

Nenamerne škodljive spremembe v živilih, ki vplivajo na zdravje potrošnikov, so pogosto rezultat nespoštovanja, brezbrižnosti ali omejenih možnosti vzdrževanja kakovosti živil kot tudi posledica pomanjkanja znanja, če naravno prisotnih škodljivih snovi sploh ne prepoznamo ali se jih ne zavedamo pravočasno.

V okoliščinah, zaradi katerih hrane ni več dovolj in nastopi lakota, zavestno in za lastno preživetje nadomeščanje običajnih sestavin živila, ki jih ni ali so nedostopne, ne moremo enačiti s potvarjanjem živil.
Kaj žene posameznika ali podjetja, da goljufa potrošnika, škodljivo vpliva na njegovo zdravje ali ga celo izpostavlja smrti? Zakaj je drznost močnejša od strahu pred kaznijo, denarno ali moralno, ki sledi tveganju odkritja nelegalnih in kriminalnih dejanj? Človeška zavest, ki nas razvojno uvršča nad ostala živa bitja? Žal ima zavest poleg prednosti tudi temne plati. Človek živilu na prikrit način namerno dodaja ali odvzema posamezne sestavine ali spreminja obstoječe naravne lastnosti živila in ga trži, pri čemer gre najpogosteje za pridobitništvo.

Zgoraj izpostavljeni primeri potvorb živil kažejo, da se ponudniki poslužujejo namernih goljufij tudi zaradi doseganja predpisanih specifikacij za parametre, ki določajo kakovost živil. Sodobnejši povod za potvorbe živil pa temelji na potrošniškem trgu z zahtevami po nižjih cenah živilskih izdelkov. Ta dobavitelje privede do razpotja med etično držo in mejo donosnosti, pri čemer je ocena tveganja razkritja zlorab žal prevečkrat nižja v primerjavi s tveganjem izgube zaupanja potrošnikov.

Čeprav se želimo prehranjevati čimbolj zdravo in kakovostno, zaradi intenzivnega tempa življenja svojega jedilnika skoraj ne krojimo več sami, temveč nam ga vsiljuje trg. Zaupati, tvegati, sploh pomišljati? Potrošnik, pripet s prehransko verigo, se pred živili, ki mu jih ponujajo pohlepneži, le stežka brani.

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Nekateri pristopi za izkoriščanje heteroze pri navadni pšenici (*Triticum aestivum* L.)

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IZVLEČEK

Heterozo najpogosteje povezujemo s superiornostjo prve filialne generacije nad parentalno. Z gospodarskega stališča pa je še posebej pomembno, da se lahko ta superiornost izraža kot višji produktivni potencial sorte. Zaradi počasne rasti višine povprečnega pridelka zrnja navadne pšenice (*Triticum aestivum* L.) na svetovni in državni ravni, je uporaba hibridov pri tej gospodarsko pomembni poljščini vedno bolj pomembna. Pogoj za pridelavo hibridnega semena je tujeprašnost, ki jo je pri navadni pšenici možno doseči z indukcijo moške sterilnosti. V preteklosti so bili za indukcijo moške sterilnosti v materni komponenti hibridne sorte predlagani pristopi, kot je uporaba moško sterilne citoplazme (CMS sistem) prenesene iz *Triticum timopheevi* Zhuk.. Zaradi svoje kompleksnosti se pristopi na genetski osnovi v praksi niso nikoli dovolj uveljavili. Danes omogočajo sredstva za kemično hibridizacijo razvoj hibridnih sort, ki presegajo pridelek zrnja standardnih linijskih sort tudi za več kot dvajset odstotkov. Alternativo kemični indukciji moške sterilnosti predstavljajo transgeni pristopi. Pridelava hibridnega semena navadne pšenice je tehnološko zahteven postopek o gospodarski upravičenosti katerega odločajo raven heteroze, višina pridelka hibridnega semena na enoto površine in prodajna cena pšenice.

Ključne besede: Heteroza, hibridna sorta, indukcija moške sterilnosti, navadna pšenica, sredstva za kemično hibridizacijo

SEVERAL APPROACHES FOR HETEROSIS EXPLOITATION IN COMMON WHEAT (*Triticum aestivum* L.)

ABSTRACT

Heterosis is commonly associated with the superiority of the first filial generation over the parental generation. From the economic point of view it is important that the superiority could be expressed as a higher productive potential of the variety. Given the slow growth of the average grain yield of common wheat (*Triticum aestivum* L.) at global and national level, the use of hybrids for production of this economically important crop, is gaining the importance. The most important condition for hybrid seed production in common wheat is cross-pollination that can be achieved by induction of male sterility. In the past, different approaches for induction of male sterility in common wheat have been proposed, for example the use of cytoplasmic male sterility (CMS system) transferred from *Triticum timopheevi* Zhuk.. Due to its complexity, the genetic approaches have never been exercised in the practice. Today the chemical hybridizing agents allow development of hybrid varieties that can top the yield of standard inbred varieties for more than twenty percent. Transgenic approaches represent the alternative to the chemical induction of male sterility. Hybrid wheat seed production is therefore technologically complex process that depends on the level of heterosis, hybrid seed yield per area unit and the market price of wheat.

Key words: Heterosis, hybrid variety, male sterility induction, common wheat, chemical hybridizing agents

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1 UVOD

Rast svetovnega prebivalstva močno vpliva na dogajanje povezano s pridelavo pšenice (*Triticum* spp.). V zadnjih petih letih se je svetovna poraba pšenice dvignila iz 610 milijonov ton na 661 milijonov ton, trend krčenja pridelovalnih površin pa se je nadaljeval (International grains council, 2011). Izrazito krčenje površin namenjenih pridelavi pšenice je značilno za Evropo. Leta 1980 je v Evropi pridelava pšenice potekala na 87.645.967 ha, leta 2009 pa na 61.089.167 ha (FAO, 2011). Poleg izrazitega krčenja pridelovalnih površin vpliva negativno na oskrbo s pšenico v Evropi še upočasnitev rasti višine povprečnega pridelka zrnja. V Veliki Britaniji se je povprečni pridelek zrnja pšenice po letu 1948 zvišal za trikrat, vendar se je rast po letu 2000 bistveno upočasnila (Angus, 2009). Za osrednjo Evropo se ocenjuje, da je letni prispevek žlahtnjenja k rasti višine povprečega pridelka zrnja navadne pšenice padel na 0,5 do 0,6 odstotka. Zaradi odvisnosti od tujih žlahtniteljskih programov je lahko v Sloveniji ta prispevek dejanko še nižji (Drezner, 2010). Počasno rast višine povprečnega pridelka zrnja pšenice v Sloveniji potrjujejo tudi podatki Statističnega urada Republike Slovenije (2011), ki kažejo, da se le ta v zadnjih nekaj letih ni bistveno spremenila in je še vedno pod 5 t/ha.

Možnost za hitrejšo rast višine povprečnega pridelka zrnja navadne pšenice (*Triticum aestivum* L.), ki je med vsemi pšenicami za svetovno gospodarstvo najpomembnejša predstavlja izkoriščanje heteroze. Bujnejši rastlinski habitus prve filialne generacije (F_1 generacija), ki je nastala z nadzorovanim križanjem dveh genetsko različnih homozigotnih staršev je bila prva asociacija, ki se je nanašala na heterozo. Superiornost heterozigotnega stanja F_1 generacije nad homozigotnim stanjem starševske generacije pa je naredila izkoriščanje heteroze gospodarsko pomembno. Pri strnih žitih se za izkoriščanje heteroze uporabljajo hibridne sorte in sintetiki (Fehr, 1987). Danes se na slovenski sortni listi nahajajo le linijske sorte navadne pšenice, ki nastanejo z večkratno samooploditvijo potomstva nastalega s križanjem najmanj dveh genetsko različnih staršev. Za razliko od linijskih sort, se pri hibridnih sortah navadne pšenice neposredno uporablja prva generacija potomstva križanja dveh genetsko različnih staršev. Razvoj hibridnih sort navadne pšenice je danes omejen predvsem na razvite zahodne države (Kindred in Gooding, 2005).

Za pridelavo hibridnega semena je potrebna tujeprašnost in ločenost moških in ženskih socvetij na isti rastlini, tako da je na enostaven način možno doseči nadzorovano križanje dveh genetsko različnih staršev. V primeru navadne pšenice, ki spada med samoprašne rastlinske vrste je nadzorovano križanje dveh genetsko različnih staršev možno doseči z indukcijo moške sterilnosti v materni komponenti hibridne sorte. Indukcija moške sterilnosti pomeni, da pelod ni viabilen in posledično se rastlina ni sposobna samooploditi (Schachschneider, 1997). Da lahko pride do nastanka hibridnega semena so poleg indukcije moške sterilnosti pomembne še floralne lastnosti starševskih komponent. V primeru, da se po učinkoviti indukciji moške sterilnosti v materni komponenti krovna pleva in predpleva ne razpreta pod večjim kotom kot pri normalnem cvetenju, ne more priti do prave tujeprašnosti (ksenogamija) in s tem do nastanka hibridnega semena (De Vries, 1971). Izkoriščanje heteroze pri navadni pšenici je torej zahteven postopek, o gospodarski upravičenosti katerega odločajo raven heteroze, izplen hibridnega semena na enoto površine in prodajna cena pšenice.

2 HETEROZA ZA PRIDELEK ZRNJA

Raven heteroze se določa, kot povprečna starševska heteroza (potomec se primerja s povprečjem obeh staršev), heterobeltiozis (potomec se primerja z boljšim staršem) in standardna heteroza (potomec se primerja s standardno sorto) ter se navadno izraža v odstotkih. Ob upoštevanju običajne gostote setve za navadno pšenico lahko znaša heterobeltiozis za pridelek zrnja od 2,8 do 40,7 odstotkov, standardna heteroza za pridelek zrnja pa od -3,8 do 32,1 odstotkov. V primeru, da se raven heteroze določa za posamezno rastlino lahko heterobeltiozis za pridelek zrnja znaša tudi 160,4 odstotkov (Bruns in Peterson, 1998; Cisar in Cooper, 2002). Raven heteroze je odvisna od kombinacijske sposobnosti starševskih komponent. Ločimo splošno in specifično kombinacijsko sposobnost. Splošna kombinacijska sposobnost predstavlja povprečno vrednost, ki jo starševska komponenta doseže pri križanju z vsemi očetovskimi komponentami, specifična kombinacijska sposobnost pa pove kako reagira ta starševska komponenta pri križanju s posamezno očetovsko komponento. Po Borojeviću (1992) splošna kombinacijska sposobnost predstavlja aditivni učinek genov, specifična kombinacijska sposobnost pa neaditivni učinek genov. Glede na raziskave Cukadarja in Ginkla (2001) obstajajo med starševskimi komponentami in hibridnimi sortami naslednje povezave:

- Splošna kombinacijska sposobnost starševskih komponent ima na pridelek zrnja hibridnih sort navadne pšenice večji vpliv od specifične kombinacijske sposobnosti.
- Možno je vzgojiti hibridne sorte z visoko povprečno starševsko heterozo za pridelek zrnja, vendar to še ne pomeni, da te hibridne sorte dosegajo višje pridelke zrnja od vodilnih linijskih sort.
- Med višino pridelka zrnja hibridne sorte in povprečnim pridelkom zrnja obeh starševskih komponent obstaja zelo močna povezava. Visokoproduktivne starševske komponente omogočajo razvoj visokoproduktivnih hibridnih sort.

Obstoj šibke povezave med povprečno starševsko heterozo za pridelek zrnja, splošno in specifično kombinacijsko sposobnostjo za pridelek zrnja ter genetsko razdaljo starševskih komponent je potrdila raziskava Maria Corbellinija in sodelavcev, ki je bila objavljena leta 2002. Raziskava je obsegala 100 F_1 hibridov, njihove vrednosti za povprečno starševsko heterozo za pridelek zrnja so znašale od -16,6 do 31,1 odstotka in so bile znotraj mejnih vrednosti podobnih raziskav. Na osnovi šibke povezave med povprečno starševsko heterozo za pridelek zrnja in genetsko razdaljo starševskih komponent lahko sklepamo, da je za uspešno izkoriščanje heteroze pri navadni pšenici potreben širok izbor starševskih komponent. Za doseganje visoke ravni heteroze je perspektivna uporaba sorodnikov navadne pšenice, ki imajo podobno genomsko strukturo. Pri križanju navadne pšenice s piro (*Triticum spelta* L.) bi lahko dosegli bistveno višjo raven heteroze kot pri križanju navadne pšenice z navadno pšenico (Qixin in sod., 1997).

3 INDUKCIJA MOŠKE STERILNOSTI

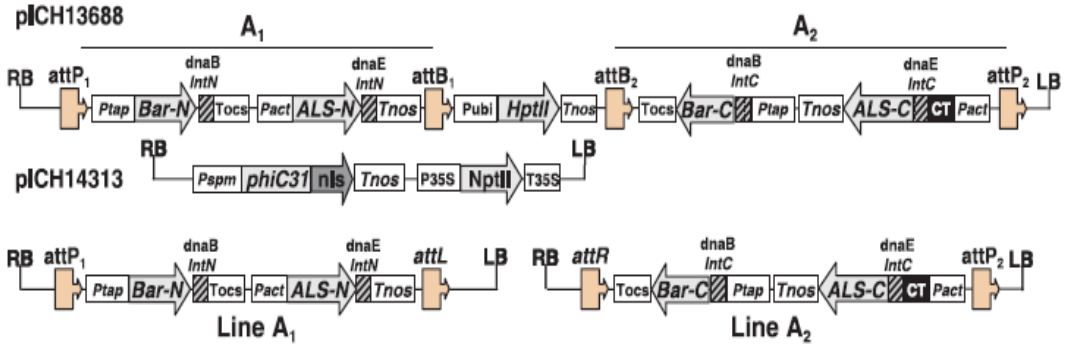
Za indukcijo moške sterilnosti pri navadni pšenici je bilo v preteklosti predlaganih več pristopov na genetski osnovi; kromosomska moška sterilnost (XYZ sistem), jedrna moška sterilnost (NMS sistem) in različne oblike citoplazemsko-genetske moške sterilnosti (CMS sistem). Od navedenih pristopov je najbolj proučen sistem citoplazemsko-genetske moške sterilnosti (Driscoll, 1972; Ogihara, 1999; Cisar in Cooper, 2002). Pri navadni pšenici je bila citoplazemsko-genetska moška sterilnost odkrita v petdesetih letih prejšnjega stoletja. O stabilni obliki citoplazemsko-genetske moške sterilnosti so prvič poročali leta 1962 na osnovi križanja navadne pšenice s timofejevo pšenico (*Triticum timopheevi* Zhuk). Istega leta so poročali tudi o genih za obnovo fertilnosti v F_1 generaciji (Rf geni), ki so jih prav tako odkrili v timofejevi pšenici (Mahajan in Nagarajan, 1998). Vzgoja hibridnih sort navadne pšenice z uporabo citoplazemsko-genetske moške sterilnosti poteka na naslednji način (Borojević, 1992; Cisar in Cooper, 2002; Ivančič, 2002):

1. Vnos citoplazemsko-genetske moške sterilnosti v linijo, ki bo predstavljala materno komponento hibridne sorte (linija A). Pri navadni pšenici se, kot vir citoplazemsko-genetske moške sterilnosti najpogosteje uporablja timofejeva pšenica (*Triticum timopheevi* Zhuk.). Poleg omenjene vrste predstavljajo zanimiv vir citoplazemsko-genetske moške sterilnosti za navadno pšenico tudi nekateri predstavniki rodu *Aegilops* L.
2. Vzdrževanje moško sterilne linije A s pomočjo fertilnega analoga (linija B). Vzdrževanje poteka, kot križanje moško sterilne linije A z linijo B.
3. Vnos Rf genov v opraševalca (linija R).
4. Pridelava hibridnega semena s križanjem linije A z linijo R.

Pristopi na genetski osnovi so se v praksi izkazali za preveč kompleksne. CMS sistem na primer zahteva pet do šest povratnih križanj za vnos moško sterilne citoplazme vrste *Triticum timopheevi* Zhuk. v materno komponento hibridne sorte, poleg tega pa povzroča probleme z obnovo fertilnosti v F_1 generaciji (McRae, 1985). Danes se za pridelavo hibridnega semena navadne pšenice uporabljajo sredstva za kemično hibridizacijo. Sredstva za kemično hibridizacijo omogoča indukcijo moške sterilnosti v enem koraku, vendar pa je delovanje aktivne snovi odvisno od številnih dejavnikov.

Da pridelava hibridnega semena navadne pšenice ne bi bila odvisna od dejavnikov na katere ni mogoče vplivati je bilo v zadnjih letih predlaganih nekaj transgenih pristopov za indukcijo moške sterilnosti (Gils in sod., 2008). Precej obetaven je dvokomponentni sistem vzgoje hibridnih sort (angl. split gene approach), ki vključuje dve lastnosti, moško sterilnost in odpornost na herbicid. Dvokomponentni sistem temelji na moško sterilnem fenotipu, ki je odporen na herbicide na osnovi sulfonilsečnine in imidazolinonov. To omogoča vzdrževanje moško sterilne materne komponente z aplikacijo herbicida. V tem sistemu se uporabljata genetski determinanti za toksičen encim barnaza, ki je pod kotrolo za tapetum peloda specifičnega promotorja in za mutirano acetolaktat sintazo (ALS), ki je pod kontrolo riževega promotorja. Obe genetski determinanti sta deljeni a se nahajata na istem fragmentu (A1 oz. A2), tako da le heterozigoti vsebujejo

vse fragmente za tvorbo funkcionalnih proteinov. Fragmenti se nahajajo na identičnih lokusih na homolognih kromosomih. Posamezen fragment določa le nefunkcionalen protein. Aktiven genski produkt nastane s fuzijo produktov dveh različnih fragmentov. Da lahko pride do tvorbe funkcionalnih proteinov vsebujejo posamezni fragmenti sekvence modrozelenih alg *Synechocystis* sp. (*DnaE* in *DnaB* sekvence). Genotipi, ki vsebujejo dve kopiji posameznega fragmenta (A1 ali A2) so normalno fertilni in občutljivi na sulfonilsečninske herbicide, le heterozigoti, ki vsebujejo obe vrsti genskih fragmentov so moško sterilni in odporni na sulfonilsečninske in imidazolinonske herbicide. Za gensko transformacijo sta bila pripravljena konstrukta pICH13688 in pICH14313, ki sta bila klonirana v pBIN19 binarni vektor. Genski konstrukt pICH13688 je nosil N in C terminalne sekvence *Bar-N* in *Bar-C* iz *Bacillus amyloliquifaciens* in *ALS-N*, *ALS-C* terminalne sekvence mutiranega *ALS* gena *Arabidopsis thaliana*. Konstrukt pICH14313 je bil dodan zaradi indukcije mestno specifične rekombinacije. Konstrukt pICH13688 je vseboval tarčna mesta (*attB* in *attP*) za delovanje mestno specifične rekombinaze, ki jo je determinirala sekvenca *PhiC31* na genskem konstruktu pICH14313. S pomočjo indukcije mestno specifične rekombinacije je bilo možno vzpostaviti mehanizem vzdrževanja moško sterilne materne komponente pri avtogamni rastlinski vrsti, kot je navadna pšenica. Genski konstrukt pICH13688 je vseboval tudi *Int-N* in *Int-C*, N in C terminalne sekvence, ki so pomagale pri fuziji genskih produktov A1 in A2 vstavljenih fragmentov (Gils in sod., 2008).



Slika 1: Struktura genskih konstruktov pred in po rekombinaciji (Gils in sod., 2008).
Figure 1: Structure of the constructs before and after recombination (Gils et al., 2008).

Pred vnosom konstruktov v primarni ekspresijski sistem vrste *Arabidopsis thaliana* je bil sistem fuzije ločenih N in C terminalnih sekvenc preiskušen z agroinfiltracijo v liste vrste *Nicotiana benthamiana*. Ker je bil končni cilj doseči moško sterilnost pri enokaličnicah, posebej pri navadni pšenici, je konstrukt vseboval promotorje iz družine trav. Ekspresija *Bar-N* in *Bar-C* sekvence je bila pod kontrolo za tapetum peloda specifičnega promotorja *Ptap*. Ekspresija *ALS-N* in *ALS-C* sekvence mutirane verzije *ALS* gena *Arabidopsis thaliana* je bila pod kontrolo riževega promotorja *Pact*. Kot terminatorske sekvence so bile konstruktoma dodane *Tnos* (nopaline synthase terminator), *Tocs* (octopine synthase terminator) in T35S sekvence. Reporterski sistem je temeljil na *GUS* genu. Uspešnost integracije genskih konstruktov v rastlinski genom je bila preverjena s pomočjo polimerazne verižne reakcije in Southern blottinga (Gils in sod., 2008).

4 SREDSTVA ZA KEMIČNO HIBRIDIZACIJO

Prvi dokumentiran podatek o vplivu kemične spojine na moško sterilnost rastline izvira iz leta 1953. Takrat so opazili vpliv bakrovega hidrazida na atrofijo pelodnih zrn (Dotlacil in Apltauerova, 1978). Leta 1957 je bil ob predstavitvi dela, ki je demonstriralo uporabo kemikalije FW-450 (α, β-dikloroizobutirat) za vzgojo hibridnih sort bombaža (*Gossipium hirsutum* L.) prvič uporabljen izraz gametocid, ki bi naj označeval kemikalije, ki selektivno vplivajo na moško sterilnost rastline. Danes se pogosteje od izraza gametocid uporablja izraz sredstvo za kemično hibridizacijo (McRae, 1985). Sredstva za kemično hibridizacijo so kemikalije, ki ob uporabi v določeni fenofazi materne komponente hibridne sorte povzročijo prenehanje nastajanja cvetnega prahu ali mu odvzamejo zmožnost oploditve in tako povzročijo, da samoprašna rastlinska vrsta ni sposobna samooploditve (Ur.l. RS št. 91/2003). Na splošno velja, da lahko med aktivnimi snovmi, ki

spadajo v skupino rastnih regulatorjev s herbicidnim delovanjem najdemo veliko potencialnih sredstev za kemično hibridizacijo. Prve aktivne snovi z znanim gametocidnim delovanjem izhajajo iz skupine halogeniranih alifatskih kislin. Med halogeniranimi alifatskimi kislinami izstopata po gametocidnem delovanju α, β-dikloroizobutirat in 2,2-dikloropropionska kislina. Med prve kemikalije, ki so bile proučevane kot potencialna sredstva za kemično hibridizacijo spada tudi etefon (2-kloroetilfosfonska kislina), ki se ga danes uporablja kot sredstvo za redčenje plodičev v sadjarstvu (McRae, 1985). Za serijo fenil oksonikotinatov (RH-531, RH-532, RH-2956, RH-4667, RH-5148, RH-0007) velja, da so bile to prve patentirane kemikalije, ki se jih je želelo uporabiti v komercialni proizvodnji hibridnega semena navadne pšenice. Med serijo fenil oksonikotinatov se najpogosteje omenja uporabo aktivne snovi fenridazon (1-(4-klorofenil)-1,4-dihidro-6-metil-4-oksopiridazin-3-karboksilna kislina) (Hewstone in sod., 1992). V prvo generacijo patentiranih kemikalij z gametocidnim delovanjem spada tudi aktivna snov 3-(p-klorofenil)-6-metoksi-s-triazin-2,4-(1h,3h)-dion-trietanolamin pripravek DPX 3778). Za prvo generacijo sredstev za kemično hibridizacijo je značilno fitotoksično delovanje, nespecifično delovanje, velika odvisnost aktivne snovi od zunanjih dejavnikov ter nizek izplen hibridnega semena na enoto površine (Johnson in Brown, 1978). Predstavniki druge generacije sredstev za kemično hibridizacijo so predvsem heterociklične karboksilne kisline s poudarkom na piridazinskem strukturnem tipu, ki so bile razvite izključno za doseganje atrofije pelodnih zrn. Primeri teh aktivnih snovi so klofencet (2-(4-klorofenil)-3-etil-2,5-dihidro-5-oksopiridazin-4-karboksilna kislina), sintofen (1-(4-klorofenil)-1,4-dihidro-5-(2-metoksietoksi)-4-oksokinolin-3-karboksilna kislina), azetidin-3-karboksilna kislina in aktivne snovi na osnovi piridin monokarboksilatov ter benzenove kisline (Ciha in Ruminski, 1991; Wong in sod., 1995; Chakraborty in Devakumar, 2006). Za klofencet in sintofen je značilno izrazito selektivno delovanje, vendar je za obe aktivni snovi dokazano rakotvorno delovanje, zaradi česar je področje njune uporabe zelo omejeno. V Evropski skupnosti ima uporabno dovoljenje izdano le aktivna snov sintofen, ki se uporablja kot pripravek CROISOR® 100 (Saaten Union, 2011). Razvoj sodobnih sredstev za kemično hibridizacijo se nadaljuje, predvsem v smeri doseganja boljše selektivnosti gametocidnega delovanja ter manjšega vpliva na okolje. Perspektivna je uporaba N-acilanilinov, analogov aminokislin in strukturnih himer aktivnih snovi s herbicidnim delovanjem (Chakraborty in Devakumar, 2005).

5 APLIKACIJA SREDSTVA ZA KEMIČNO HIBRIDIZACIJO

Sredstva za kemično hibridizacijo so najpogosteje v formulaciji, ki omogoča enostavno absorbcijo aktivne snovi, kot je na primer koncentrat za emulzijo (EC formulacija). Delovanje sredstva za kemično hibridizacijo je odvisno od higroskopičnosti aktivne snovi, porabe aktivne snovi na enoto površine, koncentracije aktivne snovi, časa aplikacije, genotipa in zunanjih dejavnikov (temperatura zraka, relativna zračna vlaga, hitrost vetra...) (Blouet in sod., 1999). O učinkovitem delovanju sredstva za kemično hibridizacijo lahko govorimo, ko je v materni komponenti hibridne sorte dosežena vsaj 98 odstotna moška sterilnost (Ur.l. RS št. 91/2003). Za starejše aktivne snovi z gametocidnim delovanjem je značilna visoka poraba aktivne snovi na enoto površine. Dotlacil in Apltauerova (1978) sta s pripravkom Ethrel (39,6 % 2-kloroetilfosfonske kisline) dosegla 90 odstotno moško sterilnost pri porabi omenjenega pripravka višji od 15 l/ha. Naknadne raziskave so pokazale, da so tako visoki odmerki povezani s fitotoksičnim delovanjem in kopičenjem ostankov v tleh. Za predstavnike druge generacije sredstev za kemično hibridizacijo je značilna višja higroskopičnost, kar omogoča nižjo porabo aktivne snovi na enoto površine. Aktivna snov sintofen aplicirana v obliki pripravka CROISOR® 100 izraža učinkovito delovanje že pri nižjem odmerku od 1 kg/ha (Wong in sod., 1995).

Slika 2: Učinkovita kemična indukcija moške sterilnosti.
Figure 2: Effective chemical induction of male sterility.

Določanje časa aplikacije sredstva za kemično hibridizacijo je zaradi številnih dejavnikov, ki vplivajo na delovanje aktivne snovi pogosto težavno. Pri aktivnih snoveh, ki so bile razvite za doseganje atrofije pelodnih zrn kot je na primer sinofen, sovpada optimalen čas aplikacije s premejotično fazo razvoja navadne pšenice (Wong in sod., 1995). Pri uporabi pripravka CROISOR® 100, ki vsebuje aktivno snov sintofen se je izkazalo, da je najvišji učinek možno doseči s tretiranjem v času, ko znaša dolžina klasa na glavnem poganjku od 15 do 20 mm. Na čas aplikacije sredstva za kemično hibridizacijo torej vpliva tudi način delovanja aktivne snovi. Sredstva za kemično hibridizacijo se po svojem vplivu na razvoj pelodnih zrnih, delijo v naslednje skupine (Blouet in sod., 1999):

- Med mikrosporogenezo pride do indukcije mejotskih anomalij v maternih celicah mikrospor.
- Med palinogenezo je razvoj mikrospor spremenjen z disfunkcijo celic tapetuma.
- Do delovanja sredstva za kemično hibridizacijo pride pozneje v razvoju. Pelodna zrna se normalno razvijejo vendar se prašnice ne razpočijo, oziroma pelodna zrna niso sposobna kalitve na brazdi pestiča.

Pri aplikaciji sredstva za kemično hibridizacijo je potrebno upoštevati razdaljo, ki jo lahko cvetni prah navadne pšenice prepotuje od opraševalca do brazde pestiča materne komponente. Ob upoštevanju te razdalje lahko razmerje v širini pasu očetovske in materne komponente znaša od 1 : 2 do 1 : 4, pri čemer se kot optimalna širina pasu materne komponente šteje 4 m (Cisar in Cooper, 2002).

Pri potrjevanju semenskega posevka hibridnih sort, kjer je bila moška sterilnost materne komponente dosežena s sredstvi za kemično hibridizacijo se lahko upošteva odstotek moške sterilnosti materne komponente, ki mora dosegati 98 odstotkov ali stopnjo hibridnosti, ki mora dosegati 95 odstotkov. Odstotek moške sterilnosti materne komponente se določi s formulo $(S_c - S_f) / S_c \times 100$, pri čemer je S_c število semen na klas netretirane rastline, S_f pa število semen na klas tretirane rastline (Chakraborty in Devakumar, 2005). Stopnja hibridnosti predstavlja delež hibridnosti v semenu, vključno s hibridi prve generacije, ki ne pripadajo hibridni sorti, razen semena iz samooplodnje in semena drugih sort (Ur.l. RS št. 91/2003). Zaradi kratkega obdobja med žetvijo semenskega posevka hibridne sorte in setvijo ozimin se v praksi za potrjevanje semenskega posevka hibridnih sort navadne pšenice pogosteje uporablja

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določanje odstotka moške sterilnosti. Stopnjo hibridnosti se določi po žetvi semenskega posevka v laboratoriju, odstotek moške sterilnosti pa pred žetvijo semenskega posevka na polju. Poleg tega pri slednji metodi odpadejo stroški zaradi laboratorijske analize (Cisar in Cooper, 2002).

6 ZAKLJUČEK

Na prostem trgu, ki velja za vse članice Evropske skupnosti, torej tudi za Republiko Slovenijo se cena pridelka pšenice (*Triticum* spp.) oblikuje na žitnih borzah. Na volatilnost žitnega trga oziroma nihanje cene pridelka posamezen pridelovalec nima vpliva in je tako prepuščen dogajanju, ki ga z nobeno makro in mikroekonomsko analizo ni mogoče predvideti. Poleg volatilnosti trga vplivajo na ekonomičnost pridelave pšenice še stroški, ki se pa zaradi rasti cene fosilnih goriv nenehno zvišujejo. Dvig ekonomičnosti pridelave pšenice je možen z znižanjem stroškov, ki pa negativno vpliva na zanesljivost oskrbe s pšenico zaradi stagnacije ali padca produktivnosti. Tako se po obdobju hiperprodukcije v osemdesetih in devetdesetih letih prejšnjega stoletja ponovno poudarja napredek pri rasti višine povprečnega pridelka zrnja navadne pšenice (*Triticum aestivum* L.), ki je med vsemi pšenicami za svetovno gospodarstvo najpomembnejša. Možnost za hitrejšo rast višine povprečnega pridelka zrnja navadne pšenice predstavlja izkoriščanje heteroze, ki se najpogosteje povezuje s superiornostjo prve filialne generacije nad parentalno generacijo. Z gospodarskega stališča je še posebej pomembno, da se lahko ta superiornost izraža, kot višji produktivni potencial. Pri navadni pšenici se za izkoriščanje heteroze uporabljajo hibridne sorte. Za pridelavo hibridnega semena je potrebno doseči nadzorovano križanje dveh genetsko različnih staršev. V primeru navadne pšenice, ki spada med samoprašne rastlinske vrste je nadzorovano križanje dveh genetsko različnih staršev možno doseči z indukcijo moške sterilnosti v materni komponenti hibridne sorte. Za indukcijo moške sterilnosti pri navadni pšenici so bili v preteklosti predlagani pristopi na genetski, kemični in transgeni osnovi. V praksi se je uveljavila uporaba sredstev za kemično hibridizacijo, ki omogoča indukcijo moške sterilnosti v enem koraku. Perspektivnost izkoriščanja heteroze pri navadni pšenici dokazujejo tudi rezultati Kmetijskega inštituta Slovenije, ki so pokazali, da je s francoskimi hibridnimi sortami navadne pšenice možno doseči več, kot do 20 odstotkov višje pridelke v primerjavi s standardnimi linijskimi sortami. Poleg introdukcije hibridnih sort navadne pšenice potekajo na Kmetijskem inštitutu Slovenije tudi raziskave povezane z razvojem okolju prijaznejših sredstev za kemično hibridizacijo, testiranjem kombinacijske sposobnosti starševskih komponent in proučevanjem uporabe predstavnikov heksaploidne skupine pšenic za izboljšanje izkoriščanja heteroze pri navadni pšenici. Pričakovan rezultat raziskovalnega dela je razvoj visoko produktivne dednine prilagojene slovenskim pedoklimatskim razmeram.

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Vpliv selena in izključitve UV-B sevanja na pridelek semen buč golic

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IZVLEČEK

Raziskan je bil vpliv okoljskega sončnega sevanja z in brez UV-B dela spektra ter foliarnega gnojenja z raztopino selenata na pridelek bučnic buče golice (*Cucurbita pepo* L.) Pridelek semen na enoto površine je bil večji, ko je bil s filtriranjem UV-B sevanja odstranjen vpliv tega dela spektra na rastline, v primerjavi z vplivi celotnega sončnega spektra. Na osnovi rezultatov se ugotavlja, da je manjši pridelek v razmerah celotnega sončnega spektra odvisen od antioksidativnih poškodb, ki jih povzroča UV-B sevanje, saj je foliarno tretiranje z raztopino selenata zmanjšalo negativen vpliv tega sevanja.

Ključne besede: buče, bučnice, olje, selen, UV-B sevanje

ABSTRACT

IMPACT OF SELENIUM AND UV-B RADIATION ON THE YIELD OF NAKED PUMPKIN SEEDS

The impact of ambient and filtered solar UV-B radiation and of selenium foliar treatment on the yield of naked seeds in pumpkins, *Cucurbita pepo* L. was determined. Seed yield was higher when solar UV-B radiation was filtered out. The results suggested that the reduced yield under solar UV-B radiation was related to the oxidative damage, as selenium foliar treatment increased the yield under ambient radiation conditions.

Key words: pumpkins, naked pumpkin seeds, oil, selenium, UV-B radiation

1 UVOD

Buče spadajo v red *Cucurbitales*, ki vsebuje eno samo družino *Cucurbitaceae* (bučevke) (Jakop in sod., 2003). Najpomembnejše in najbolj razširjene vrste so: navadna buča (*Cucurbita pepo* L.), orjaška buča (*Cucurbita maxima* Duch.) in muškatna buča (*Cucurbita moschata* Duch). Iz navadnih, njivskih krmnih buč so bile vzgojene sorte in hibridi za olje, sorte vrtnih jedilnih buč in sorte okrasnih buč (Kocjan Ačko, 1999). Sorte se med seboj razlikujejo po obliki rasti (plezajoča ali sedeča stebla), po obliki plodov (podolgovati, okrogli, ploščati, gobasti, hruškasti itn.) in namenu uporabe (Osvald in Kogoj-Osvald, 1994). Uporabni so skoraj vsi deli: bučno meso, včasih z lupino vred, semena (golic in belic) in cvetovi.

Bučno seme je bogato s pomembnimi hranilnimi sestavinami, kot so vitamin E (tokoferoli α, β, γ ,δ), betakaroteni, minerali in mikroelementi. Od vseh sestavin, ki jih najdemo v bučnicah (in posredno v bučnem olju), je največ linolne kisline, ki je kot druge nenasičene maščobne kisline esencialna maščobna kislina, nujno potrebna za presnovo v telesu (Jakop in sod., 2003). Semena lahko zaužijemo kot prigrizek, raziskave pa kažejo na njihov dober učinek pri zdravljenju prostate (Sacilik, 2007). 10 gramov bučnih semen pri odraslem človeku zadosti 17 % dnevnih potreb po beljakovinah, 17 % potreb po kaliju in povprečno 7,5-14 % potreb po pomembnih elementih: magneziju, cinku, selenu (če so bučne rastline dobro preskrbljene s selenom, lahko 10 g bučnih semen zagotovi kar 18,4 % dnevnih potreb po selenu), bakru, kromu in molibdenu. Bučno seme vsebuje pomembno količino linolne kisline. 10 gramov semen pri 1 do 8-

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letnem otroku pokrije 15,4 % dnevnih potreb po ključno pomembni n-6 maščobni kislini (Glew in sod., 2006).

Golice (bučna semena brez ovoja), na široko pridelujejo v Sloveniji, v južnih avstrijskih območjih (Štajerska) in sosednjih pokrajinah ter na Madžarskem. Buče, gojene na avstrijskem Štajerskem, imajo visoko vsebnost zelenih semen brez ovoja. Olje, ki ga iztisnemo iz bučnih semen, je pogosto uporabljeno kot solatno olje (Murkovic in Pfannhauser, 2000). Je temno zelene barve, z močnim, značilnim okusom, ter pomemben vir prehranskih rastlinskih sterolov in vitamina E (Kreft in sod., 2002). Ni primerno za kuhanje in cvrtje zaradi barve in visoke vsebnosti (do 78 %) nenasičenih maščobnih kislin (Kocjan Ačko, 1996). Bučno olje je zdravilno, predvsem če ga pripravljamo po hladnem postopku (Černe, 1988). Ko je olje izločeno, ostanejo pogače ali tropine, ki so bogate z beljakovinami in v nekaterih primerih tudi s selenom (Stibilj in sod., 2004).

Selen je kemijski element, ki je v majhnih količinah nujno potreben za pravilno delovanje človeškega in živalskega organizma (dolgotrajno uživanje 2,5-3 mg ali več dnevno pa vodi do kroničnih zastrupitev). Pomanjkanje selena je povezano z nekaterimi rakastimi obolenji, boleznimi srca (bolezen Keshan, odkrita na Kitajskem je bila otrokom smrtno nevarna), vnetji sklepov ter zmanjšano odpornostjo na bolezni in viruse. Povezan je tudi s presnovo maščob, saj selen vsebuje encim glutation peroksidazo, ki vsebuje selen in se brez ustrezne količine tega elementa ne more sintetizirati. Selen najdemo v žitih, ribah, mesu in drobovini (Haavisto in sod., 1996). Če ga na določenih območjih primanjkuje, ga lahko rastlinam dodajamo z mineralnimi gnojili, foliarnim nanosom ali namakanjem semen, v prehrani ljudi pa z različnimi prehranskimi nadomestki. Slovenija ima srednjo stopnjo pomanjkanja selena (Stibilj in sod., 2004).

Vsebnost selena v semenih, olju in oljnem kolaču buče (*Cucurbita pepo* L.) so analizirali Kreft in sod. (2002). Selen je vključen predvsem v beljakovinah, pri stiskanju olja pa ostanejo beljakovine praktično v celoti v tropinah oz. v kolaču (selen je torej bolj koncentriran v oljnem kolaču kot v celotnih semenih). Pri olju je bila vsebnost selena pod mejo zaznavanja z uporabljeno metodo (manj kot 0,001 mg kg^{-1}), bučno olje je torej siromašen vir selena. Če primerjamo priporočeni dnevni odmerek (RDA) za selen, ki znaša 55 µg/dan, ocenjujemo, da lahko le majhen del dnevnega vnosa pokrijemo z oljnim kolačem iz slovenskih buč (Kreft in sod., 2002). Stibilj in sod. (2004) pa so s foliarno fertilizacijo povečali vsebnost selena v bučnih semenih in tako dobili semena, ki so bogat vir prehranskega selena, kar je lahko uporabno izhodišče za obogatene prehranske izdelke (Stibilj in sod., 2004).

V raziskavi o delovanju selena kot anti- in pro-oksidanta, pri npr. ljulki je ugotovljeno, da je selen deloval pri nižjih koncentracijah antioksidantno, pri višjih pa je deloval prooksidantno in posledično imel dvojni učinek na metabolizem ter rast ljulke. Pozitivni učinek na rast rastlin pri nizki stopnji gnojenja s selenom se je pokazal pri kasnejšem spravilu rastlin. Spodbujevalni učinek selena lahko tako pripišemo pospešeni antioksidaciji, ki je izničila stres zaradi staranja rastlin (Hartikainen in sod., 2000).

Na rast in razvoj rastlin ter poškodbe DNK vpliva ultravijolično sevanje (UV), ki ga oddaja sonce. UV je elektromagnetno valovanje, katerega valovna dolžina je krajša od valovne dolžine vidne svetlobe in daljša od valovne dolžine rentgenskih žarkov. Razdeli se na UV-A; UV-B ter UV-C območja. UV sevanje se delno absorbira v ozonski plasti zemeljskega ozračja, ki ima sposobnost vsrkanja vsega organizmom najbolj škodljivega UV-C sevanja in delno UV-B sevanja, površje dosežejo večinoma UV-A žarki. Zaradi ožanja ozonskega plašča v stratosferi se veča UV–B sevanje na zemeljsko površino, kar lahko vpliva na strukturo in funkcije ekosistemov neposredno (takojšnji škodljivi vplivi na rast in razvoj rastlin in drugih organizmov v ekosistemu) ali posredno (sekundarne posledice v ekosistemih, ki se izražajo pri rastlinski morfogenezi in sekundarnem rastlinskem metabolizmu) (Rozema in sod., 1997). Učinki povečanega UV-B sevanja so torej večplastni: opazni upad pridelka pri gospodarsko pomembnih poljščinah, poškodbe na fotosistemu I (PS I) in fotosistemu II (PS II), motnje pri karboksilatnem encimu, poškodbe DNK, oksidativni stres in ultrastrukturne spremembe (Valkama in sod., 2003). Posledice povečanega UV-B na kulture kmetijskih rastlin vključujejo zmanjšanje pridelka, spremembe pri tekmovanju med vrstami, zmanjšanje fotosintetične aktivnosti, občutljivost oz. dovzetnost za bolezni in spremembe v strukturi ter pigmentaciji. Ob visokem UV-B sevanju se zmanjšajo vegetativni in reproduktivni parametri, kar se odraža v zmanjšani kvantiteti poganjkov (Gao in sod., 2003).

Pri kopenskih gojenih rastlinah ni naravnega okolja, kjer vidna svetloba ne bi vsebovala tudi UV sevanja, prav tako noben zaščitni pigment ne more absorbirati 100 % sprejetega UV-B sevanja. UV sevanje tako lahko povzroča določeno stopnjo oksidativne škode (pirimidinski hidrati) in na prečnih vezeh (DNK-beljakovina in DNK-DNK). Najpomembnejše poškodbe pa povzroča pojavljanje različnih tipov pirimidinskih dimerov, saj je zaradi njihovega učinka na transkripcijo prisotnost le-teh izjemno toksična (mehanizem za njihovo učinkovito odstranitev je ključna funkcija vsakega živega organizma, izpostavljenega sončni svetlobi). Za popravilo DNK sta dve glavni kategoriji mehanizmov: poškodbe se lahko neposredno reverzno

popravijo ali pa se poškodbe odstranijo iz genoma, obstoječe vrzeli pa se popravijo z uporabo nepoškodovanega dela vijačnice DNK, ki služi kot matrica. V primeru pirimidinskih dimerov pri večini organizmov delujeta oba omenjena mehanizma, s katerima je mogoče popraviti dimere (Britt, 1999).

Raziskava je pokazala, da so bile rastline soje (*Glycine max* L.), ki so rasle pod povečanima UV-B in temperaturo, skupaj ali v kombinaciji, podvržene negativnim spremembam na morfologijo cvetov in peloda, produkcijo peloda, klitje in dolžino peclja, ne glede na tretiranje s CO_2 (Koti in sod., 2005). Bombaž (*Gossypium*) je poljščina, za vlakna pomembna za trgovino in gospodarstvo številnih držav. Raziskovalci so opravili poskus z dodajanjem UV-B sevanja na rastline bombaža med rastno sezono: z dodajanjem dodatnega 9,5 % UV-B sevanja bombaž utrpi naslednje negativne posledice: pri rasti se višina zmanjša za 14 %, listna površina za 29 % in skupna biomasa za 34 %. Prav tako se je zmanjšala kvaliteta vlaken, ekonomski donos pa je upadel za 72 % (Gao in sod., 2003).

Obstajajo tudi rastline, ki uspevajo v nizki vodi in so obdržale sposobnost sintetiziranja UV absorpcijskih spojin, t. j. različnih glikoflavonov, kar jim omogoča zaščito pred UV-B sevanjem (Germ in sod., 2002).

Znanstveniki so raziskali tudi vpliv UV-C sevanja na mikrobne populacije in propadanje tkiva *Cucurbita*-e *pepo*, pri čemer so z uporabo germicidnih luči za 1, 10 in 20 minut izpostavili vzorce tkiva cukinija (*Cucurbita pepo* L. cv. Tigress) ultravijoličnim C žarkom (UV-C). Vzorci, ki so bili 10 in 20 minut izpostavljeni UV-C, so pokazali pomembno zmanjšanje mikrobne aktivnosti in s tem manjše propadanje tkiva (pri shranjevanju na 5 ali 10 ºC). Pri vzorcih, ki so jih 10 in 20 minut dnevno 12 dni obsevali z UV-C pri temperaturi 10 ºC, je bilo mogoče opaziti rahlo radiacijsko poškodbo (sprememba barve v rdeče-rjavo) na površju vzorca, medtem ko tisti pri 5 ^{o}C niso kazali nobenih poškodb (Erkan in sod., 2001).

Italijanski raziskovalci so se ukvarjali z vplivom sončnega sevanja oz. sevalno energijo pri pridelavi cukinov (*Cucurbita pepo* L.). Rast rastline in njen pridelek, ko so na voljo voda in hranila v zadostnih količinah ter nanju ne vplivajo pleveli, škodljivci, bolezni in lastnosti zemlje, sta odvisna izključno od sposobnosti rastlin uporabiti sprejeto sončno energijo, ki se akumulira v rastlinski masi. Uporaba sevanja (RUE) meri učinkovitost uporabe sevalne energije, ki jo uporabimo za napovedovanje pridelka in interpretiranje razlik pri pridelavi kot posledico različnih klimatskih razmer. Variabilnost v RUE se razlaga s fizikalnimi parametri (pomanjkanje pritiska izhlapevanja, temperatura, vodni stres) in z biološkimi parametri (rastlinska fenologija, stopnja izmenjave ogljikovega dioksida in vsebnost dušika v listih) (Rouphael in Colla, 2005).

Odsotnost vidne svetlobe ne vpliva na težo in dimenzijo plodov, na število ali težo semen, na vsebnost lipidov in pigmentov pri bučah, kar so pokazale raziskave možnega vpliva fotosinteze plodov ali semen na pridelek plodov, semen in olja buč (Kreft in sod., 2011). Ugotovili so, da plodovi buč, vzgajani v temi, niso bili statistično značilno različni od kontrole (plodovi vzgajani na svetlobi), kljub temu, da ima stena plodov značilno fotosintetsko aktivnost, meritve niso pokazale aktivnosti v razvojnem stadiju semen. V bučnih plodovih, za razliko od semen soje, je vsebnost kisika nizka, bučna semena v nobenem stadiju razvoja ne fotosintetizirajo (embriji soje so fotosintetsko aktivni in proizvajajo kisik. Fotosinteza priskrbi energijske zaloge za sintezo maščob). Vsi asimilati, ki so bili shranjeni v semenih, se transportirajo iz drugih delov rastlin, energija, potrebna za pretvorbo ogljikovih hidratov v lipide, prihaja iz transportiranih kemičnih virov. Ker so zaloge kisika nizke, je tudi dihanje omejeno (Kreft in sod., 2011).

Ugotovljeno je, da selen in UV-B sevanje vplivata na pridelek plodov in nekatere fiziološke parametre pri bučah (Germ in sod., 2005). Zanimalo nas je, kako selen in UV-B sevanje vplivata tudi na pridelek bučnic ter če je med obema dejavnikoma interakcija.

2 MATERIAL IN METODE

2.1 Rastlinski material

Semena *C. pepo* L. smo 3. maja 2003 posejali v laboratoriju v sterilnih razmerah. Mlade rastline smo 19. maja prenesli na laboratorijsko polje Biotehniške fakultete Univerze v Ljubljani (320 m n.m.v., 46^{o}35'S, 14 ^{o}55'V). Posajene so bile na parcelicah z merami 1,5 m x 1,5 m (dve rastlini na parcelo, štiri ponovitve), 20. junija smo rastline foliarno tretirali s selenom in pokrili s folijami. 8. avgusta smo plodove buč pobrali, 15. avgusta odprli, vzeli semena, jih posušili ter izmerili maso sušine bučnic.

2.2 Rastne razmere

Poskusi so vključevali okoljsko sevanje brez tretiranja s selenom (UV1Se0), okoljsko sevanje in selenovo škropivo (UV1Se1), odvzem UV-B okoljskega sevanja prav tako brez selenovega tretiranja (UV0Se0) in odvzem UV-B okoljskega sevanja z dodanim selenovim škropivom (UV0Se1). Valovne dolžine pod 320 nm smo izločili tako, da smo parcele pokrili z 0,15 mm debelo Mylar folijo. Kontrolne rastline, ki so prejemale okoljsko sončno sevanje, pa smo pokrili z 0,15 mm debelo polietilensko folijo, ki je prepuščala UV-B sevanje in

le minimalno zmanjšala ostale valovne dolžine. 49. dan po setvi (kar sovpada z začetkom cvetenja) smo izključili UV-B sevanje in nanesli selen v obliki foliarnega škropljenja z natrijevim selenatom s koncentracijo 1,5 mg l^{-1}. Kontrolne rastline smo poškropili z destilirano vodo, ki ni vsebovala nobenih zaznavnih količin selena.

2.3 Meritve

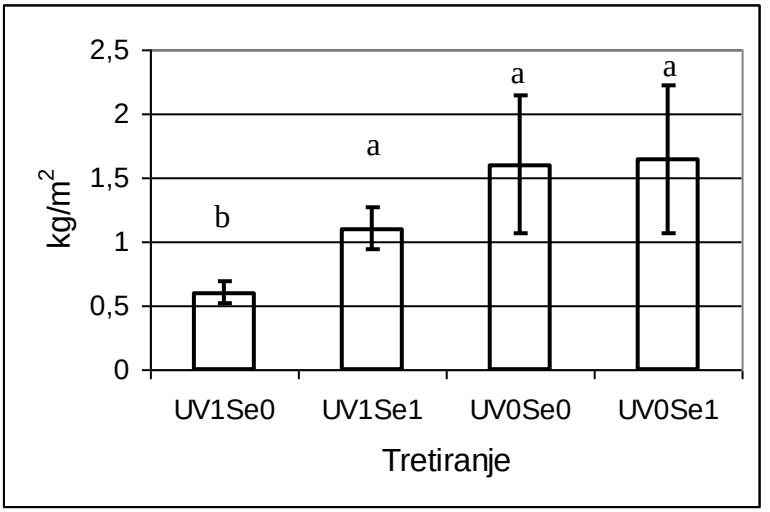
Pridelek buč smo izmerili ob koncu poskusa. Plodove buč smo pobrali tako, da smo odrezali pecelj 5 cm od ploda in jih stehtali. Preučevan je bil pridelek suhih bučnih semen pri kombinacijah obeh dejavnikov, torej vpliv UV-žarkov (običajen odmerek in zmanjšan odmerek) ter selena (foliarno nanesenega in brez). Izvedli smo torej skupno štiri kombinacije tretiranj.

2.4 Statistična analiza

Vse meritve so bile opravljene na štirih do šestih paralelnih vzorcih. Podatke smo vnesli v multifaktorsko ANOVA-o (Statgraphics Version 4). Statistična značilnost P = 0,01.

3 REZULTATI

Eksperiment je pokazal, da je najnižji pridelek suhih bučnih semen pri izpostavitvi rastline buče okoljskemu UV sevanju, pri čemer rastline niso bile tretirane s selenom (Slika 1.). Edino ta podatek se od ostalih statistično pomembno razlikuje na nivoju značilnosti P=0,01. Glede pridelka suhih bučnic so se rastline ugodno odzvale na zaščito pred UV-B sevanjem, pri naravnem UV-B sevanju pa na foliarno škropljenje z raztopino selena.



Slika 1: Pridelek suhe snovi bučnih semen (kg/m^2) pri bučah, ki so rasle pri različnem UV-B in Se tretiranju. Okoljsko sevanje (UV1Se0), okoljsko sevanje in selenovo foliarno tretiranje (UV1Se1), izključitev UV-B okoljskega sevanja (UV0Se0), izključitev UV-B okoljskega sevanja in selenovo foliarno tretiranje (UV0Se1). Odklonske daljice kažejo 95% interval zaupanja. Stolpci, označeni z enakimi črkami, se statistično pomembno ne razlikujejo (P=0,01).

Fig. 1: Yield of dry matter (kg/m2) of seeds of pumpkins grown at different UV-B and Se treatments. Natural solar radiation (UV1Se0), natural solar radiation and Se foliar spraying (UV1Se1), solar radiation with excluded UV-B radiation (UV0Se0), solar radiation with excluded UV-B radiation and foliarly sprayed with Se solution (UV0Se1). Bars shows interval of 95% confidence. Columns marked with the same letters were not significantly different.

4 RAZPRAVA IN SKLEPI

Rezultati poskusa so zlasti pomembni s stališča prihodnjega pridelovanja. Pri gojenju buč je torej za večji pridelek bučnih semen smiselna foliarna aplikacija selena, predvsem kadar so rastline pri pridelavi izpostavljene naravnemu UV-B sevanju. S tem se poveča količina pridelka, z vidika kakovosti pa se poveča vsebnost selena v mesu buč in suhih bučnih semenih. Tretiranje s selenom torej občutno poveča pridelek buč pri izpostavitvi UV-B sevanju. Ta raziskava je pokazala občutljivost pridelka buč na

sedanjo stopnjo UV-B okoljskega sevanja v Sloveniji. Zaviralni učinki UV-B sevanja na rast so bili ugotovljeni tudi za ajdo in druge poljščine. Stimulacijski učinek foliarnega nanosa selena za pridelek bučnic, ki smo ga ugotavljali v tej raziskavi, je skladen s podobnimi raziskavami pri ljulki, solati, krompirju in pri plodovih buč (Germ in sod., 2005; Germ, 2006).

Poškodbe, ki jih povzroča UV-B sevanje, so posledica tvorbe prostih radikalov. Da bi rastline lahko preživele njihove škodljive vplive, morajo tvoriti molekule, s katerimi lahko zmanjšajo vpliv prostih radikalov.

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Agris category code: h10, f60

Kateri biofizikalni in biokemični dejavniki lahko pripomorejo k večji odpornosti zelja (*Brassica oleraceae* L. var. *capitata*) na napad gospodarsko najpomembnejših škodljivcev

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IZVLEČEK

Raziskava o vplivu nekaterih biofizikalnih (vsebnost epikutikularnega voska) in biokemičnih (obarvanost listov, skupni polifenoli in antioksidacijski potencial) dejavnikov na odpornost zelja (*Brassica oleracea* L. var. *capitata*) proti poškodbam, ki jih povzročajo kapusovi bolhači (*Phyllotreta* spp.), kapusove stenice (*Eurydema* spp.) in tobakov resar (*Thrips tabaci*), je potekala v letu 2010 na Laboratorijskem polju Biotehniške fakultete v Ljubljani. V poljski poskus je bilo vključenih 20 genotipov zelja in sicer 9 zgodnjih, 5 srednje zgodnjih in 6 srednje poznih genotipov (glede na dolžino rastne dobe), 3 rdeči in 17 belih genotipov (glede na barvo listov) ter 14 hibridov in 6 sort (glede na poreklo). Statistična analiza je pokazala, da biofizikalna in biokemična sestava listov zelja najbolj vpliva na odpornost te vrtnine na napad kapusovih bolhačev. Ti namreč kažejo šibko preferenco do zgodnjega in rdečega zelja ter do hibridov, ki imajo visoko vsebnost epikutikularnega voska (r^2 = –0,6137, r^2 = –0,7603 in r^2 = –0,6812). Prav tako smo pri kapusovih bolhačih ugotovili močno negativno korelacijo med antioksidacijskim potencialom in obsegom poškodb pri srednje poznem zelju (r^2 = –0,7185), pri rdečem zelju (r^2 = –0,7811) in pri sortah zelja (r^2 = –0,7802).

Ključne besede: kapusovi bolhači, kapusove stenice, tobakov resar, poškodbe, epikutikularni vosek, barva, polifenoli, antioksidacijski potencial, zelje

ABSTRACT

WHICH BIOPHYSICAL AND BIOCHEMICAL FACTORS MAY CONTRIBUTE TO HIGHER RESISTANCE OF CABBAGE (*Brassica oleraceae* L. var. *capitata*) TO ATTACK OF THE MOST IMPORTANT PESTS

Research on the impact of certain biophysical (epicuticular wax content) and biochemical (colour, total polyphenols and antioxidative potential) factors on the resistance of cabbage (*Brassica oleracea* L. var. *capitata*) against damage caused by flea beetles (*Phyllotreta* spp.), cabbage stink bugs (*Eurydema* spp.) and onion thrips (*Thrips tabaci*) was carried out in 2010 at the Experimental field of the Biotechnical Faculty. In a field trial the following 20 cabbage genotypes were included: 9 early, 5 mid-early, 6 mid-late (regarding the longevity of growing period), 3 red, 17 white (regarding the colour), 14 hybrids and 6 varieties (regarding genetic origin). Statistical analysis showed that the biophysical and biochemical composition of cabbage leaves has the greatest impact on resistance of this vegetable to flea beetles attack. Flea beetles showed only weak preference to early and red cabbage, and to the hybrids, which have a high epicuticular wax content (r^2 = –0.6137, r^2 = –0.7603, and r^2 = –0.6812). It has also been found a strong negative relationship between the antioxidative potential and extent of damage in the mid-late cabbage (r^2 = –0.7185), red cabbage (r^2 = –0.7811) and cabbage varieties (r^2 = –0.7802).

Key words: flea beetles, cabbage stink bugs, onion thrips, damage, epicuticular wax, colour, polyphenols, antioxidative potential, cabbage

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1 UVOD

V integriranem načinu pridelave vrtnin je na voljo le omejeno število sredstev za zatiranje škodljivih žuželk. Zato je po mnenju Ciepiela in sod. (1999) izbira ustreznih kultivarjev pomemben dejavnik v boju proti fitofagnim škodljivcem. Odporni kultivarji so združljivi s kemičnim, integriranim in zvečine tudi z biotičnim varstvom rastlin. Trije osnovni mehanizmi odpornosti, ki jih je opisal in pozneje dopolnil Painter (1941), so: antiksenoza, antibioza in toleranca (Smith, 1989).

Antiksenoza je oblika odpornosti, pri kateri rastlina zaradi svojih morfoloških, fizikalnih, strukturnih ali biokemičnih lastnosti odvrača določeno vrsto žuželk. Laski npr. vplivajo na obnašanje žuželk na listnem površju, na njihovo premikanje in prehranjevanje (Goertzen in Small, 1993). K antiksenotični odpornosti pripomorejo tudi voščene prevleke na kutikuli, ki sicer varujejo rastline pred izsušitvijo (Bodnaryk, 1992). Med biokemičnimi lastnostmi rastlin sodijo med najvplivnejše sladkorji, aminokisline, fosfolipidi, glikozidi, alkaloidi, terpeni in hitro hlapljiva eterična olja, ki jih izločajo nekatere rastline (Schoonhoven, 1982). Antibioza je opisana kot mehanizem napadene rastline, ki negativno vpliva na metabolične procese fitofagnih žuželk (Kogan, 1994). Ta oblika odpornosti povzroča visoko smrtnost ličink in komaj razvitih žuželk, slabšo razvitost žuželk in zmanjšano plodnost, njihove morfološke nepravilnosti in nenormalno vedenje. Vzroki antibioze so lahko biofizikalni ali biokemični, lahko pa so tudi posledica prehranjenosti rastline (Panda in Kush, 1995). Toleranca je zmožnost rastline, da gosti škodljivca in da prikrije poškodbe, ki jih je ta povzročil, oziroma da njegova navzočnost ne vpliva na videz ali na pridelek rastline. Prag tolerance je običajno genetsko določen, napadena rastlina pa se brani tako, da odvrže ali nadomesti napadeno tkivo (Stowe, 1998). Pri tem niso vsi mehanizmi odpornosti jasno ločeni med seboj, ampak se prepletajo. Tako na primer biofizikalnih in biokemičnih obrambnih mehanizmov, kot je prehranjenost rastline, ne moremo vedno pripisati le antiksenozi, ampak jih lahko povežemo tudi z antibiozo (Huang in sod., 2003). Prav tako včasih težko ločimo med antiksenozo in antibiozo, zato ker je v posameznih primerih težko določiti vpliv nekaterih kemičnih elementov in toksinov na odpornost.

Temeljne in aplikativne raziskave o naravni odpornosti zelja na napad različnih škodljivcev v Sloveniji potekajo že slabo desetletje. Pričujoči prispevek je nadaljevanje raziskav o vplivu različnih biofizikalnih in biokemičnih sestavin zelja na odpornost posameznih kultivarjev na kapusove bolhače (*Phyllotreta* spp.), kapusove stenice (*Eurydema* spp.) in tobakovega resarja (*Thrips tabaci* Lindeman).

2 MATERIAL IN METODE

Poljski poskus je bil postavljen na Laboratorijskem polju Biotehniške fakultete. Sadike zelja, ki so bile vzgojene v rastlinjaku so bile ročno presajene na prosto v zadnji dekadi aprila 2010. Razdalja med sadikami je znašala 30 x 40 cm. Posamezna parcela je bila dolga 8,2 m. Poskus je bil zastavljen v štirih ponovitvah z 20 genotipi zelja iz treh skupin (zgodnji [Z], srednje zgodnji [SZ] in srednje pozni [SP]), ki so bile oblikovane glede na dolžino rastne dobe zelja (Z – od 55 do 70 dni, SZ – od 80 do 90 dni in SP – od 110 do 140 dni). Med temi genotipi je bilo 14 hibridov ('R1-Cross F1', 'Hinova F1' [oba SP], 'Pandion F1', 'Sunta F1', 'Delphi F1', 'Tucana F1', 'Ixxion F1', 'Autumn queen F1', 'Destiny F1', 'Green rich F1' [vsi Z], 'Red dinasty F1', 'Cheers F1', 'Fieldforce F1', 'Vestri F1' [vsi SZ]) in 6 sort ('Futoško' [SZ], 'Kranjsko okroglo', 'Ljubljansko', 'Holandsko rdeče', 'Varaždinsko' [vsi SP], 'Erfurtsko rdeče' [Z]).

Zelja med rastno dobo nismo škropili z insekticidi, medtem ko so bili preostali agrotehnični ukrepi izvedeni v skladu s standardno komercialno prakso, značilno za pridelavo te vrtnine.

Biofizikalne in biokemične analize listov so bile opravljene na Katedri za tehnologije, prehrano in vino, Oddelka za živilstvo. Epikutikularni voski so bili ekstrahirani po metodi Bodnaryka (1992), modificirani pa po Trdan in sod. (2008a). Antioksidacijski potencial smo določili z metodo DPPH (2,2 difenil-1pikril-hidrazil). Radikal DPPH absorbira svetlobo pri 517 nm. V reakciji z antioksidantom (redukcija) DPPH razpade, zaradi česar se zmanjša absorbcija, zmanjševanje absorbance pa je proporcionalno s koncentracijo antioksidantov v vzorcu (Vidrih in Kač, 2000). Za določitev koncentracije skupnih fenolnih snovi smo dodali Folin-Ciocalteujev reagent, ki v alkalni raztopini reducira fenolne snovi. Masno koncentracijo skupnih fenolnih snovi smo izračunali iz umeritvene krivulje, ki smo jo predhodno pripravili iz standardne referenčne raztopine različnih koncentracij galne kisline (Molyneux, 2004). Za merjenje barve listov smo uporabili kromometer Minolta CR-200b, povezan z DATA DP 100. Sistem temelji na CIE (Commission Internationale I'Eclairage) L* a* b* načinu določanja barve.

Poškodbe, ki so jih povzročili škodljivci na listih zelja smo ocenili ob tehnološki zrelosti zelja, in sicer s 6-stopenjsko lestvico (Stoner in Shelton, 1988) za stenice in resarja ter s 5-stopenjsko lestvico za bolhače (OEPP/EPPO, 2002). Rezultate povprečnih indeksov poškodb in biofizikalnih ter biokemičnih analiz smo statistično obdelali ob pomoči računalniških programov MS Excel 2000 in Statgraphics Plus 4.0.

Statistično značilno različnost (podobnost) med povprečji smo ugotavljali po metodi analize variance (ANOVA) z Newman-Keulsovim preizkusom mnogoterih primerjav. Upoštevali smo 5-odstotno tveganje (P≤0,05). Korelacije med indeksi poškodb in vrednostmi posameznih sestavin listov smo izračunali z linearno regresijsko analizo. Pred izračunom Pearsonovega koeficienta korelacij smo genotipe zelja razvrstili v skupine po metodi, ki so jo opisali Trdan in sod. (2008b).

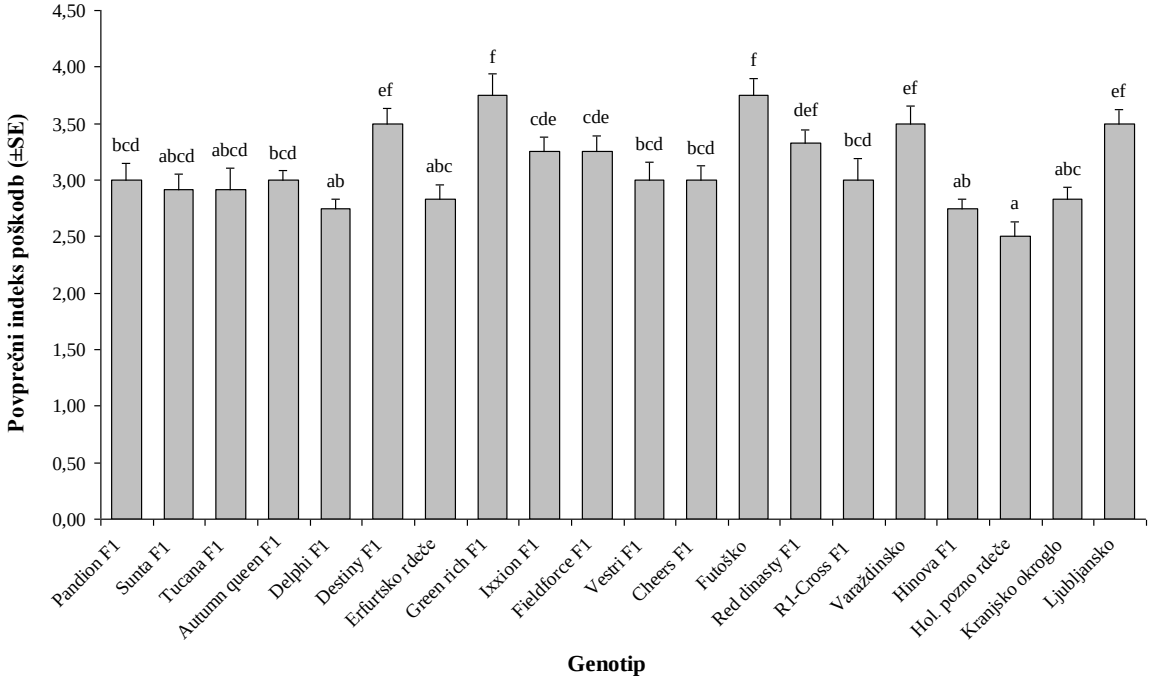
3 REZULTATI IN DISKUSIJA

Ocena poškodb

Statistična analiza je pokazala, da genotip zelja signifikantno vpliva (P≤0,0001) na obseg poškodb, ki jih povzročajo proučevani škodljivci.

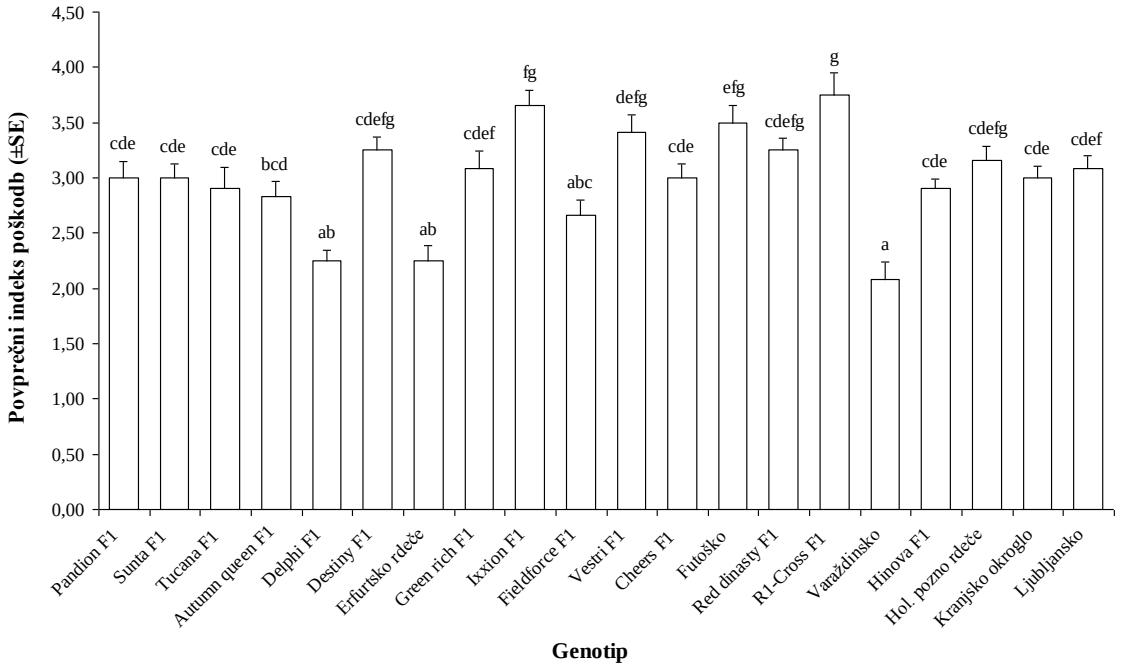
Kot najmanj občutljiva za napad bolhačev se je pokazala sorta 'Holandsko pozno rdeče' (indeks 2,5), medtem ko je najmanjši odpor do tega škodljivca pokazala sorta 'Varaždinsko' (indeks 4,0). Po drugi strani pa se je sorta 'Varaždinsko' izkazala kot najbolj odporna na sesanje kapusovih stenic (indeks 2,1). Napad stenic je najbolj prizadel hibrid 'R1-Cross F1' (indeks 3,7), sicer pa je bil povprečni indeks poškodb med genotipi zelja manjši zaradi poškodb, ki jih povzročajo stenice v primerjavi z bolhači.

Tobakovi resarji na listih zelja niso povzročili pomembnejših poškodb, saj gospodarski prag škodljivosti (povprečni indeks poškodb = 2 ali do odstotek poškodovane listne površine) ni bil dosežen pri nobenem genotipu. Signifikantno največji indeks poškodb zaradi hranjenja resarjev sta sicer imela hibrida 'Sunta F1' (indeks 1,33) in 'Cheers F1' (indeks 1,35).



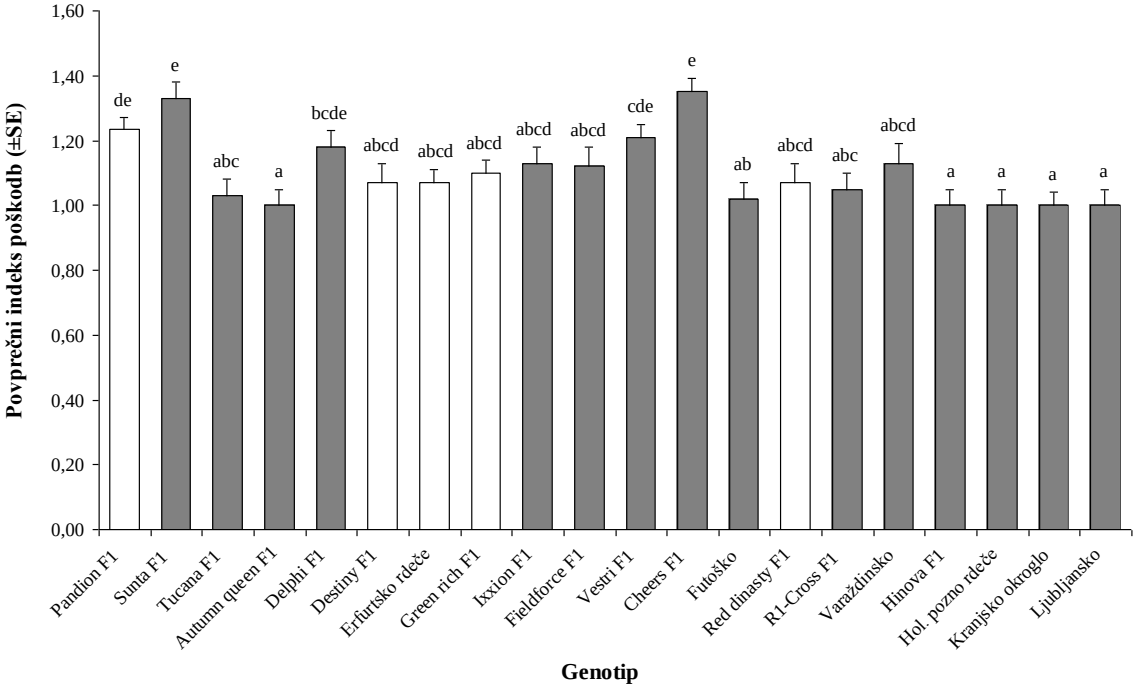
Slika 1: Povprečni indeks poškodb, ki so jih povzročili kapusovi bolhači (*Phyllotreta* spp.) na dvajsetih genotipih zelja

Figure 1: Mean index of damage caused by flea beetles (*Phyllotreta* spp.) on 20 genotypes of cabbage



Slika 2: Povprečni indeks poškodb, ki so jih povzročile kapusove stenice (*Eurydema* spp.) na dvajsetih genotipih zelja

Figure 2: Mean index of damage caused by cabbage stink bugs (*Eurydema* spp.) on 20 genotypes of cabbage



Slika 3: Povprečni indeks poškodb, ki jih je povzročil tobakov resar (*Thrips tabaci* Lindeman) na dvajsetih genotipih zelja

Figure 3: Mean index of damage caused by onion thrips (*Thrips tabaci* Lindeman) on 20 genotypes of cabbage

Povezava med biofizikalnimi in biokemičnimi analizami ter poškodbami

Povprečna masa epikutikularnega voska se je med analiziranimi genotipi gibala med 0,11 (sorta 'Ljubljansko') in 0,97 µg/cm^2 (hibrid 'Hinova F1'). Srednje pozno zelje sorte 'Ljubljansko' je tudi v prejšnjih raziskavah imelo najmanj povoščene liste (Trdan in sod., 2009). Iz statistično ovrednotenih rezultatov smo razbrali, da imajo listi hibrida 'Fieldforce F1' najnižji antioksidacijski potencial (AOP), in sicer 282,11 mg galne kisline/l. Z dietetičnega vidika AOP pomeni seštevek posameznih antioksidantov, kot so vitamin C in E, polifenoli, karotenoidi, terpenoidi, ter sledovi Zn, Cu in Se. Po nam znanih podatkih iz literature pa še ni bila narejena sistematična analiza o vplivu AOP na škodljivce vrtnin. K AOP količinsko največ prispevajo skupni polifenoli. Med genotipi v naši raziskavi je največje vrednosti tega antioksidanta dosegel hibrid 'Pandion F1' (791,45 mg galne kisline/l). Na podlagi dobljenih rezultatov bi omenjeni hibrid v humani prehrani lahko pripomogel k učinkoviti zaščiti organizma pred kroničnimi boleznimi.

S sistemom L* a* b* (+L* – svetlejši, –L* – temnejši), *a (+a* – bolj rdeč, –a* – manj rdeč), *b (+b* – bolj rumen, –b* – manj rumen), ki deluje podobno kot človeško oko, smo skušali ugotoviti, ali tudi obarvanost listov antiksenotično vpliva na škodljivce zelja. Analiza je pokazala, da ima najsvetlejše in hkrati najbolj rdeče liste hibrid 'Red dinasty F1', medtem ko ima hibrid 'Sunta F1' najmanj rdeče in najbolj rumeno obarvane liste.

Preglednica 1: Povprečne vrednosti sestavin listov dvajsetih genotipov zelja

Table 1: Mean value of leaf components in 20 genotypes of cabbage

Sestavina	Vrednost (min. – maks.)	Genotip (min. – maks.)
Vosek (µg/cm^2)	0,11 – 0,97	'Ljubljansko' – 'Hinova F1'
Antioksidac. potencial (nmol/l)	1,58 – 3,61	'A. queen F1' – 'Hol. poz. rdeče'
Σ polifenoli (mg galne kis./l)	282,11 – 791,45	'Fieldforce F1' – 'Pandion F1'
L*	35,46 – 46,63	'Red dinasty F1' – 'Futoško'
a*	–19,3 – –4,05	'Sunta F1' – 'Red dinasty F1'
b*	3,03 – 15,13	'Sunta F1' – 'Hol. poz. rdeče'

Za vseh sedem skupin genotipov zelja smo potrdili negativno korelacijo med povprečno maso epikutikularnega voska in obsegom poškodb na listih zaradi prehranjevanja vseh treh proučevanih vrst škodljivih žuželk. Do podobnih rezultatov smo prišli tudi v predhodnih raziskavah (Trdan in Žnidarčič, 2004). Močno negativno korelacijo med poškodbami, ki so jih povzročili bolhači, in vsebnostjo voska smo ugotovili pri rdečih genotipih zelja (r = –0,7603). Nekoliko nižja, vendar še vedno značilna pa je bila korelacija pri zgodnjih genotipih (r = –0,6137) in pri hibridih (r = –0,6811). Primerljive vrednosti za stenice niso bile statistično značilne. Pri tobakovem resarju pa smo šibko povezavo ugotovili le pri rdečem zelju (r = –0,4236).

Statistična analiza ni pokazala povezave med poškodbami in vsebnostjo skupnih polifenolov v zeljnih listih.

Izrazito negativno pa sta kolerirala AOP in povprečni indeks poškodb, ki jih povzročajo bolhači na rdečem zelju (r = –0,7802), na sortah (r = –0,7802) in na zgodnjem zelju (r = –0,5711). AOP in poškodbe zaradi napada stenic so bile v značilni povezavi le pri srednje zgodnjem zelju (r = –0,4187). AOP na tobakovega resarja ni imel omembe vrednega vpliva.

Od barvnih komponent sta samo L* in a* značilno vplivali na poškodbe, in sicer so svetlejši listi srednje poznega zelja bolj odporni na napad bolhačev (r = –0,4237), medtem ko so rdečkasto obarvani listi istega genotipa zelja bolj dovzetni za istega škodljivca (r = –0,5044). Vpliv barve pri odpornosti kapusnic na napad škodljivih žuželk je bil doslej le redko proučevan. V eni od takšnih raziskav so potrdili večjo odpornost zelenorumeno obarvanih zeljnih glav na napad tobakovega resarja (Fail in sod., 2008).

Preglednica 2: Koeficienti korelacije med povprečnimi indeksi poškodb, ki so jih povzročili kapusovi bolhači (*Phyllotreta* spp.) in povprečnimi vrednostmi 6 sestavin listov dvajsetih genotipov zelja

Table 2: Correlation coefficients among indexes of damage caused by flea beetles (*Phyllotreta* spp.) on 20 genotypes of cabbage and the different values of 6 leaf components

Genotip	Vosek	Σpolifenoli	AOP	L*	a*	b*
Z	–0,6137*	–0,2352	–0,5711*	0,1214	0,0542	0,1162
SZ	–0,4775*	0,1921	0,0654	–0,0113	0,0543	–0,0389
SP	–0,2252	0,2131	–0,7185	–0,4237*	0,5044*	0,1031
Rdeč	–0,7603**	–0,0248	–0,7812**	–0,2801	0,2183	0,4915*
Bel	–0,5956*	–0,0134	–0,2626	0,0303	0,2007	–0,0356
Hibrid	–0,6811*	0,0652	–0,1388	–0,0622	0,1868	–0,0463
Sorta	–0,1941	–0,1027	–0,7802**	0,0361	0,1602	0,2944

Legenda: **statistično značilna povezava pri 99-odstotni stopnji zaupanja, *statistično značilna povezava pri 95-odstotni stopnji zaupanja.

Preglednica 3: Koeficienti korelacije med povprečnimi indeksi poškodb, ki so jih povzročile kapusove stenice (*Eurydema* spp.) in povprečnimi vrednostmi 6 sestavin listov dvajsetih genotipov zelja

Table 3: Correlation coefficients among indexes of damage caused by cabbage stink bugs (*Eurydema* spp.) on 20 genotypes of cabbage and the different values of 6 leaf components

Genotip	Vosek	Σpolifenoli	AOP	L*	a*	b*
Z	–0,2062	0,2251	–0,0812	0,1393	–0,0085	0,1208
SZ	–0,1396	0,3706	0,4187*	0,1342	0,0036	–0,0293
SP	–0,1647	0,3394	0,0767	–0,1479	–0,1049	0,0684
Rdeč	–0,3038	0,1252	0,1634	0,1018	–0,1465	–0,1297
Bel	–0,2514	0,2707	–0,0879	0,0728	0,0041	0,0446
Hibrid	–0,1153	0,1575	0,0976	0,0018	0,0814	–0,0675
Sorta	–-0,3582	0,3855	0,2185	0,2369	–0,2634	0,1878

Legenda: **statistično značilna povezava pri 99-odstotni stopnji zaupanja, *statistično značilna povezava pri 95-odstotni stopnji zaupanja.

Preglednica 4: Koeficienti korelacije med povprečnimi indeksi poškodb, ki jih je povzročil tobakov resar (*Thrips tabaci* Lindeman) in povprečnimi vrednostmi 6 sestavin listov dvajsetih genotipov zelja

Table 4: Correlation coefficients among indexes of damage caused by onion thrips (*Thrips tabaci* Lindeman) on 20 genotypes of cabbage and the different values of 6 leaf components

Genotip	Vosek	Σpolifenoli	AOP	L*	a*	b*
Z	–0,1794	0,0162	–0,0544	–0,2166	–0,1046	0,1394
SZ	–0,3412	–0,0425	–0,1298	0,0781	–0,3619	–0,0025
SP	–0,0495	–0,2046	–0,1179	–0,3144	0,3671	0,1011
Rdeč	–0,4236*	0,0024	–0,2218	–0,0896	0,2392	0,0087
Bel	–0,0923	–0,0013	–0,0439	–0,0801	0,0432	0,0794
Hibrid	–0,1013	0,0171	–0,0713	–0,0533	–0,0246	0,1224
Sorta	–0,2771	–0,2157	–0,0514	–0,0945	0,2295	–0,0186

Legenda: **statistično značilna povezava pri 99-odstotni stopnji zaupanja, *statistično značilna povezava pri 95-odstotni stopnji zaupanja.

4 SKLEPI

Rezultati raziskave bodo predvsem dobrodošli za slovenske pridelovalce zelja pri izboru primernih sort oziroma hibridov. Hkrati pa rezultati dajejo tudi koristne informacije za dopolnjevanje strategije Dobre kmetijske prakse varstva rastlin in integrirane pridelave vrtnin v Sloveniji, saj ima naša država s svojo nacionalno kmetijsko politiko dolžnost in odgovornost tudi do skupne kmetijske politike v Evropski uniji.

5 ZAHVALA

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CONTENT ANALYSIS OF THE PAPERS IN THE ACTA AGRICULTURAE SLOVENICA
VSEBINSKA OBDELAVA PRISPEVKOV V ACTA AGRICULTURAE SLOVENICA let. 97 št. 2

Tomaž BARTOL[a], Karmen STOPAR[b],

SUBJECT INDEX BY AGROVOC DESCRIPTORS
PREDMETNO KAZALO PO DESKRIPTORJIH AGROVOC

SUBJECT INDEX BY AGRIS CATEGORY CODES
VSEBINSKO KAZALO PO SKUPINAH ZNANJA (PREDMETNIH KATEGORIJAH)

NAVODILA AVTORJEM

Prispevki

Sprejemamo izvirne znanstvene članke, predhodne objave in raziskovalne notice s področja agronomije, hortikulture, rastlinske biotehnologije, raziskave živil rastlinskega izvora, agrarne ekonomike in informatike ter s sorodnih področij v slovenskem, angleškem in nemškem jeziku, znanstveno pregledne članke samo po poprejšnjem dogovoru. Objavljamo prispevke, podane na simpozijih, ki niso bili v celoti objavljeni v zborniku simpozija. 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.

Pri prispevkih v slovenskem jeziku morajo biti preglednice, grafikoni, slike in priloge dvojezični, povsod je slovenščina na prvem mestu. Naslovi grafikonov in slik so pod njimi. Slike in grafikoni so v besedilu. Priloženi morajo biti tudi jasno označeni izvirniki slik. Na avtorjevo željo jih vračamo, s tem da je želja pisno sporočena ob oddaji gradiva in ponovno v teku 30 dni po izidu. Latinske izraze pišemo ležeče. V slovenščini uporabljamo decimalno vejico, v angleščini decimalno piko. Prispevki v angleščini morajo imeti povzetek v slovenščini in obratno. Prispevki v nemščini morajo imeti tudi povzetka v slovenščini in angleščini.

Prispevki naj bodo strnjeni, kratki, praviloma največ 12 strani. Uporabljamo Microsoft Word 97 (Windows); pisava Times New Roman, velikost strani 16,2 x 23,5 cm, velikost črk besedila 10, v obsežnih preglednicah je lahko 8; izvlečki in metode dela Arial velikost 8, levi in desni rob 2,1 cm, zgornji rob 1,3 cm, spodnji rob 1,6 cm,

Prva stran

Na prvi strani prispevka na desni strani označimo vrsto prispevka v slovenščini in angleščini, sledi naslov prispevka, pod njim avtorji. Ime avtorjev navedemo v polni obliki (ime in priimek). Vsak avtor naj bo označen z indeksom, ki ga navedemo takoj pod avtorji, in vsebuje polni naslov ustanove ter znanstveni in akademski naslov; vse v jeziku prispevka. Navedemo sedež ustanove, kjer avtor dela. Če je raziskava opravljena drugje, avtor navede tudi sedež te inštitucije. Na željo avtorjev bomo navedli naslov elektronske pošte.

Pod naslovi avtorjev je datum prispetja in datum sprejetja prispevka, ki ostaneta odprta. Sledi razumljiv in poveden izvleček z do 250 besedami. Vsebuje namen in metode dela, rezultate, razpravo in sklepe. Sledijo ključne besede.

Izvlečku v jeziku objave sledi naslov in izvleček s ključnimi besedami v drugem jeziku.

Viri

V besedilu navajamo v oklepaju avtorja in leto objave: (priimek, leto). Če sta avtorja dva, pišemo: (priimek in priimek, leto), če je avtorjev več, pišemo: (priimek in sod., leto). Sekundarni vir označimo z "navedeno v" ali "cv.". Seznam virov je na koncu prispevka, neoštevilčen in v abecednem redu. Vire istega avtorja, objavljene v istem letu, razvrstimo kronološko z a, b, c. Primer: 1997a. Navajanje literature naj bo popolno: pri revijah letnik, leto, številka, strani; pri knjigah kraj, založba, leto, strani. Za naslove revij je dovoljena uradna okrajšava, za okrajšanimi besedami naj bodo vedno pike. Navedbo zaključimo s piko. Za primere upoštevajte objave v Zborniku BFUL.

Oddaja

Avtorji prispevke oddajo v dveh izvodih, enega z dvojnim razmakom med vrsticami in največ 35 vrst na strani, in na disketi. Priložijo tudi izjavo s podpisi vseh avtorjev, da avtorske pravice v celoti odstopajo reviji.

Prispevke recenziramo in lektoriramo. Praviloma pošljemo mnenje prvemu avtorju, po želji lahko tudi drugače. Če uredniki ali recenzenti predlagajo spremembe oz. izboljšave, vrne avtor popravljeno besedilo v 10 dneh v dveh izvodih, enega z dvojnim razmakom. Ko prvi avtor vnese še uredniške pripombe, odda popravljeno besedilo v enem izvodu in na disketi ter vrne izvod z uredniškimi popravki.

Prispevke sprejemamo vse leto.

NOTES FOR AUTHORS

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Papers in Slovenian language should have tables, graphs, figures and appendices in both languages, Slovenian language being the first. Titles of graphs and figures are below them. Figures and graphs are part of the text. Clearly marked origins of figures should be added; they can be returned if author desires. Latin expressions are written in italics. Decimal coma is used in Slovenian and decimal point in English. Papers in English should contain abstract in Slovenian and *vice versa*. Papers in German should contain abstracts in German, Slovenian and English.

The papers should be condensed, short and usually should not exceed 12 pages. Microsoft Word 97 (Windows) should be used, fonts Times New Roman, paper size 16.2 x 23.5 cm, font size in main text 10; in large tables size 8 could be used, abstracts and material and methods Arial size 8, right and left margin 2.1 cm, upper margin 1.3 cm and lower margin 1.6 cm.

First page

The type of the paper should be indicated on the first page on the right side in Slovenian and English language following by title of the paper and authors. Full names of authors are used (first name and surname). Each name of the author should have been added an index, which is put immediately after the author(s), and contains address of the institution and academic degree of the author, in the language of the paper. The address of the institution in which the author works is indicated. If the research was realised elsewhere, the author should name the headquarters of the institution. E-mail is optional.

Under the address of the authors some space for dates of arrival and acceptance for publishing should be left. A comprehensive and explicit abstract up to 250 words follows indicating the objective and methods of work, results, discussion and conclusions. Key words follow the abstract.

The abstract in the language of the paper is followed by the title, abstract and key words in another language.

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