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Assessment of phenotypic variability of saccharides in soybean genotypes suitable for growing in Europe

Procjena fenotipske varijabilnosti saharida kod genotipova soje prikladnih za uzgoj u Europi

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ABSTRACT

Given the great potential of soybean (*Glycine max* (L.) Merr.) as a staple crop for food, feed and pharmaceutical industry, improving the genetic basis of seed quality is one of the main breeding aims on a global scale. An important quality trait is saccharide composition since it affects digestibility and nutritional value of soybean seed, affecting the usability of this agricultural commodity. This 3-year research (2010-2012) investigated soybean variability by measuring saccharide contents (glucose, fructose, sucrose, raffinose, stachyose) in high-yielding genotypes (maturity groups 00-II) suitable for almost all European regions, while taking into consideration the effect of weather conditions. Statistical analyses included calculating the basic measures of descriptive statistics, analysis of variance (ANOVA), determining correlation and using the Nearest Neighbour clustering to construct a dendrogram based on the Euclidean distance. Results showed the existence of diversity in saccharide content with the influence of genotype, year and their interaction being statistically significant. The lack of correlation between investigated parameters indicates the use of indirect selection is not possible. Nevertheless, the determined divergence indicates the given set of genotypes is suitable for use in future crossing programmes aiming to produce cultivars with more beneficial sugar content in comparison to the existing ones.

Keywords: breeding, *Glycine max* (L.) Merr., sugar content, seed quality

SAŽETAK

Uzimajući u obzir izniman potencijal soje (*Glycine max* (L.) Merr.) kao sirovine za farmaceutsku industriju te proizvodnju ljudske i stočne hrane, poboljšavanje genetske osnove za kvalitetu zrna soje jedan je od osnovnih oplemenjivačkih ciljeva na globalnoj razini. Značajno svojstvo kvalitete soje je sastav saharida jer utječe na probavljivost i nutritivnu vrijednost zrna o čemu ovisi iskoristivost soje kao sirovine. Cilj ovog trogodišnjeg (2010. - 2012.) istraživanja bio je procijeniti varijabilnost odabranih linija soje mjerenjem sadržaja saharida (glukoze, fruktoze, saharoze, rafinoze i stahioze) u visokoprinosnim genotipovima (grupe zriobe 00 - II) pogodnim za uzgoj u većini područja Europe. Pri tome je uzet u obzir i utjecaj uvjeta okoline. Statistička obrada podataka uključivala je izračun osnovnih mjera deskriptivne statistike, analizu varijance (ANOVA), korelacijsku analizu te je konstruiran dendrogram temeljen na Euklidskoj udaljenosti prema metodi najbližeg susjeda. Rezultati su pokazali postojanje različitosti testiranog materijala prema sadržaju saharida pri čemu su genotip i godina bili statistički značajan izvor varijabilnosti. Nedostatak korelacije između testiranih parametara ukazuje na nemogućnost indirektne selekcije. Ipak, utvrđena divergentnost potvrđuje opravdanost uporabe materijala u budućim programima križanja čiji je cilj stvaranje kultivara s povoljnijim sadržajem saharida u odnosu na postojeće.

Ključne riječi: *Glycine max* (L.) Merr., kvaliteta zrna, oplemenjivanje, sadržaj šećera

INTRODUCTION

The significance of soybean emanates mainly from the chemical composition of its seed which is why improving the genetic basis of soybean seed quality is one of the main breeding aims globally (Fehr and Curtiss, 2004). Although the most important seed quality parameters in soybean are protein and oil concentrations, saccharide profile is significant since sugars affect quality, digestibility and nutritional value of soybean seed. Soybean seed contains 33% of carbohydrates on average, of which, depending on the genotype (Lowell and Kuo, 1989), 16.6% are soluble sugars (Hymowitz and Collins, 1974). Five main sugars in soybean seed are glucose, fructose, sucrose, raffinose and stachyose. Among these, sucrose and stachyose are the predominant ones, while glucose, fructose and raffinose are present in lower amounts (Hou et al., 2009). Glucose, fructose and sucrose are beneficial in soybean seed intended for human consumption because they can be easily digested and give soybean food products their characteristic sweet taste. Oligosaccharides, i.e. mainly galactooligosaccharides (stachyose and raffinose), on the other hand, limit soybean food products' nutritional value and reduce metabolisable energy that can be obtained from soybean meal since they are indigestible and cause irritation of the gastrointestinal tract in humans and animals (Félix et al., 2013; Gulewicz et al., 2014; Qiu et al., 2015). In consequence, the use of soybean seed as food and feed is limited, but the demand for 'food grade' soybean is on the rise due to the increasing awareness of the health benefits connected with soy food consumption. Lowering oligosaccharides would, therefore, make soybean products more amenable to human consumption and consequently increase the share of soybean seed used as feed, thus increasing the market for seed production.

Although galactooligosaccharides are considered to be anutritive factors, their role is not necessarily negative. They serve as transport metabolites (Jones et al., 1999) and have been proposed to play a positive role in cold acclimation and desiccation tolerance during seed maturation (Dey and Dixon, 1985; Jones et al., 1999; Bentsink et al., 2000). Furthermore, galactooligosaccharides are thought to have positive effects on health. Galactooligosaccharides ferment into low chain fatty acids in human intestines with prebiotic qualities, which makes soybean seed interesting for pharmaceutical as well as functional food industry (Roberfroid, 2007; Zheng et al., 2012).

Modifying soybean seed sugar content according to particular end use necessitates investigating the variability of available soybean germplasm. The genotypes in this research were chosen to represent 00 to II maturity groups (MGs) suitable for growing in almost all European regions (Kurasch et al., 2017). Statistically significant differences in sugar composition and content, as well as variability among soybean genotypes that justify selection, were determined in many studies (Hymowitz and Collins, 1974; Geater and Fehr, 2000; Hollung et al., 2005; Hou et al., 2009; Mozzoni et al., 2013). Geater et al. (2000) concluded that the differences in total sugar concentration among tested genotypes were constant in all environments. Moreover, heritability for monosaccharides is considered to be moderate to high and high for oligosaccharides (Mozzoni and Chen, 2019; Kim et al., 2005, 2006). Contrary to Geater and Fehr (2000) and Geater et al.'s (2000) studies, Taira (1990) found the environment to be the larger source of variation among their plant materials. Determined maternal and additive effects in the case of soybean saccharides (Openshaw and Hadley, 1978; Maughan et al., 2000), together with the often occurring absence of genotype x environment interaction (Geater et al., 2000) can facilitate selection and breeding process aiming to improve sugar profiles in soybean seed. Furthermore, Chen (2004) considers there is a significant potential for improvement since breeding for increased sugar content was at most times efficient.

Regardless of the goal, genetic variability for traits of interest is always the main prerequisite of a successful breeding programme. For introgressing the existing variability into breeding programmes, accessions first need to be thoroughly described (Tara Satyavathi et al., 2006). Ristova et al. (2010) noticed that the genetic base of the material used in Southeast European countries is relatively narrow and there is very little reliable information regarding the source of germplasm introduction, pedigree and breeding procedures applied. Tavaud-Pirra et al. (2009) concluded that there is very little information on the diversity of European soybean germplasm in general but based on the long history of successful breeding it can be assumed that significant variability between accessions exists. Furthermore, while soybean does have a narrow genetic base (Gizlice et al., 1994), Mulato et al. (2010) think that the potential for creating new and improved soybean cultivars in the future is very large because, throughout history, only a small number of accessions from germplasm collections were used for developing elite cultivars.

According to Burton (1997) and Miladinović et al. (2008), genetically distant elite parental lines have the highest likelihood of creating superior progeny. For determining such parents, breeders use conventional methods such as morphological descriptors and agronomic traits along with contemporary chemical, biochemical and molecular analyses. This enables the creation of diverse, recombinant population. Quantification of seed quality traits by chemical analyses is crucial for phenotype evaluation and can offer predictive estimates of trait variability. Predictive estimates are even more accurate if the effects of the environment are taken into consideration. Furthermore, since the frequency of adverse weather events (for e.g. distribution, intensity and frequency of heat waves and droughts) has been increasing over the last 20 years (Jug and Güttler, 2015; Jug et al., 2018), continuous and intensive research is necessary to create stable European varieties with high seed quality which would become an integral part of conservation agriculture. This is particularly important since European countries import 95% of the annual demand for soybean seed, meal and oil from overseas causing an enormous trade deficit in these commodities. Nevertheless, the increase in harvested area in Europe over the past few years (FAOSTAT, 2018) is proof that large land areas favourable for soybean cropping (Kurasch et al., 2017; Krön et al., 2015) and high-quality germplasm from European breeding houses together with the additional incentives for soybean production from European governments (Dima, 2016) motivated the interest in this valuable crop. For keeping these positive trends in the future and make soybean even more appealing to European farmers both in its agronomic and economic aspects, intensification of soybean research, determining diversity for most important traits as well as the exchange of materials between different breeding institutions across Europe are imperative.

Following the trends in soybean variety development, this research concentrated on soybean seed sugar profiles with the aim to determine phenotypic variability, examine sources of variation and interrelationships between different studied parameters which would all together facilitate breeding effectiveness.

MATERIALS AND METHODS

The 3-year trial (2010-2012) was set up in the experimental fields of the Agricultural Institute Osijek (Osijek, Republic of Croatia) in a randomised complete block design with 2 replications. Plant material included 22 soybean (*Glycine max* (L.) Merr.) genotypes (Table 2) belonging to MGs 00 to II which are, according to Kurasch et al. (2017), suitable for growing in almost all European regions. Among chosen genotypes, 2 were introduced from foreign breeding programmes (NS-1 originating in Serbia, SG-1originating in Canada), 1 is from a different domestic breeding institution (ZG-1), while the rest were created at the Agricultural Institute Osijek.

Since all 3 years (2010-2012) in which the field trial was set up differed in weather conditions, it enabled the preliminary screening for saccharide content stability. The mean monthly air temperatures (°C) and the distribution of total monthly precipitation (mm) along with the respective long-term averages (LTA) (1961-1990) for soybean growing season (April-September) at Osijek, Republic of Croatia, are presented in Figures 1 and 2. It is apparent from Figure 1 that average monthly temperatures were higher than LTA in all 3 experimental years. Figure 2 shows that 2010 was the most humid and had higher precipitation values than the LTA, while 2011 and 2012 were below the precipitation LTA.

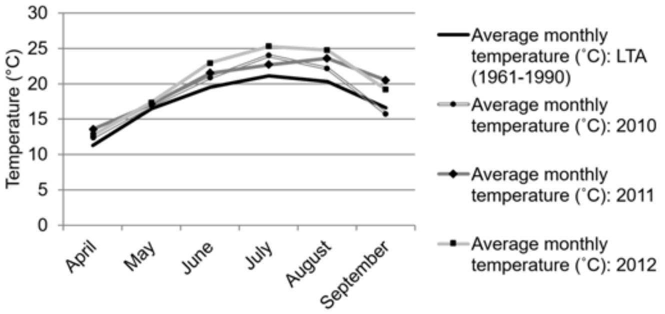

Figure 1. LTA (1961-1990) monthly temperatures and average monthly temperatures (°C) in years 2010-2012 for soybean growing season (April-September) in Osijek, Republic of Croatia (Croatian Meteorological and Hydrological Service)

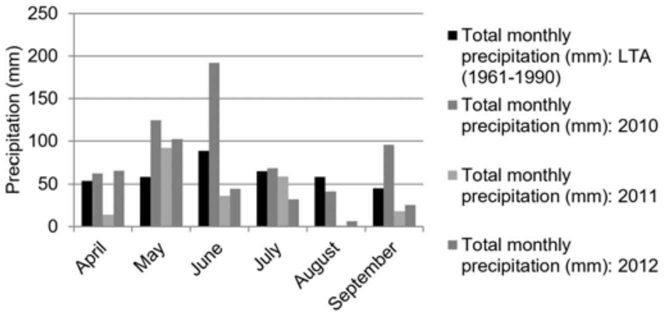

Figure 2. Total monthly precipitation LTA and total monthly precipitation (mm) in years 2010–2012 for soybean growing season (April–September) in Osijek, Republic of Croatia (Croatian Meteorological and Hydrological Service)

Each year, the experimental plot size was 10 m^2, between row distance 50 cm, and seed distance within a row 2-3 cm. During the vegetation, all currently accepted agricultural management practices were applied.

The trial plots were harvested every trial year at full harvesting maturity (R8) (Fehr et al., 1971) after which composite dry grain samples per genotype and replication were used for chemical analyses.

Saccharide concentrations were determined by high-performance liquid chromatography (HPLC) method according to Giannoccaro et al. (2006). Aqueous sample extracts were used to identify saccharides by their retention time, and quantification was done according to the peak area using the external-standard procedure. Stachyose, raffinose, sucrose, glucose, fructose, total saccharide and total oligosaccharide concentration values were expressed in % of seed dry matter (DM).

The first step in the statistical analysis was calculating the basic measures of descriptive statistics (range, average value, the coefficient of variation) for each trait in Microsoft Office Excel 2010. Microsoft Office Excel 2010 was also used for determining the phenotypic correlation coefficient and regression for genotypes averaged over years. The analysis of variance, Duncan's multiple range post hoc test ($P \leq 0.01$) and the pair-wise similarity based on the Euclidean distance were all conducted in software STATISTICA 12.0 (StatSoft Inc., USA). The cluster analysis, presented by a dendrogram constructed using the single linkage method (nearest neighbour clustering), grouped genotypes into classes according to the similarity in their saccharide concentrations. According to Antalikova et al. (2008), this method is suitable for identification of divergence among genotypes based on pedigree, morphological or agronomic traits and can be useful in confirming the results of ANOVA in preliminary plant breeding research.

RESULTS AND DISCUSSION

A highly significant ($P \leq 0.01$) influence of genotype, year and their interaction on the expression of studied traits was evident after ANOVA, but the year had no significant influence on total saccharide content (Table 1). Geater et al. (2000) determined somewhat different sources of variation while researching sucrose, raffinose, stachyose and total sugars in 16 small-seeded soybean cultivars used

Table 1. ANOVA (mean squares and significance) for analysed traits (TS total saccharides, TO total oligosaccharides, G glucose, F fructose, S sucrose, R raffinose, ST stachyose) in 22 soybean genotypes (Osijek, Republic of Croatia, 2010–2012)

Source of variation	TS	TO	G	F	S	R	ST
Genotype (G)	0.095**	2.403**	0.125**	0.623**	7.392**	1.432**	5.280**
Year (Y)	2.216^{ns}	0.374**	0.006**	0.010**	0.586**	0.125**	0.175**
G x Y	4.170**	0.644**	0.010**	0.009**	0.751**	0.196**	0.251**

** - Highly significant; ^{ns} - Non-significant (P≤0.01)

for making *natto* ie. genotypes differed significantly only in the case of total sugars and raffinose while raffinose is the only saccharide for which the significant year effect was determined. In a study by Taira (1990), raffinose and stachyose variability among 67 genotypes produced locally in Japan was mostly influenced by genotype, while environment had a bigger impact than genotype on the variability of total saccharides and sucrose. Both genotype and year were statistically significant sources of variation for sucrose concentration in the research by Maughan et al. (2000) conducted on a F2 population consisting of 149 individual plants, developed from an interspecific cross between an adapted high-sucrose large-seeded G. *max* breeding line (MG V) and a low sucrose small-seeded G. *soja* plant (MG IV). Although it was evident from the results of this research that total saccharide concentration was mostly under genetic control, concentrations of individual saccharides, as well as total oligosaccharides were significantly influenced by the year as well (Table 1). This meant that, in the given set of genotypes, breeding for total saccharide content is much more predictable than altering sugar profiles.

The range for total saccharide concentrations (Table 2) was much narrower (5.69-7.68%) than the range (2.19 to 18.4%) determined for 23 soybean cultivars in the research by Geater and Fehr (2000). The average value for the total saccharide concentration in this research (6.62%) was below the minimal value (7.26%) in the research by Hou et al. (2009) who investigated a much larger set of genotypes, ie. 241 genotypes originating across the world. Ranges, as well as average concentrations for glucose (0.16-0.29%; 0.23%), fructose (0.11-0.28%; 0.18%), sucrose (2.32-3.46%; 2.99%), raffinose (0.66-1.10%; 0.85%) and stachyose (1.64-2.41%; 1.93%) determined in this research were in the lower part of the ranges determined by Hou et al. (2009). Wide concentration ranges for glucose, fructose, sucrose, stachyose and raffinose were determined by Hymowitz and Collins (1974) investigating 194 soybean cultivars ranging in maturity from Group 00 to Group IV and Hartwig et al. (1997) investigating 20 high oil genotypes and 20 high protein genotypes. Hymowitz and Collins (1974) concluded that germplasm tested in their research contains sufficient variability for altering total sugar or soluble sugar concentrations. Geater et al. (2000) determined 6.2-7.1% range for sucrose, 0.49-0.58% range for raffinose and 4.7-4.9% range for stachyose while investigating 16 small-seeded soybean cultivars. The reason why lower saccharide concentrations were determined in this research (Table 2) than in all other mentioned studies may lie in the number, but also in the origin of investigated genotypes. For example, genotypes in the Hou et al. (2009) study mostly originated from areas where soybean is predominantly used for human consumption and therefore has specifically tailored sugar profiles. In the creation of the genotypes used in this research, the accent in breeding was put on increasing the protein concentration, which is reported to be in negative correlation with total saccharide (Krober and Carter, 1962; Openshaw and Hadley, 1981), raffinose and sucrose (Hartwig et al., 1997) concentrations. In Europe, protein concentration is still the most important seed quality because soybean seed is still mainly being used as animal feed.

Table 2. Basic measures of descriptive statistics and Duncan's post hoc test for analysed traits (TS total saccharides, TO total oligosaccharides, G glucose, F fructose, S sucrose, R raffinose, St stachiose) in 22 soybean genotypes suitable for growing in Europe (Osijek, Republic of Croatia, 2010–2012)

Genotype	MG	TS	TO	G	F	S	R	ST
		Average (2010-2012)						
OS-L-453	I	5.86^{jk}	2.44^{l}	0.21^{ef}	0.13^{k}	2.78^{hi}	0.66^{l}	1.78^{jk}
OS-L-793	0	6.75^{ef}	2.78^{fgh}	0.22^{e}	0.21^{d}	3.02^{ef}	0.94^{de}	1.81^{hij}
OS-L-788	0-I	6.07^{i}	2.61^{jk}	0.21^{e}	0.13^{jk}	2.83^{gh}	0.71^{jk}	1.89^{g}
OS-L-513	0-I	5.69^{k}	2.40^{l}	0.19^{fg}	0.17^{ef}	2.65^{j}	0.75^{hi}	1.64^{l}
OS-L-526	0	5.73^{k}	2.80^{fg}	0.16^{h}	0.11^{l}	2.32^{k}	0.74^{ij}	2.06^{c}
OS-L-794	0	5.96^{ij}	2.49^{l}	0.22^{e}	0.14^{ij}	2.78^{hi}	0.69^{k}	1.78^{jk}
OS-L-554	0-I	6.34^{h}	2.64^{ijk}	0.24^{cd}	0.21^{d}	2.84^{gh}	0.87^{f}	1.76^{k}
OS-L-581	0	6.85^{e}	2.69^{hij}	0.24^{cd}	0.16^{fghi}	3.41^{ab}	0.66^{l}	2.03^{cde}
OS-L-711	00	6.51^{g}	2.79^{fg}	0.24^{cd}	0.17^{efgh}	2.97^{f}	0.79^{g}	1.99^{e}
OS-L-712	00-0	6.09^{i}	2.72^{ghi}	0.19^{g}	0.16^{efgh}	2.62^{j}	0.69^{kl}	2.03^{cde}
OS-L-806	0	7.12^{d}	3.00^{cd}	0.26^{b}	0.20^{d}	3.03^{ef}	1.01^{bc}	2.00^{de}
OS-L-821	0-I	7.08^{d}	2.91^{e}	0.23^{d}	0.22^{c}	3.07^{e}	0.97^{de}	1.94^{f}
OS-L-805	0	6.75^{ef}	3.18^{b}	0.18^{g}	0.15^{hi}	2.75^{i}	0.98^{cd}	2.19^{b}
OS-L-442	0-I	6.08^{i}	2.57^{k}	0.21^{e}	0.17^{e}	2.63^{j}	0.74^{ij}	1.83^{hi}
OS-L-874	0-I	7.58^{ab}	2.97^{de}	0.29^{a}	0.28^{a}	3.41^{ab}	1.10^{a}	1.85^{gh}
OS-L-875	0-I	7.11^{d}	2.74^{fgh}	0.26^{b}	0.22^{c}	3.36^{b}	0.94^{e}	1.78^{jk}
OS-L-877	00-0	7.43^{bc}	3.08^{c}	0.26^{b}	0.20^{d}	3.40^{ab}	1.03^{b}	2.03^{cd}
OS-L-800	0	6.60^{fg}	2.72^{ghi}	0.24^{cd}	0.17^{efg}	3.03^{ef}	0.86^{f}	1.85^{h}
OS-L-899	0-I	7.31^{c}	3.04^{cd}	0.25^{bcd}	0.25^{b}	3.28^{c}	1.09^{a}	1.93^{f}
ZG-1	II	6.73^{ef}	2.82^{f}	0.21^{e}	0.15^{ij}	3.18^{d}	0.75^{hi}	2.07^{c}
NS-1	I	6.26^{h}	2.58^{k}	0.22^{e}	0.15^{ghi}	2.88^{g}	0.78^{gh}	1.79^{ijk}
SG-1	0	7.68^{a}	3.40^{a}	0.25^{bc}	0.17^{efg}	3.46^{a}	0.97^{de}	2.41^{a}
Average (Genotypes)		6.62	2.79	0.23	0.18	2.99	0.85	1.93
Minimum		5.69	2.40	0.16	0.11	2.32	0.66	1.64
Maximum		7.68	3.40	0.29	0.28	3.46	1.10	2.41
CV (%)		9.18	8.94	13.42	23.42	10.47	16.93	8.85
$Q_{(0.01)}$		0.169	0.083	0.015	0.014	0.069	0.033	0.040

Genotype means with the same superscript letter are not significantly different at $P \leq 0.01$

The existence of phenotypic variability among tested genotypes was confirmed by the coefficient of variation (CV). In average, relative variability (Table 2) was the highest in the case of fructose (23.42%), followed by raffinose (16.93%) and glucose (13.42%). The lowest CV was determined for stachyose (8.85 %) but total oligosaccharide CV was very near (8.94%). In a population of 92 $F_{5:7}$ recombinant elite lines developed by crossing the line MD 96-5722 with Spencer, Akond et al. (2015) determined somewhat different order of variability, ie. sucrose had higher CV (12.37%) in comparison to raffinose (11.11%) and stachyose (6.73%).

In all, CV values determined for saccharide concentration in this research were relatively high in comparison to other soybean seed quality parameters. For example, Matoša Kočar et al. (2017) determined CV values of 1.81% for protein concentration and 2.26% for oil concentration in a 3-year field trial conducted in the same agroecological region but with a somewhat different set of genotypes. CV values for fatty acids determined in a similar set of genotypes tested over the same time period (2010-2012) ranged from 2.48% to 7.01% (Matoša Kočar et al., 2018). This indicates that there is potentially more room for progress in altering saccharide profiles than other important seed quality traits.

The influence of weather condition was evident from the significant differences in average concentration values over years (Table 3). The differences were lacking only for total saccharides. Sucrose and stachyose had the lowest average concentrations recorded in 2012 (2.54%; 1.58% respectively) which had higher average monthly temperature values for soybean growing season in comparison to the LTA values, as well as values recorded in 2010 and 2011(Figure 1). This coincides with the results in Wolf et al. (1982) research in which authors concluded that sucrose and stachyose concentrations decreased with the rise of average year temperature but temperature had no effect on glucose, fructose and raffinose. In this research, total oligosaccharides had the highest concentrations in both 2010 and 2011 and lowest in 2012, glucose and fructose concentrations were the highest in 2012 and lowest in 2010 and 2012 respectively, while raffinose had the highest concentration values in 2011, and lowest in 2010 (Table 3). Since, according to Wolf et al. (1982) temperature had no effect on glucose, fructose and raffinose, significant differences between years in concentrations of mentioned saccharides in this research (Table 1), could therefore be influenced by some other environmental effect if not temperature, but further investigation is needed to determine possible causes.

The year effect was also confirmed by coefficients of variation (CV) which differed between years (Table 3). The highest CV values were determined in 2012 for all parameters except fructose concentration, and the lowest in 2010 for all except glucose (2011) and fructose (2012) concentrations. This can lead to a conclusion that high temperatures and the lack of precipitation determined in 2012 (Figure 1 and 2) were favourable for the expression of variability of all parameters except fructose. At the same time, such conditions were unfavourable for saccharide accumulation since 2012 was the year the lowest average concentration values were determined for total oligosaccharides, sucrose and stachyose. It is evident that high variability was followed by low average values for all saccharide parameters except glucose which had both maximum variability and the maximum average value in 2012 (Table 3).

Since understanding correlations among important characters can facilitate proper interpretation of results (Kumar et al., 2015), determining relationships between different saccharides is considered beneficial for planning more efficient breeding programmes. For example, the significant positive phenotypic correlation between raffinose and stachyose as well as the significant negative correlation between sucrose and mentioned oligosaccharides would enable effective indirect selection procedure for decreased raffinose and stachyose (Neus, et al., 2005). This means that only single saccharide concentration needs to be determined and followed during the selection process which would lower the expenses and reduce time invested in analyses. Besides Neus et al. (2005), who determined significant

Table 3. Average yearly concentration values (% DM), Duncan's post hoc test and CV (%) for analysed traits (TS total saccharides, TO total oligosaccharides, G glucose, F fructose, S sucrose, R raffinose, St stachyose) in each trial year (2010-2012) for 22 soybean genotypes suitable for growing in Europe (Osijek, Republic of Croatia, 2010–2012)

		TS	TO	G	F	S	R	ST
	2010	6.63^{a}	2.92^{a}	0.21^{b}	0.04^{c}	3.34^{a}	0.65^{c}	2.27^{a}
Average	2011	6.57^{a}	2.93^{a}	0.19^{c}	0.23^{b}	3.08^{b}	0.98^{a}	1.93^{b}
	2012	6.65^{a}	2.52^{b}	0.29^{a}	0.26^{a}	2.54^{c}	0.93^{b}	1.58^{c}
	2010	8.13	8.64	23.24	42.94	11.19	16.29	7.44
CV (%)	2011	13.58	11.52	17.01	28.06	15.22	23.65	8.42
	2012	21.83	22.83	23.69	27.32	21.90	33.36	25.13
	$Q_{(0.01)}$	0.063	0.031	0.006	0.006	0.025	0.012	0.015

Year means with the same superscript letter are not significantly different at $P \leq 0.01$

and very strong positive relationship between raffinose and stachyose as well as significant and very strong negative relationship between sucrose and raffinose and stachyose together, the significant strong positive correlation between raffinose and stachyose was also determined by Hou et al. (2009) in 241 plant introductions obtained from the USDA Soybean Germplasm Collection. The significant negative correlation between sucrose on one side and raffinose and/or stachyose on the other was found in studies by Hymowitz et al. (1972) in 60 soybean plant introductions, forage crop introductions and varieties belonging to Maturity groups 00 to IV, Hartwig et al. (1997) in 20 genotypes with medium to high oil content, 2 genotypes with high seed protein content and 18 genotypes with high seed yield and high seed protein, and Mozzoni et al. (2013) in an F3-derived population from the cross between wild type soybean and high-sucrose/low-oligosaccharides type soybean. Unfortunately, the relationships between saccharides can differ and are not always favourable for indirect selection. For example, Geater et al. (2000) determined no significant correlation between raffinose, stachyose and sucrose in 16 small-seeded cultivars and lines developed by Iowa State University. Hartwig et al. (1997) determined a significant moderate positive correlation between raffinose and sucrose in 12 widely grown cultivars adapted to latitudes between 30° and 37° and 8 advanced breeding lines, while Hou et al. (2009) determined the relationship between sucrose and raffinose to be significant, positive and strong. Although many authors determined the existence of significant relationships among different saccharides in the soybean seed, the only 2 significant correlation coefficients in this research were determined between sucrose and fructose and sucrose and raffinose (Table 4). The first (-0.17*) represented no or negligible relationship, and the second (0.23*) weak positive relationship.

In order to get a better insight into divergence between genotypes according to their saccharide concentrations, the cluster analysis was conducted. The pair-wise similarity based on the Euclidean distance divided all genotypes into 2 main groups with the distance of 0.56 between them (Figure 3). One group included only the Canadian cultivar SG-1 and the other group all 21 remaining genotypes, further divided into subgroups. Foreign genotype NS-1 originally from Serbia and genotype originating from the different domestic breeding house (ZG-1) were grouped together with genotypes from the Agricultural Institute Osijek. This was expected since genotypes from Pannonian Plain, to which these belong to, are known to be genetically very similar (Perić et al., 2014). Nevertheless, the formation of groups and subgroups in the cluster analysis confirms previously determined divergence among 22 soybean genotypes concerning their saccharide concentrations.

Table 4. Phenotypic correlations coefficient among analysed traits (TS total saccharides, TO total oligosaccharides, G glucose, F fructose, S sucrose, R raffinose, St stachyose) of 22 soybean genotypes (Osijek, Republic of Croatia, 2010–2012)

	TS	TO	G	F	S	R	ST
TS	1						
TO	0.80^{ns}	1					
G	0.54^{ns}	0.09^{ns}	1				
F	0.33^{ns}	0.01^{ns}	0.41^{ns}	1			
S	0.73^{ns}	0.77^{ns}	0.09^{ns}	-0.17*	1		
R	0.68^{ns}	0.53^{ns}	0.37^{ns}	0.78^{ns}	0.23*	1	
St	0.45^{ns}	0.78^{ns}	-0.17*	-0.55^{ns}	0.73^{ns}	-0.11^{ns}	1

* - Significant; ^{ns} - Non-significant ($P \leq 0.05$)

Since oligosaccharides are indigestible and can cause intestinal irritation while glucose, fructose and sucrose are easily digestible and give soybean end products their characteristic sweet taste (Jones et al., 1999; Gulewitz et al., 2014), if the goal is to produce soybeans for animal or human consumption, it is necessary to create cultivars with decreased raffinose and stachyose concentrations but at the same time increased glucose, fructose and sucrose concentrations. In this research, genotypes OS-L-513 (2.40%), OS-L-453 (2.44%) and OS-L-794 (2.49%) had the lowest concentrations of oligosaccharides, but at the same time their glucose, fructose and sucrose concentrations were in the middle of the range (Table 2). The highest oligosaccharide concentration was determined for SG-1 (3.40%) which should, therefore, be excluded from the breeding programmes whose main aim is creating cultivars for human or animal consumption (Table 2). On the other hand, genotype OS-L-874 is the most suitable for human or animal consumption because it has a very high level of desirable monosaccharides (glucose and fructose) and disaccharide sucrose while the amounts of indigestible oligosaccharides are not among the highest.

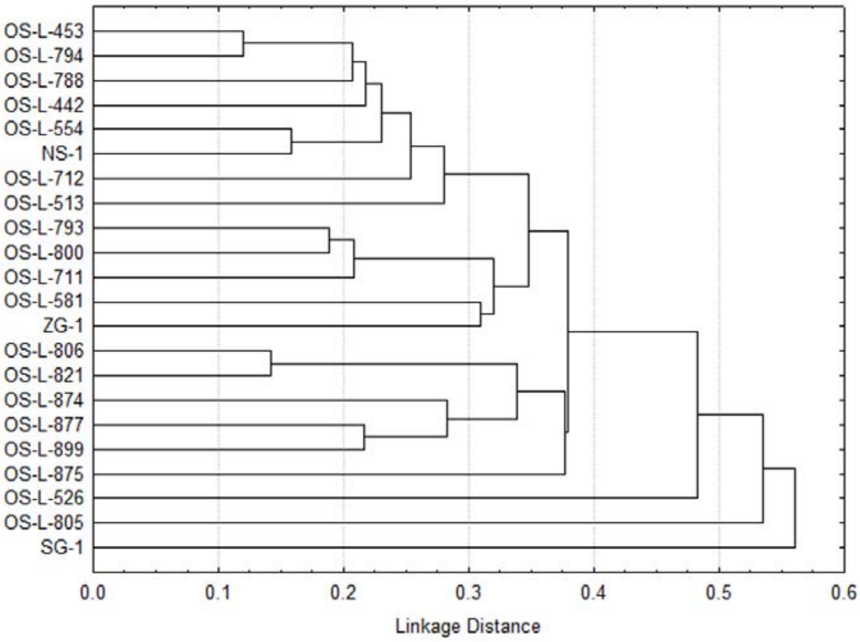

Figure 3. The Nearest Neighbour dendrogram based on the Euclidean distance between 22 soybean genotypes evaluated according to the total and individual saccharide concentration values

CONCLUSIONS

The results of this 3-year study determined the significant variability of total saccharide concentration, total oligosaccharide concentration and the concentrations of glucose, fructose, sucrose, stachyose and raffinose among 22 soybean genotypes suitable for growing in Europe, with genotype and genotype and year interaction having a significant effect on all tested parameters and year on all except total saccharides. Significant genotype effect ensures there is sufficient choice for parent selection, while significant environmental effects mean there is a need for the development of location-specific varieties. Since total saccharide concentration was not under the influence of year but all other parameters were, it can be concluded that altering the amount of total saccharides in soybean seed is much more predictable than altering the saccharide profile. Nevertheless, because of the confirmed differences between genotypes in all tested parameters, genotypes which are more suitable as parents in breeding programmes aiming to improve saccharide profiles could be determined. Although the lack of significant correlation between analysed parameters determined in this set of genotypes can prevent the reduction of breeding costs, the results are beneficial for determining future breeding strategies. These research findings could enable the alteration of total and individual saccharide concentration values in European soybean germplasm, thus improving digestibility and taste of soybean seed for food and feed industries as well as improving the final soybean products for end consumers. Furthermore, as the genetic base is becoming narrower, studies which define germplasm in local genebanks, as well as their susceptibility to environmental factors are enabling the introduction of diversity by means of material exchange between different breeding institutions across Europe.

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