Characterization and interrelationships of soybean [*Glycine max* (L.) Merrill] yield components during dry and humid seasons

Karakterizacija i povezanost komponenti prinosa soje [*Glycine max* (L.) Merrill] tijekom sušne i vlažne godine

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Abstract

Global climate changes have caused a significant weather oscillation. The objectives of this study were to determine the influence of weather conditions on the association of soybean seed yield and yield components, to find out the magnitude and effect of each component and their reaction to environmental stress. Seventy-four soybean varieties from nine geographical origins were studied during two seasons (2015 with less rainfall and increased temperatures and 2016 with increased humidity and moderate temperatures) at the Croatian Centre for Agriculture, Food and Rural Affairs in Osijek, Croatia. Correlation and path analysis were used to examine the association of the studied traits. The variance analysis revealed significant (P<0.01) effect of genotype, year and their interactions on all examined traits. During both seasons the seed yield was in significant and positive correlation with seed number per plant, pods number per plant, seed number per pod and 1,000-seed weight. All the traits mentioned had a stronger correlation with the yield in the dry 2015 compared to the year 2016. The seed number per plant had the highest correlation coefficients and a high and positive direct effect on seed yield in both years. Therefore, the selection of high yielding genotypes based on this trait can be done directly regardless of the variable weather conditions. The hierarchical clustering of varieties resulted in eight clusters in both years, confirming high genetic variability of the examined varieties. In 2016 one cluster that mainly consisted of varieties typical for the breeding programs of this region was singled out.

Keywords: correlation, hierarchical clustering, path analysis, seed yield, soybean, weather oscillation, yield component

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Sažetak

Globalne klimatske promjene uzrokuju sve veće vremenske oscilacije. Ciljevi ovog istraživanja bili su odrediti utjecaj vremenskih čimbenika na povezanost prinosa zrna soje i komponenti prinosa, ispitati jačinu i učinak svake komponente te njihovu reakciju na okolišni stres. Tijekom dvije vegetacijske sezone (2015. sa sušnim uvjetima i povišenim temperaturama i 2016. s vlažnim vremenskim uvjetima i umjerenim temperaturama) pri Hrvatskom centru za poljoprivredu, hranu i selo u Osijeku proučavane su sedamdeset četiri sorte soje podrijetlom iz devet različitih geografskih izvora. Za ispitivanje povezanosti proučavanih svojstava korišteni su korelacijska i path analiza. Analiza varijance pokazala je značajan (P<0,01) učinak genotipa, godine i njihove interakcije na sva ispitivana svojstva. Tijekom obje sezone prinos sjemena je bio u signifikantnoj i pozitivnoj korelaciji s brojem zrna po biljci, brojem mahuna po biljci, brojem zrna po mahuni i masom 1000 zrna. Navedena svojstva su imala jaču korelaciju sa prinosom u sušnoj 2015. u usporedbi s 2016. godinom. Broj zrna po biljci je imao najviši koeficijent korelacije te visok i pozitivan izravni učinak na prinos u obje godine pa se izbor genotipova visokih prinosa na temelju ovog svojstva može obaviti izravno bez obzira na promjenjive vremenske uvjete. Hijerarhijsko grupiranje sorti rezultiralo je s osam klastera u obje godine, potvrđujući visoku genetsku varijabilnost ispitivanih sorti. U 2016. godini izdvojen je jedan klaster koji se uglavnom sastojao od sorti karakterističnih za uzgojni program ove regije.

Ključne riječi: hijerarhijsko grupiranje, komponente prinosa, korelacija, path analiza, prinos zrna, soja, vremenske oscilacije

Introduction

Soybean is the most important food and feed crop in the world due to its high protein and oil production. Cultivated form, *Glycine max* (L.) Merr. is a tetraploid (2n=40), it belongs to the family *Leguminosae,* the genus *Glycine* and the subgenus *Soja*. The soybean has recently increased its share in agricultural, medicinal and industrial sectors. The leading world producers are the United States, Brazil, Argentina, India and China (FAOSTAT, 2016). According to FAO reports (2016), the world production of soybean is around 121 million ha with an average yield of 2.75 t/ha. In the Republic of Croatia soybean is grown on 88,867 ha with an average yield of 2.2 t/ha (Croatian Bureau of Statistics, 2016). As the world demand for soybean increases, the breeders have to bridge the limitations of soybean production, such as low yield, chemical compound demands, disease susceptibility, adaptability to very variable weather conditions, such as drought and high temperatures, etc. Soybean seed yield is the product of many components and their interactions that can affect yield directly or indirectly. Therefore, it is important to evaluate the contribution of each trait, and to give more attention to those with higher influence (Malek et al., 2014). According to Vratarić and Sudarić (2008), for the successful selection of high-yielding genotypes the association between the yield components and seed yield itself needs to be known, as well as the relationship between the yield components and the changes of individual components under the influence of the environment. Unpredictable

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changes in weather conditions necessitate the need to conduct the research during two or more years to derive logical conclusion regarding genotype selection, especially when the experiments are conducted on the same geographical location (Ghodrati, 2013). The influence of climatic changes, particularly precipitation and temperature regimes have a direct and often negative influence on the quantity and quality of field crop yields (Kovačević et al., 2013; Tucak et al., 2016). The objectives of this research were to investigate yield components responsible for yield improvement and their relations during two seasons of opposite weather conditions, to determine the magnitude and the direction of the effect for each trait as well as their reaction to environmental stress. Additionally, the genetic variability of the examined soybean germplasm in two seasons has been assessed with an aim to find out if there is any segregation regarding the different geographical origin.

Materials and methods

Seventy-four soybean varieties originating from nine countries: Austria (1), Canada (15), Croatia (25), Czech Republic (1), France (6), Hungary (5), Serbia (11), Switzerland (3) and the USA (7) were used as the experimental material. At the beginning of the experiment (2015) all the soybean varieties from the List of Varieties of the Republic of Croatia (35) were included in tested collection, which means that the trial included the varieties of good adaptability to the growing conditions and ranged from very early (000) to late (II). The field experiment was carried out at the experimental field of Croatian Centre for Agriculture, Food and Rural Affairs (CCAFRA), Institute for Seed and Seedlings in Osijek (N 45°33', E 18°40') during two consecutive growing seasons (2015-2016). At the experimental site the soil type was Eutric brown with silty loam texture. The soil properties are shown in Table 1.

Table 1. Soil properties at the experimental site

Humus content	AL-P_2O_5	AL-K_2O	pH (H_2O)
1.72%	22.9 mg/100 g soil	26.7 mg/100 g soil	6.4

Field trials were sown with Wintersteiger plot machine on 17 April 2015 and 13 April 2016. The experiment was set up according to randomized complete block design with two replicates. Each plot consisted of four rows, each 5 m long. Row to row spacing was 50 cm and plant-to-plant distance in rows was 5 cm. All the recommended agronomic practices were followed in both seasons. Harvesting was done in full maturity. At this stage ten plants from each replicate were randomly selected from the two middle rows. The data were collected on each plant that was harvested by threshing machine Wintersteiger LD 350. The obtained data were used to calculate averages for each replicate. The following traits were measured: plant height (PTH) (cm), pods number per plant (PNPL), seed number per plant (SNPL), seed number per pod (SNPD) (calculated as the ratio of seed number per plant and pods number per plant), 1,000-seed weight (TSW) (g) (calculated as the ratio of seed weight per plant and the number of seeds per plant), the number of days to the

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beginning of flowering (NDBF) (counted from the emergence to the date when 50% plants have at least one flower open), the number of days to maturity (NDM) (counted from the emergence to the date when 95% pods matured), protein (PRTC) and oil content (OILC) (expressed as a percentage on a dry matter basis) and seed yield per plant (SYPP) (g). The protein and oil content were determined with a Near infrared spectrometer IM 9500 (Perten Instruments AB, Sweden). The IM 9500 uses near-infrared transmission to analyse samples with the wavelength range 570-1,100 nm.

The weather data for the soybean growing seasons (April-September) during the study period (2015 - 2016) and the long-term average (LTA) for the experimental site Osijek are presented in Table 2 (Source: Meteorological and Hydrological Institute of Croatia, 2017). In 2015 the average air temperature during growing season was 1.6 °C higher compared to the LTA. Both temperature and precipitation had higher and adverse oscillation, particularly during intensive soybean growth, pod development and filling. The highest differences in average month temperatures in comparison to LTA were recorded in July (+2.9 °C) and August (+3.1 °C). In the same year in April (-45 mm), June (-65 mm) and July (-34 mm) total monthly precipitation was significantly lower compared to the LTA values. This indicates that the 2015 season had very stressful growing conditions. In the season 2016, the variation of average monthly temperatures was less pronounced. Compared to the LTA, the season of 2016 had 0.8 °C higher average temperature. The rainfall in the period April - September 2016 was 428 mm or 42 mm above the LTA and 112 mm higher than 2015 season.

The differences between varieties in examined traits were tested with the factorial analysis of variance (P<0.01). Pearson's correlation coefficients were used to determine the relationships among the examined traits in both years. The path analysis was carried out according to Akintunde (2012). By using these two methods complementary, the misleading cases of high correlation between two traits, where high correlation is actually an indirect product of other traits, can be avoided (Dewey and Lu, 1959; Iqbal et al., 2010). Hierarchical clustering was carried out on all examined traits. The difference between varieties was calculated as Euclidean distance and the varieties were grouped according to unweighted pair-group method (UPGMA). The statistical analyses were carried out with SAS 9.1.3. for Windows and Enterprise Guide 5.1. of the SAS System for Windows (SAS Institute Inc., 2012).

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Table 2. The average monthly air temperatures and total monthly precipitation from April to September during 2015 and 2016 at the experimental site Osijek

Month	Average monthly air temperature (°C)			Total monthly precipitation (mm)		
	2015	2016	LTA[a]	2015	2016	LTA[a]
April	12.1	13.1	11.5	13	40	58
May	17.8	16.5	16.6	113	63	71
June	20.8	21	19.8	17	100	82
July	24.6	22.8	21.7	26	111	60
August	23.7	20.6	20.9	106	72	59
September	17.9	18.1	16.7	41	43	56
Average/Total*	19.5	18.7	17.9	316*	428*	386*

[a]LTA – long-term average (Osijek, 1899-2015, source: http://klima.hr/klima.php?id=k1&param=srednjak&Grad=osijek)

Results and discussion

The mean, range and variability (CV) of the examined traits for 74 soybean varieties in both years are shown in Table 3. The most variable trait in 2015 was SYPP (27.8%), while the most variable trait in 2016 was PTH (20%). The high difference in SYPP variability between season 2015 (27.8%) and 2016 (15.1%) could be a consequence of the stressful environmental condition in 2015 (Table 2). According to Ray et al. (2015), globally, 32–39% of the yield variability could be explained by climate variability. Besides SYPP, the variability of SNPL in the examined set of varieties was also highly affected by unfavourable environmental conditions in 2015. As a consequence of such conditions, SNPL variability was 6.9% higher in 2015 in comparison to 2016. Higher variability among soybean varieties in SYPP and SNPL in 2015 in comparison to 2016 can be explained as a varietal specificity or varietal ability to adapt to unfavourable environmental conditions.

Among the examined traits, PTH (23.6%) and TSW (11.2%) showed the highest increase in mean values in 2016 compared to 2015. In addition, the SYPP in 2016 was also higher in comparison to 2015, but only for 1.7 g. The decrease in yield components can be attributed to drought and stressful weather conditions (Srebrić and Perić, 2014). On the other hand, SNPL, SNPD, NDBF, and OILC were higher in 2015 in comparison to 2016.

The factorial analysis of variance showed that genotype (G), year (Y), and genotype x year (GxY) interaction had a significant effect on all studied traits (Table 3). Salimi et al. (2012) have also shown a significant difference among genotypes for yield and

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yield traits under drought stress condition. Although GxY interaction was significant for all examined traits, the highest effect of GxY has been recorded for SNPL (Table 3). By analysing soybean yield components during the three year testing on 10 genotypes, Ghodrati (2013) also determined a significant effect for the year, genotype and their interactions for all studied traits, merely the highest GxY effect had biological yield and seed yield.

Yield per se is a very complex quantitative trait influenced by many different genes and environment. All associations between yield and yield components are considered to have a high importance for indirect selection, so the rational approach has to be made towards enlargement of yield components (Moe and Girdthai, 2013). In both years SYPP was in significant and positive correlation with SNPL, PNPL, SNPD and TSW (Table 4). However, all the mentioned traits had stronger correlation with SYPP in the dry 2015 compared to 2016 (Table 4). In 2016 PRTC and OILC were also in correlation with SYPP while in 2015 the correlation between SYPP and PRTC and OILC, respectively, has not been found (Table 4).

In both years SNPL was in a positive and significant correlation with PNPL and SNPD (Table 4). In addition, SNPL also had a high positive direct effect on the yield in both years (Path coefficients 0.95 and 0.55 in 2015 and 2016, respectively) (Table 5, Table 6). Therefore, the selection of high yielding genotypes based on this trait can be done directly regardless of the weather conditions. Kobraee and Shamsi (2011) also reported that SNPL in stress conditions had the highest direct effect.

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Table 3. Mean square values, means, range and coefficient of variation for ten traits of 74 soybean varieties

Traits	Genotype (G)	Year (Y)	G x Y	Mean	Range	CV (%)	Mean	Range	CV (%)
	Source of variation			2015			2016		
PTH	776.4**	33568.8**	173.2**	68.97	24-110	17.67	90.27	26-142	20.01
PNPL	347.9**	1254.3**	151.6**	62.13	25-116	20.13	66.24	30-123	14.59
SNPL	2459.3**	1783.1**	1281.8**	152.88	49-304	23.37	147.97	63-312	16.47
SNPD	0.09**	3.5**	0.05**	2.46	1.22-3.53	8.42	2.24	1.11-3.77	8.42
TSW	1183.1**	25932.1**	240.5**	149.59	86.1-272.4	12.37	168.31	97.2-239	11.42
NDBF	20.4**	1885.2**	3.8**	36.49	32-47	8.09	31.45	28-39	5.86
NDM	509.8**	154.7**	36.3**	122.28	101-147	9.43	123.73	104-147	9.57
PRTC	15.2**	14.6**	1.3**	38.31	34-45.9	5.55	38.76	34.9-46.2	4.98
OILC	3.8**	31.7**	0.6**	21.29	16.4-23.3	4.84	20.63	16.3-22.3	5.1
SYPP	76.2**	209.4**	33.2**	22.97	5.6-47.6	27.8	24.66	8.8-48.4	15.13

PTH - plant height (cm), PNPL - pods number per plant, SNPL - seed number per plant, SNPD - seed number per pod, TSW – 1,000-seed weight (g), NDBF - number of days to beginning of flowering, NDM - number of days to maturity, PRTC - protein content (%), OILC-oil content (%), SYPP - seed yield per plant (g), CV - coefficient of variation (%); **level of significance P<0.01

PNPL also achieved high correlation coefficients to the yield in both years (Table 4). Merely its effects were different. In 2015 its main positive course of affecting was indirectly through SNPL (Table 5). In 2016 its direct effect was positive and high with also high indirect effect via SNPL (Table 6). SNPD showed highly significant positive correlation with SYPP in 2015 and significant, positive but low correlation in 2016. In 2015, on the contrary to correlation, it had negative direct effect and highly positive indirect effect through SNPL, in 2016 its direct effect was positive with also high indirect effect via SNPL.

Concerning the TSW in 2015, the correlation coefficient with yield was highly significant and positive with high value and also with highly positive direct effect, while in 2016 it had significant but very low correlation, positive direct effect but also notable indirect negative effects from traits of SNPL, PTH, PNPL, NDBF and SNPD towards the seed yield. The positive direct effect of the TSW and its significant negative indirect effects through SNPD and PNPL like in 2016 are in agreement with the results by Malek et al. (2014).

In 2015 PRTC and OILC were not in correlation with SYPP. On the other hand, in 2016 both traits were in significant correlation to SYPP, but PRTC was in negative and OILC in positive correlation to SYPP (Table 4). The negative association of PRTC with yield was manifested indirectly through PNPL and SNPL while the positive association of OILC was achieved also indirectly through TSW (Table 6).

PTH and NDBF did not show any association with SYPP in both years. NDM correlated with yield only in 2015, it had positive but very low value and affected yield mainly indirectly through SNPL. Trait association in soybean was also studied by Kumar et al. (2014) who reported that high yield effect, indicated by path analysis was achieved by 1,000-seed weight. Baraskar et al. (2015) identified the 1000-seed weight and days to maturity as very important yield components, whereas Ali et al. (2015), while analysing the correlation of soybean traits, noted the importance and positive association of number of days to flowering, number of days to maturity, number of pods per plant and 1,000-seed weight with seed yield. By analysing the association of yield and component characters, Aditya et al. (2011) identified the number of pods per plant as the highest correlated trait with seed yield. According to Malek et al. (2014), the significant soybean yield components are the number of pods per plant, seeds per pod and 1,000 seed weight which define the seed yield. Although the seed number per plant is a product of the number of pods per plant and the number of seeds per pod, in the conducted research this observation is crucial by itself. Because of sowing during the unfavourable 2015 season, the pods number per plant had a very low and negative direct effect and its main course of affecting the yield was increasing indirectly through seed number per plant.

Table 4. Pearson's correlation coefficients for nine traits and seed yield per plant in 2015 and 2016 (n = 74)

Traits	YR	PTH	PNPL	SNPL	SNPD	TSW	NDBF	NDM	PROTC	OILC
PNPL	'15	0.27*								
	'16	0.43**								
SNPL	'15	0.19	0.93**							
	'16	0.37**	0.85**							
SNPD	'15	-0.07	0.23*	0.56**						
	'16	0.02	-0.07	0.42**						
TSW	'15	0	0.22	0.2	0.06					
	'16	-0.41**	-0.37**	-0.46**	-0.25*					
NDBF	'15	0.49**	0.19	0.12	-0.11	-0.27*				
	'16	0.69**	0.47**	0.41**	0	-0.36**				
NDM	'15	0.70**	0.34**	0.27*	-0.05	-0.01	0.57**			
	'16	0.66**	0.42**	0.26*	-0.19	-0.12	0.65**			
PRTC	'15	-0.42**	-0.21	-0.25*	-0.22	0.14	-0.4**	-0.53**		
	'16	-0.29*	-0.32**	-0.33**	-0.12	-0.06	-0.21	-0.29*		
OILC	'15	0.2	0.15	0.18	0.144	0.1	0.07	0.24*	-0.79**	
	'16	-0.15	0.059	0.09	0.08	0.32**	-0.21	-0.05	-0.75**	
SYPP	'15	0.15	0.85**	0.89**	0.47**	0.6**	-0	0.23*	-0.13	0.17
	'16	0.11	0.63**	0.72**	0.29*	0.27*	0.17	0.19	-0.42**	0.34**

YR – year of testing, PTH - plant height (cm), PNPL - pods number per plant, SNPL - seed number per plant, SNPD - seed number per pod, TSW - 1000-seed weight (g), NDBF - number of days to beginning of flowering, NDM - number of days to maturity, PRTC - protein content (%), OILC-oil content (%), SYPP - seed yield per plant (g); **level of significance P<0.01, *level of significance P<0.05

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Table 5. Direct (bold) and indirect effect of nine traits (independent variables) on seed yield per plant (g) (dependent variable) in 74 soybean varieties grown in 2015 by path analysis

Traits	PTH	PNPL	SNPL	SNPD	TSW	NDBF	NDM	PRTC	OILC
PTH	-0.011	-0.033	0.179	0.003	0.002	0.003	0.021	-0.006	-0.003
PNPL	-0.003	-0.123	0.884	-0.01	0.098	0.001	0.01	-0.003	-0.003
SNPL	-0.002	-0.114	0.948	-0.025	0.089	0.001	0.008	-0.004	-0.003
SNPD	0.001	-0.029	0.531	-0.044	0.027	-0.001	-0.001	-0.003	-0.002
TSW	0	-0.027	0.193	-0.003	0.439	-0.002	0.000	0.002	-0.002
NDBF	-0.005	-0.024	0.12	0.005	-0.12	0.007	0.017	-0.006	-0.001
NDM	-0.008	-0.042	0.262	0.002	-0.005	0.004	0.029	-0.007	-0.004
PRTC	0.005	0.027	-0.245	0.01	0.063	-0.003	-0.016	0.014	0.013
OILC	-0.002	-0.019	0.173	-0.006	0.046	0.001	0.007	-0.011	-0.017

PTH - plant height (cm), PNPL - pods number per plant, SNPL - seed number per plant, SNPD - seed number per pod, TSW – 1,000-seed weight (g), NDBF - number of days to beginning of flowering, NDM - number of days to maturity, PRTC - protein content (%), OILC-oil content (%)

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Table 6. Direct (bold) and indirect effect of nine traits (independent variables) on seed yield per plant (g) (dependent variable) in 74 soybean varieties grown in 2016 by path analysis

Traits	PTH	PNPL	SNPL	SNPD	TSW	NDBF	NDM	PRTC	OILC
PTH	0.015	0.203	0.207	0.005	-0.318	-0.001	-0.009	0.005	0.004
PNPL	0.006	0.474	0.468	-0.02	-0.29	-0.001	-0.006	0.005	-0.001
SNPL	0.006	0.404	0.549	0.124	-0.36	-0.001	-0.004	0.006	-0.002
SNPD	0	-0.035	0.248	0.274	-0.201	0	0.003	0.002	-0.002
TSW	-0.006	-0.176	-0.253	-0.07	0.783	0.001	0.002	0.001	-0.008
NDBF	0.01	0.223	0.229	0	-0.286	-0.002	-0.009	0.004	0.005
NDM	0.01	0.199	0.144	-0.052	-0.097	-0.001	-0.014	0.005	0.001
PRTC	-0.004	-0.15	-0.183	-0.034	-0.05	0	0.004	-0.017	0.018
OILC	-0.002	0.028	0.05	0.023	0.25	0	0.001	0.013	-0.024

PTH - plant height (cm), PNPL - pods number per plant, SNPL - seed number per plant, SNPD - seed number per pod, TSW - 1000-seed weight (g), NDBF - number of days to beginning of flowering, NDM - number of days to maturity, PRTC - protein content (%), OILC-oil content (%)

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The cluster analyses based on all ten traits were carried out separately for the years 2015 and 2016, and the results are shown in Figure 1. In both years, UPGMA procedure defined eight clusters, indicating notable genetic divergence. Classifications based on morphological traits of different soybean varieties are in agreement with previous reports by Ojo et al. (2012). In 2015 among the eight clusters, two large clusters were separated, both together included 83.7% of the total number of accessions. These two clusters had no clear relationship between genetic diversity and geographical origin. These results are in accordance with the research of Malik et al. (2011) who explained that accessions from one origin entered into more than one cluster lead to discrepancy in genetic diversity and geographical origin, while accessions from different geographical origins showed tendency to be clustered in one part of the dendrogram.

In 2016 three large clusters were separated and all three included 90.6% of the total number of accessions. The largest cluster 3 was mainly consisted of the varieties from the eastern and south-eastern parts of the Pannonian Plain (Croatia, Serbia and Hungary). The exceptions from these 27 varieties were the two from the USA. The moderate conditions of 2016 affected the pattern of this cluster. The varieties of this group with an average number of days to maturity (133), bred in this part of region have shown similar performance, which is in accordance with the findings of Vratarić and Sudarić (2008) that for this part of the region the optimal maturity group of soybean is from 130 to 135 days. It is also indicated in the conclusions of Ristova et al. (2010) and Perić et al. (2014).

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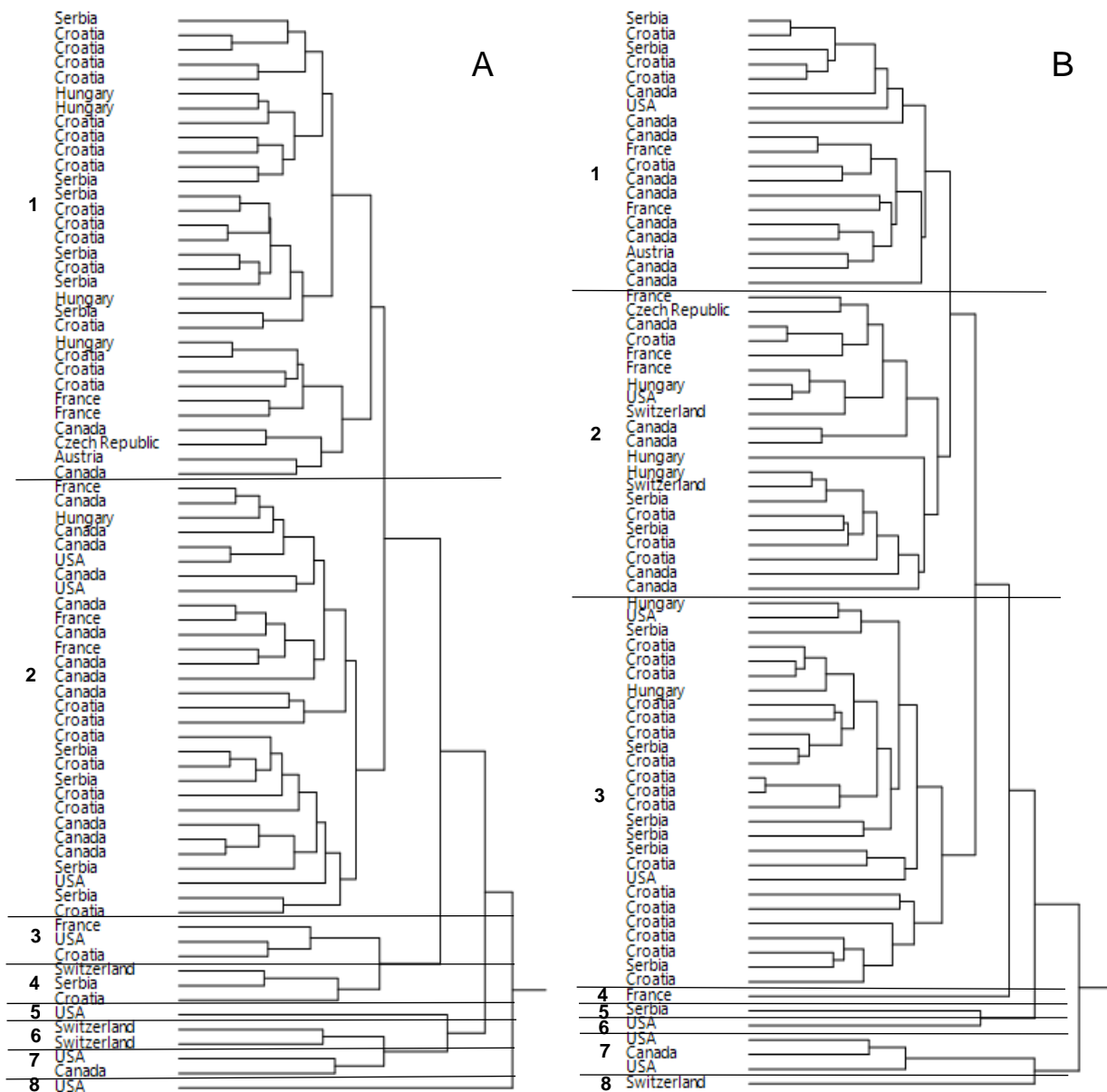
Figure 1. Dendrogram of 74 soybean varieties (geographical origin is shown) based on nine studied traits and yield in a year 2015 (A) and 2016 (B)

Conclusions

In conclusion, the present study revealed a high level of genetic variation in nine traits and seed yield among the tested soybean varieties. On the association between traits the valuable predictors of varietal adaptability to weather oscillation or drought could have been singled out. As the best predictor for the selection of adaptable genotypes in oscillating weather conditions, the number of seeds per plant was demonstrated as the best trait. With this primary predictor, pods number per plant and seed number per pod could also be used as secondary and additional

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predictors for the selection of high yielding varieties. The 1,000-seed weight was shown as a better predictor in drought conditions. Hierarchical clustering based on examined traits in unfavourable 2015 did not show any relatedness between the countries of origin, but in the moderate 2016 season, one cluster that consists mainly of the varieties typical for the breeding programs of this region was formed.

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