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Original scientific paper

ISOFLAVONE CONCENTRATIONS IN SOYBEANS SUITABLE FOR GROWING IN EUROPE

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Isoflavones are nutraceuticals with many different medical benefits found abundantly in soybean (*Glycine max* (L.) Merr.) seeds. The prerequisite of utilising this valuable source of bioactive compounds and creating quality stock for the pharmaceutical and functional food industries is the screening of available soybean germplasm for isoflavone content. The objectives of this research were to determine the isoflavone concentrations (total isoflavones, daidzein, genistein, glycitein) in 22 high-yielding soybean genotypes, to investigate their variability and explore the effect of different weather conditions on isoflavone phenotypes. Field trials were set up as a randomised complete block design with two replicates in three consecutive years (2010 - 2012) at the Agricultural Institute Osijek (Osijek, Croatia). Chosen genotypes belonged to 00 - II maturity groups (MGs) suitable for growing in almost all European regions. Results showed the existence of genetic diversity among the tested plant material. The influence of genotype and year were both statistically significant. The divergence determined by the analysis of variance (ANOVA) and confirmed by the pair-wise similarity based on the Euclidean distance, confirmed that this set of genotypes was suitable for the use in future crossing

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programmes aiming to produce cultivars superior in isoflavone content in comparison to the existing ones.

Keywords: Glycine max, seed, isoflavones, environment

INTRODUCTION

European countries import 95% of the annual demand for soybean (*Glycine max* (L.) Merr.) grains, meal and oil from overseas causing an enormous trade deficit in these commodities. As a result, the governments in Europe are starting to introduce additional incentives aiming to stimulate the growing of the soybean crop (DIMA, 2016). Government stimulation together with large land areas favourable for soybean cropping (KURASCH *et al*., 2017; KRÖN *et al*., 2015) and high quality germplasm from European breeding houses motivated interest in this valuable crop, which is apparent from the increase in harvested area over the past few years (FAOSTAT, 2018). In order to keep the positive trends in the future, growing soybean production in Europe must be followed by continuous and intensive research in order to create varieties which wolud make soybean more appealing to European farmers both in its agronomic and economic aspects. Although most of the breeding efforts in the past have been focused on seed yield, seed quality is not of less importance, especially when 2 billion people today suffer from malnutrition (VARSHNEY *et al*., 2018). Soybean seeds, rich in proteins, oils, and nutraceutical compounds with many different medical benefits (United Soybean Board, 2014), can greatly contribute to improving human and animal diets and health as well as provide a quality stock for pharmaceutical and functional food industries.

One group of such nutraceuticals present in the soybean seeds are isoflavones, and soybean is considered to be the most abundant natural source of these compounds in the human and animal diet (BHAGWAT *et al*., 2008). Isoflavones are the main components of flavonoids and the most common form of phytoestrogens, ie. non-steroidal compounds with estrogen-like biological properties (KIM *et al*., 2012). They are known to have potential benefits in preventing the development of specific cancers, high blood cholesterol levels, heart disease, osteoporosis and hot flashes in menopausal women (CHALVON-DEMERSAY *et al*., 2017; KOU *et al*., 2017; ZHENG *et al*., 2016; ZHONG *et al*., 2016; DASTMALCHI and DHAUBHADEL, 2014). In plants, isoflavones encourage infection and nodulation by *Bradyrhizobium* enabling fixation of atmospheric nitrogen (PREGELJ *et al*., 2011) and help in abiotic and biotic stress resistance (MENG *et al*., 2011).

The most important isoflavones are genistein, daidzein and glycitein (MESSINA, 1999). As a quantitative trait controlled by many minor genes (GUTIERREZ-GONZALEZ *et al*., 2011), isoflavone content in soybean seed largely depends on genotype, environment and their interaction (ADIE *et al*., 2015; AKOND *et al*., 2013; KIM *et al*., 2012), even to the extent that the smallest changes in the microclimate can cause significant changes in the isoflavone concentrations. Nevertheless, isoflavone heritability was estimated to be medium (AKOND *et al.*, 2014) to high (GUTIERREZ-GONZALEZ *et al*., 2011) while CVEJIĆ *et al*. (2011) and ADIE *et al*. (2015) reported that genotype effects were high enough to enable efficient improvement of isoflavone contents.

The objectives of this research were to screen 22 high-yielding soybean genotypes for isoflavone concentrations as well as to determine their variability and investigate the effect of weather conditions on soybean seed isoflavones. The genotypes were chosen to represent 00 to II maturity groups (MGs) commonly sown in Central and Southeastern Europe but suitable for growing in almost all European regions according to KURASCH *et al*. (2017). Kurasch *et al*. (2017), researched 75 soybean genotypes from five MGs (000 – II) at 22 locations in 10 European countries (Belgium, Netherlands, Germany, France, Switzerland, Austria, Italy, Croatia, Serbia, Romania) in order to identify mega-environments for soybean growing since no harmonized MG classification existed in Europe prior to this. They've found that MGs 00 to II matured at all locations except the two northernmost, in Germany and Netherlands, which confirms that these MGs have wide adaptability and can be grown in most of Europe.

Determining seed isoflavones is important for the development of cultivars which could help encourage the use of soybean as a significant source of nutraceuticals in the functional food market as well as in the pharmaceutical industry. If we considered the increased frequency of adverse weather events in the recent years, global climate changes as well as the fact that isoflavone contents depend largely on even the smallest fluctuations in microenvironments, it is imperative to identify soybean genotypes which express higher trait stability in different cropping areas.

MATERIALS AND METHODS

Plant materials and trial design

The three-year trial (2010-2012) investigating 22 MGs 00 to II soybean (*Glycine max* (L.) Merr.) genotypes (Table 1) was set up in the experimental fields of the Agricultural Institute Osijek (45°32’ N, 18°44’ E, Osijek, Croatia) in a randomized complete block design with two replicates. Among chosen genotypes, two were introduced from foreign breeding programmes (NS-1, SG-1) and remainig 20 originated from national breeding institutions located in Zagreb (ZG-1) and Osijek (OS-Lines). The experimental plot size was 10 m^2, between row distance was 50 cm, and seed distance within a row was 2-3 cm. During the vegetation, all currently accepted agricultural management practices were applied.

Weather conditions

The mean monthly air temperatures (°C) and the distribution of the total monthly amount of precipitation (mm) for the soybean growing season (April – September) in years 2010, 2011 and 2012 along with the respective long-term averages (LTA) (1961–1990) at the location Osijek, Croatia are presented in Figures 1 and 2. All three years differed in terms of weather conditions which enabled the screening for isoflavone content stability. It is apparent from Figure 1 that average monthly temperatures were higher than LTA in all three experimental years. Figure 2 shows that conditions in 2010 were much more humid during the soybean growing season than in 2011 and 2012. The first trial year (2010) had more precipitation than LTA, while 2011 and 2012 were below the precipitation LTA.

Isoflavone extraction and quantification

The trial plots were harvested each year at full harvesting maturity (R8) (FEHR *et al*., 1971) after which isoflavones were extracted from soybean seeds and concentrations of genistein, daidzein and glycitein were determined using the high-performance liquid chromatography (HPLC) method according to VYN *et al.* (2002). Aglycon amounts expressed in mg 100^{-1} g of dry matter (DM) were calculated from the area under the peak by the internal standard method. All measurements were conducted in triplicate, and the value for total isoflavones was calculated as the sum of genistein, daidzein and glycitein concentrations.

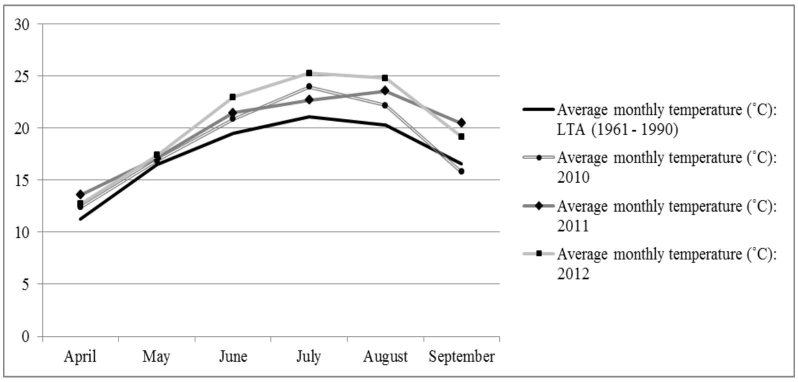

Figure 1. LTA monthly temperatures and average monthly temperatures (˚C) in years 2010–2012 for soybean growing season (April–September) in Osijek, Croatia (Croatian Meteorological and Hydrological Service).

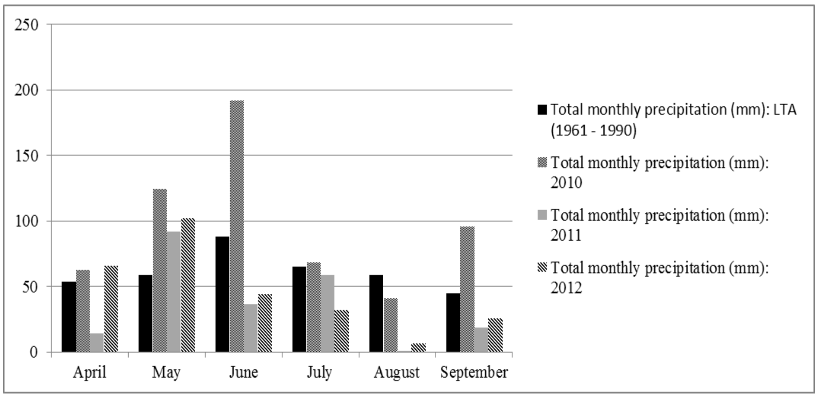

Figure 2. Total monthly precipitation LTA and total monthly precipitation (mm) in years 2010–2012 for soybean growing season (April–September) in Osijek, Croatia (Croatian Meteorological and Hydrological Service).

Statistical analyses

The results of the analysis were statistically processed with software Statistica 12.0 (StatSoft Inc., USA, 2013). Statistical data analysis included the analysis of variance (ANOVA) and the least significant difference (LSD) test (values were tested at 5% and 1%) after which the pair-wise similarity based on the Euclidean distance, i.e. the cluster analysis was done grouping genotypes into classes according to the similarity in terms of their isoflavone concentration. The cluster analysis results are presented by a dendrogram constructed using the single linkage method (also known as the nearest neighbour clustering) which is, according to ANTALIKOVA *et al*. (2008), suitable for identification of divergence among genotypes on the basis of their pedigree, morphological or agronomic traits and therefore it can be used for further confirming the results of ANOVA in preliminary plant breeding research.

RESULTS AND DISCUSSION

The isoflavone concentrations of individual soybean genotypes are listed in Tables 1 and 2. The average total isoflavone cconcentration as a sum of genistein, daidzein and glycitein was 164.58 mg 100^{-1} g DM, varying between 124.06 (SG-1) and 286.20 (OS-L-711) mg 100^{-1} g DM (Table 1). Since isoflavone concentrations are highly susceptible to external and genotype effects (HASANAH *et al*., 2015; MURPHY, 2009; LOZOVAYA *et al*., 2005), earlier research papers reported both, significantly lower values and significantly higher values.

Table 1. Average amount values, minimal and maximal values and coefficient of variation (CV, %) for total isoflavones and daidzein (mg 100^{-1} g DM) in 22 soybean genotypes suitable for growing in Europe (Osijek, Croatia, 2010–2012).

Genotype	MG	Total isoflavone concentration (mg 100^{-1} g DM) 2010	2011	2012	*Average*	Daidzein concentration (mg 100^{-1} g DM) 2010	2011	2012	*Average*
OS-L-453	I	152.87	232.49	164.89	*183.41*^{cd}	51.97	90.89	66.78	*69.88*^{a}
OS-L-793	0	163.53	133.23	143.63	*146.80*^{gh}	53.94	39.81	33.08	*42.28*^{h}
OS-L-788	0-I	199.74	117.27	188.96	*168.66*^{e}	57.93	42.57	39.89	*46.79*^{fg}
OS-L-513	0-I	230.47	91.97	124.05	*148.83*^{g}	69.35	28.09	30.63	*42.69*^{h}
OS-L-526	0	116.30	148.39	144.05	*136.25*^{j}	42.14	41.96	50.88	*44.99*^{g}
OS-L-794	0	150.24	130.71	144.76	*141.90*^{hi}	53.95	39.85	47.72	*47.17*^{f}
OS-L-554	0-I	191.21	119.33	128.11	*146.22*^{gh}	54.66	36.61	35.50	*42.26*^{h}
OS-L-581	0	171.76	212.30	156.91	*180.32*^{d}	55.57	85.56	59.03	*66.72*^{b}
OS-L-711	00	286.65	498.01	73.92	*286.20*^{a}	87.29	105.36	11.58	*68.08*^{ab}
OS-L-712	00-0	372.48	254.80	153.18	*260.16*^{b}	97.99	42.32	39.86	*60.06*^{c}
OS-L-806	0	166.61	189.96	90.12	*148.89*^{g}	63.51	91.33	12.93	*55.93*^{d}
OS-L-821	0-I	155.09	113.19	142.46	*136.91*^{ij}	46.69	43.08	29.31	*39.69*^{ij}
OS-L-805	0	160.59	156.79	92.37	*136.59*^{j}	42.60	47.65	19.88	*36.71*^{k}
OS-L-442	0-I	196.95	214.10	103.10	*171.39*^{e}	60.27	71.04	23.53	*51.62*^{e}
OS-L-874	0-I	237.49	125.42	149.25	*170.72*^{e}	80.22	43.63	31.64	*51.83*^{e}
OS-L-875	0-I	187.98	151.82	139.83	*159.88*^{f}	49.00	48.26	25.23	*40.83*^{hi}
OS-L-877	00-0	204.32	180.40	100.30	*161.68*^{f}	51.04	63.36	20.67	*45.02*^{g}
OS-L-800	0	187.80	129.13	120.43	*145.79*^{gh}	53.32	47.88	37.26	*46.15*^{fg}
OS-L-899	0-I	177.66	134.53	77.63	*129.94*^{k}	53.24	43.02	11.76	*36.01*^{k}
ZG-1	II	142.78	109.45	192.61	*148.28*^{g}	45.14	54.55	76.29	*58.66*^{c}
NS-1	I	247.66	239.25	76.60	*187.83*^{c}	78.07	63.46	13.93	*51.82*^{e}
SG-1	0	143.29	124.82	104.06	*124.06*^{l}	43.48	24.14	48.69	*38.77*^{j}
Average		*192.89*^{a}	*173.06*^{b}	*127.78*^{c}	*164.58*	*58.70*^{a}	*54.29*^{b}	*34.82*^{c}	*49.27*
Minimum		*116.30*	*91.97*	*73.92*	*124.06*	*42.14*	*24.14*	*11.58*	*36.01*
Maximum		*372.48*	*498.01*	*192.61*	*286.20*	*97.99*	*105.36*	*76.29*	*69.88*
CV (%)		*29.21*	*49.91*	*27.08*	*23.97*	*25.65*	*40.18*	*51.27*	*20.56*
		$LSD_{year(0.01)}$ = 1.915; $LSD_{genotype(0.01)}$ = 5.185				$LSD_{year(0.01)}$ = 0.717; $LSD_{genotype(0.01)}$ = 1.942			

Genotype means with the same superscript are not significantly different at P = 0.01

Table 2. Average amount values, minimal and maximal values and coefficient of variation (CV, %) for total glycitein and genistein (mg 100^{-1} g DM) in 22 soybean genotypes suitable for growing in Europe (Osijek, Croatia, 2010–2012)

Genotype	MG	Glycitein concentration (mg 100^{-1} g DM)				Genistein concentration (mg 100^{-1} g DM)			
		2010	2011	2012	Average	2010	2011	2012	Average
OS-L-453	I	17.85	26.74	39.28	*27.95*[b]	83.05	114.86	58.83	*85.58*[ij]
OS-L-793	0	21.52	20.02	19.23	*20.26*[gh]	88.06	73.40	91.32	*84.26*[jk]
OS-L-788	0-I	15.91	10.39	46.29	*24.20*[cd]	125.90	64.31	102.79	*97.67*[ef]
OS-L-513	0-I	19.85	13.97	16.68	*16.83*[ij]	141.27	49.91	76.73	*89.30*[hi]
OS-L-526	0	18.81	20.12	32.52	*23.82*[cde]	55.35	86.32	60.65	*67.44*[m]
OS-L-794	0	25.41	31.97	19.12	*25.50*[c]	70.89	58.88	77.92	*69.23*[m]
OS-L-554	0-I	20.77	17. 19	12.88	*16.95*[ij]	115.78	65.53	79.72	*87.01*[ij]
OS-L-581	0	15.22	14.52	32.55	*20.77*[fg]	100.97	112.21	65.33	*92.84*[gh]
OS-L-711	00	11.27	40.22	16.84	*22.77*[def]	188.09	352.43	45.50	*195.34*[a]
OS-L-712	00-0	30.87	26.66	19.70	*25.74*[c]	243.62	185.82	93.62	*174.35*[b]
OS-L-806	0	13.39	15.17	18.07	*15.54*[j]	89.71	83.46	59.11	*77.43*[l]
OS-L-821	0-I	10.14	18.55	11.55	*13.41*[k]	98.25	51.56	101.60	*83.80*[jk]
OS-L-805	0	23.81	25.84	15.54	*21.73*[fg]	94.19	83.31	56.96	*78.15*[l]
OS-L-442	0-I	20.77	23.04	24.32	*22.71*[def]	115.91	120.02	55.25	*97.06*[f]
OS-L-874	0-I	19.39	14.52	14.58	*16.16*[j]	137.89	67.27	103.03	*102.73*[d]
OS-L-875	0-I	17.26	20.32	14.95	*17.51*[ij]	121.73	83.24	99.65	*101.54*[de]
OS-L-877	00-0	26.68	22.01	19.60	*22.77*[def]	126.61	95.03	60.03	*93.89*[fg]
OS-L-800	0	25.18	16.80	13.78	*18.58*[hi]	109.31	64.46	69.40	*81.06*[kl]
OS-L-899	0-I	17.16	17.94	15.71	*16.93*[ij]	107.27	73.57	50.16	*77.00*[l]
ZG-1	II	18.47	8.76	38.61	*21.95*[efg]	79.17	46.14	77.70	*67.67*[m]
NS-1	I	13.82	23.34	11.54	*16.23*[j]	155.76	152.44	51.13	*119.78*[c]
SG-1	0	29.55	46.29	18.43	*31.42*[a]	70.27	54.39	36.93	*53.86*[n]
	Average	*19.69*[b]	*21.56*[a]	*21.44*[a]	*20.90*	*114.50*[a]	*97.21*[b]	*71.52*[c]	*94.41*
	Minimum	*10.14*	*8.76*	*11.54*	*13.41*	*55.35*	*46.14*	*36.93*	*53.86*
	Maximum	*30.87*	*46.29*	*46.29*	*31.42*	*243.62*	*352.43*	*103.03*	*195.34*
	CV (%)	*28.15*	*41.74*	*46.08*	*21.67*	*36.77*	*68.54*	*28.21*	*34.66*
		$LSD_{year(0.01)}$ = 0.744; $LSD_{genotype(0.01)}$ = 2.015				$LSD_{year(0.01)}$ = 1.547; $LSD_{genotype(0.01)}$ = 4.189			

Genotype means with the same superscript are not significantly different at P = 0.01

MURPHY *et al*. (2009) determined 160 – 370 mg 100^{-1} g DM range for total isoflavones in $F_{4:7}$ population tested in two-year, multi-location trials in Canada, while Adie et al. (2015) determined 14.97 – 39.85 mg 100^{-1} g DM range for ten soybean lines tested during one-year trial on eight locations in Indonesia. GUTIERREZ-GONZALES *et al*. (2011) reported of extremely wide concentration range (12.4 – 317.4 mg 100^{-1} g DM) for total isoflavones determined in the population of recombinant elite lines comprising of 188 F_7 progenies in Missouri, USA whereas

ELDRIDGE and KWOLEK (1983) found the range of total isoflavones in a single genotype to be 46 – 309 mg 100^{-1} g DM. CVEJIĆ *et al.* (2011) researched 20 F_1 soybean progenies in agro-ecological conditions of Southeastern Europe where Croatia is located and found their total isoflavone concentration ranged from 156 to 366 mg 100^{-1} g DM. In the same agro-ecological region, BURSAĆ *et al.* (2017) determined the range of 211 to 524 mg total isoflavones/100 g DM for different seed coat coloured offsprings derived from the single cross between commercial variety and germplasm collection genotype with black seed coat colour.

Average daidzein, glycitein, and genistein concentrations determined in this research were 49.27 mg 100^{-1} g DM, 20.90 mg 100^{-1} g DM and 94.41 mg 100^{-1} g DM respectively (Table 1 and 2), making genistein the most abundant isoflavone component in tested germplasm, followed by daidzein and glycitein. These finds comply with the results of Lozovaya et al. (2005) where the same order of isoflavone abundance was determined for two French and three U.S. cultivars, chosen beacuse of their large differences in isoflavone concentrations and differential responses to environmental changes. SUMARDI *et al.* (2017) researched daidzein and genistein concentrations in 34 black soybeans in Indonesia and found that daidzein content was higher in 31 genotypes. In the research done by TEPAVČEVIĆ *et al.* (2010), CVEJIĆ *et al.* (2011) and BURSAĆ *et al.* (2017), total daidzein was the highest followed by total genistein and total glycitein. Contrary to the mentioned research papers, GUTIERREZ-GONZALEZ *et al.* (2011) found that glycitein was the most abundant isoflavone, followed by genistein and daidzein. Since genistein is reported to have approximately ten times higher biological activity compared to daidzein and glycitein (MORITO *et al.*, 2001), genotypes with high genistein values should be favoured in selection processes aiming to create soybean genotypes suitable for food and dietetic supplement industries. Although all genotypes had high genistein levels, OS-L-711 (195.34 mg 100^{-1} g DM) and OS-L-712 (174.35 mg 100^{-1} g DM) had very high values. The highest average concentration of total isoflavones was determined in OS-L-711 (286.20 mg 100^{-1} g DM) followed by OS-L-712 (260.16 mg 100^{-1} g DM), the highest daidzein values were found in OS-L-453 (69.88 mg 100^{-1} g DM), followed by OS-L-711 (68.08 mg 100^{-1} g DM) and OS-L-581 (66.72 mg 100^{-1} g DM), whereas glycitein concentration was the highest in SG-1 (31.42 mg 100^{-1} g DM) followed by OS-L-453 (27.95 mg 100^{-1} g DM).

Average values for total isoflavones, daidzein, glycitein and genistein in different genotypes had wide ranges which indicate the variability of the tested material (Tables 1 and 2). The existence of traits' relative variability was confirmed with the coefficient of variation (CV) which was, in average, highest for genistein (34.66%), followed by total isoflavones (23.97%), glycitein (21.67%) and daidzein (20.56%). The same order of variation, excluding total isoflavones, was determined by LEE *et al.* (2003). The highest CV value in the research done by GUTIERREZ-GONZALEZ *et al.* (2011) was determined for glycitein, followed by daidzein and genistein while SUMARDI *et al.* (2017) observed a wider range of concentration values for daidzein than for genistein. In all, isoflavones have relatively high CV values in comparison to other soybean seed quality parameters such as protein and oil concentrations, for which MATOŠA KOČAR *et al.* (2017) in a three-year field trial conducted in the same agroecological region but with a somewhat different set of genotypes determined CV values of 1.81% and 2.26% respectively. CV values for fatty acids determined in a similar set of genotypes tested in the same time period ranged from 2.48% to 7.01% (MATOŠA KOČAR *et al.*, 2018). This indicates that isoflavones are more susceptible to external and genotype effects in comparison to other seed quality traits but at the same time that there is more room for breeding progress.

Table 3. ANOVA (mean squares and significance) for analyzed traits in 22 soybean genotypes (Osijek, Croatia, 2010–2012)

Source of variation	Total isoflavones	Daidzein	Glycitein	Genistein
Genotype	9335.57**	615.86**	123.04**	6426.04**
Year	48998.31**	7102.82**	48.61**	20583.93**

The significance level is marked at $p \leq 0.05$ (*) and 0.01 (**).

The highly significant influence of genotype and environment ($P \leq 0.01$) on isoflavone variation was confirmed by ANOVA, while replications were not statistically significant (Table 3) which was in agreement with the results of GUTIERREZ-GONZALEZ *et al*. (2011) and MURPHY *et al*. (2009). Year effects were highly significant ($P \leq 0.01$) in all analysed traits, showing divergence in phenotypes caused by different environmental conditions (Table 3). The first field trial year (2010) has had average monthly soybean growing season (April – September) temperatures higher than the LTA but lower than two other consecutive years (2011, 2012). At the same time, soybean growing season's (April – September) total precipitation was in 2010 higher than the LTA and the next two field trial years (Figure 1 and 2). These conditions were favourable for isoflavone accumulation (HASANAH *et al.* 2015; CHENNUPATI *et al*., 2011; MURPHY *et al*., 2009) which is why average total isoflavones, daidzein, and genistein concentrations were higher in 2010 than in 2011 and 2012 (Table 1 and 2). The positive effect of higher soil water content during seed development was determined by LOZOVAYA *et al.* (2005) in the glasshouse experiment conducted with five soybean cultivars. They determined that 21% soil water holding capacity during the R6-R7 period resulted in 14% total isoflavone content reduction in comparison to values observed at 70% soil water holding capacity. Furthermore, the negative impact of water deficit on soybean isoflavone concentration was confirmed in many earlier studies (HASANAH *et al*., 2015; GUTIERREZ-GONZALEZ *et al*., 2010; RASOLOHERY *et al*., 2008). Third field trial year (2012) was the hottest in the period from April to September. Although 2012 had higher average monthly temperatures in comparison to the LTA, 2010 and 2011, total precipitation was low, but not the lowest. The lowest amount of total precipitation was measured in 2011 (Figure 1 and 2). Nevertheless, total isoflavones, daidzein, and genistein all had the lowest concentration values measured in 2012 (Table 1 and 2). The same situation was previously reported in CARRERA and DARDANELLI's (2015) study of variation in soybean isoflavone content affected by climatic variables in 76 environments during two years who found a 91% decrease in total isoflavone content with mean temperatures during the seed development rising from 14.1 to 26.7˚C. The decrease in isoflavone content with rising temperatures is probably due to the negative effect of high temperatures on the expression of key metabolic entry points in the phenylpropanoid pathway responsible for the formation of all isoflavonoids (CHENNUPATI *et al*., 2012). Among traits tested in the present study, glycitein had the highest amounts determined in hot and dry 2011 and the lowest amounts in the year 2010 which was the most favourable for the accumulation of all other investigated isoflavones. This lack of relationship between mean temperature and isoflavone accumulation could result from the fact that one location provides a somewhat narrower range of temperature variation (CARRERA and DARDANELLI, 2015). For example, MORRISON *et al*. (2010) evaluated 14 cultivars across 12 years at one location determining that high mean temperature during seed development did not result in isoflavone concentration decrease.

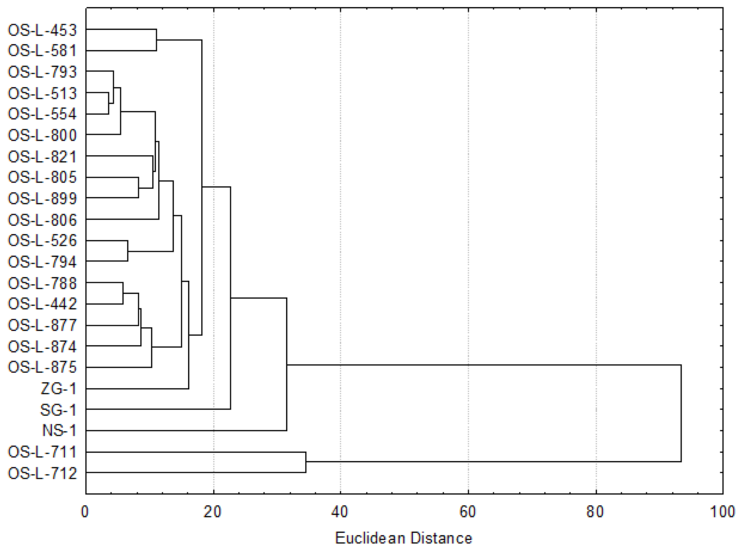

Figure 3. The dendrogram based on the Euclidean distance between 22 soybean genotypes evaluated according to the seed isoflavone concentration parameters (total isoflavones, daidzein, glycitein, genistein).

Pair-wise similarity based on the Euclidean distance between genotypes in terms of their total and individual isoflavone concentration values divided all genotypes into two main groups with the linkage distance of > 92 (Figure 3). One group included two genotypes (OS-L-711 and OS-L-712) and the other remaining 20 genotypes, further divided into subgroups. Although foreign genotypes (NS-1, SG-1) were grouped together in the second, larger set of genotypes, both of them branched out in the first two consecutive steps. The formation of two groups in the cluster analysis confirms previously determined divergence among 22 soybean genotypes.

CONCLUSIONS

The results of this three-year study determined the significant variability of total isoflavone concentration and the concentrations of daidzein, glycitein, and genistein among 22 soybean genotypes suitable for growing in Europe, with both year and genotype having a significant effect on all tested traits. Variability caused by genotype effect ensures there is enough choice for parent selection, while significant environmental effects mean the need for location specific varieties. The most abundant isoflavone component in the tested germplasm was genistein, which it is beneficial from the nutritional point of view since genistein has approximately ten times higher biological activity compared to daidzein and glycitein. Genotypes OS-L-711 and OS-L-712 had the highest average concentration of total isoflavones while being separated from all other tested genotypes based on their Euclidean distance which makes them suitable as parents in future crossings which aim to improve isoflavone concentration values.

In conclusion, these findings are valuable and beneficial in determining future breeding strategies for altering total and individual isoflavone content values in European soybean germplasm, thus enhancing the added value properties of soybean seed for the functional food market and pharmaceutical industry as well as improving the final soybean products for end consumers. Moreover, as the exchange of materials between different breeding institutions across Europe aiming to introduce diversity is becoming more frequent nowadays, studies which define germplasm in local genebanks, as well as their susceptibility to environmental factors are becoming evermore necessary.

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KONCENTRACIJE IZOFLAVONA U SOJI KOJE POGODUJU GAJENJU U EVROPI

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Izvod

Izoflavoni su nutritijenti mnogo različitih medicinskih pogodnosti koji se nalaze u obilju u zrnu soje (*Glycine max* (L.) Merr.). Preduslov korišćenja ovog dragocenog izvora bioaktivnih jedinjenja i stvaranje kvalitetnih zaliha za farmaceutsku i funkcionalnu prehrambenu industriju je skrining raspoložive germplazme soje za sadržaj izoflavona. Ciljevi ovog istraživanja bili su: utvrđivanje koncentracija izoflavona (ukupni izoflavoni, daidzein, genistein, glicitein) kod 22 genotipa soje visokog prinosa, istraživanje njihove varijabilnosti i uticaja različitih vremenskih uslova na fenotipove izoflavona. Poljski ogledi su postavljeni kao randomizirani kompletni blok sa dva ponavljanja u tri uzastopne godine (2010 - 2012) na Poljoprivrednom institutu Osijek (Osijek, Hrvatska). Izabrani genotipovi pripadali su grupama zrenja 00 - II (MG) pogodnim za gajenje u gotovo svim evropskim regijama. Rezultati su pokazali postojanje genetičke raznovrsnosti među testiranim biljnim materijalom. Uticaj genotipa i godine bili su statistički značajni. Divergentnost određena analizom varijanse (ANOVA) i potvrđena sličnošću na osnovu euklidske udaljenosti, potvrdila je da je ovaj skup genotipova pogodan za upotrebu u budućim programima ukrštanja sa ciljem da se proizvedu sorte superiorne u sadržaju izoflavona u odnosu na postojeće.

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