ISSN 1330-7142
UDK = 631.523:633.853.52

GENETIC ADVANCE IN QUANTITATIVE TRAITS OF SOYBEAN LINES WITHIN DIFFERENT MATURITY GROUPS

Marija Vratarić [(1)], Aleksandra Sudarić [(1)], Rezica Sudar [(1)], T. Duvnjak [(1)], Draženka Jurković [(2)], Zorica Jurković [(1)]

Original scientific paper
Izvorni znanstveni članak

SUMMARY

The breeding work on soybean at the Agricultural Institute Osijek has primarily been aimed at continued development of new and better cultivars with higher yield potential in the maturity range of 00 to II. Improving of soybean yield potential is based on the improvement of genetic basis of traits determining its quantity and the application of conventional breeding methods used in self-pollinated crops, such as soybean. The objective of this research was to evaluate genetic advance in quantitative performances - grain yield, protein and oil content in grain as well as oil quality of recently developed soybean elite breeding lines, which was done in comparative field tests with standards through the three-year period (2001 to 2003) in Osijek, Croatia. Tested elite breeding lines have been developed from different hybridizations within the Institute's soybean breeding program and selected on the basis of their performance. The grain yield and grain quality value of tested genotypes was estimated through analysis of level and stability of grain yield, protein content and oil content in grain. The tested materials which showed high level and good stability in all three investigated traits, were subjected to the analysis of triacylglycerols. Statistical analysis showed that most of the tested elite breeding lines had significantly higher grain yield, protein and oil content in grain relative to the standards into MGs 00, 0 and I, as well as good stability in analyzed traits. There was a significant genetic variability in triacylglycerols. Generally, these data indicate achieved genetic advance in grain yield and grain quality of domestic soybean lines into MGs 00, 0 and I.

Key-words: soybean (Glycine max (*L.**) Merrill**), grain yield, protein content, oil content, triacylglycerols, trait stability, RP-HPLC, genetic advance, maturity groups.

INTRODUCTION

The Agricultural Institute Osijek has developed a large soybean breeding program that has had an enormous influence on the development, improvement and stability of soybean production in Croatia, particularly in its eastern part. The result of this breeding work is 31 registered soybean cultivars (2 cultivars were registered in Hungary) within maturity groups 00 to II. The registered soybean cultivars are grown on large areas with about 10 Institute's cultivars covering about 70% of total area under soybean production in Croatia. They are characterized by a high yield potential and good adaptability to the local agroecological conditions. Due to these characteristics, cultivars significantly contribute to increasing and improving of soybean production in the country.
The soybean breeding program has had several directions. Firstly, the creation of new genetic variability into cultivated soybean (*Glycine max* (L.) Merrill) as the base for selection of superior germplasm, using genetically diverse materials and different selection methods. Secondly, continuous development of new and improved domestic soybean lines and cultivars for wide soybean production, with high and stable grain yield and satisfactory grain quality (protein content and oil content). Particularly valuable are the new lines with different protein and oil quality. Furthermore, the new soybean lines have high level of tolerance to main diseases occuring in our climatic conditions such as *Peronospora manshurica* (Naum.) Syd ex Gaum., *Sclerotinia sclerotiorum* (Lib.) de Bary,

(1) Prof.D.Sc. Marija Vratarić, D.Sc. Aleksandra Sudarić, D.Sc. Rezica Sudar, D.Sc. Tomislav Duvnjak, D.Sc. Zorica Jurković – The Agricultural Institute Osijek, Južno predgrađe 17, Osijek; (2) Prof.D.Sc. Draženka Jurković – University of J.J. Strossmayer in Osijek, Faculty of Agriculture in Osijek, Trg Sv. Trojstva 3, Osijek

Diaporthe phaseolorum (Cooke&Ellis) Sacc.var.*caulivora* Athow&Caldwell and *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehmayer. Artificial infection methods (inoculation) are used for development of new lines tolerant to disease *Phomopsis longicola* and this work is in progress (Duvnjak, 2004). Identification of superior lines is done through testing in multiple environments to achieve further genetic advance and further improved soybean production in Croatia (Vratarić and Sudarić, 2000; Sudarić et al., 2001; Vratarić et al., 2004). Lately, the focus of our breeding work has been the analysis of oil quality, especially in relation to the fatty acids composition and triacylglycerols (TAG) composition (Sudar et al., 1997, 2003, 2004). The fatty acids composition can be used to evaluate the stability and nutritional quality of oil (Velasco and Fernandez-Martinez, 2002), while distribution of fatty acids in triacylglycerols is interesting for development of cultivars with oil of improved characteristics (Neff et al., 1992, 2001).
The objective of paper was to test the level and stability of grain yield, protein and oil content in the seed of the newly developed elite soybean breeding lines with standards. Second objective was the analysis of TAG composition seed oil of best elite breeding (promising) lines from MGs 00, 0 and I.

MATERIAL AND METHODS

The research was conducted during the three-year period (2001 to 2003) at the experimental field of the Agricultural Institute Osijek (Croatia) and involved three sets of soybean genotypes (elite breeding lines and standards), belonging to maturity groups: 00, 0 and I. The total number (132) of tested genotypes was as follows: 15 genotypes from MG 00, 47 genotypes from MG 0 and 70 genotypes from MG I. Tested elite breeding lines have been developed from different hybridizations within the Institute's soybean breeding program and were selected on the basis of their agronomic performance. Standards for each maturity group are commercial cultivars released by the Institute and grown in Croatia. Field trials were designed as a randomized complete block design (RCBD) with 3 replications using plots of $10m^2$. Each year standard agricultural practices for soybean were applied. The occurrence and intensity of principal soybean diseases were determined during the growing season. Test plots were harvested at full maturity by small plot combine. After harvest, grain yield was measured from each plot and converted to 13% seed moisture and t/ha. Protein and oil content were determined from the grain sample per plot by Infratec 1241 Analyzer and expressed in a percentage of absolutely dry matter of grain (% in ADM). The data were analyzed by analysis of variance (ANOVA), which was also used to test genotype x environment interaction (GEI) for grain yield, protein content and oil content in grain. Stability analysis was performed by parameter S^2_{GxE} – portion of GEI variance of each genotype in total GEI variance (Plaisted and Peterson, 1959). Among tested elite breeding lines, only the elite breeding lines with the highest level and stability in all three analyzed traits (the best lines) were analyzed for triacylglycerols composition of the oil by using a Perkin-Elmer reversed-phase high-performance liquid chromatography (RP-HPLC) system series 200 equipped with isocratic pump and refractive index detector. The separation was performed on two serial connected PE Pecosphere C18 columns (83 x 4.6). The analysis was carried out with a mobile phase acetone/acetonitrile (70:30). Oil samples were dissolved in HPLC-grade acetone and 20 μL aliquots were injected into the column and eluted at a flow rate of 2.5 mL/min. Triacylglycerols from extract were identified by comparing their retention time to standards. The concentrations of TAG were estimated on the basis of their relative peak areas measured with the RI detection. The differences in TAG composition among soybean lines as well as among maturity groups were tested by analysis of variance, with an LSD at $P \leq 0.05$ and 0.01.

RESULTS AND DISCUSSION

The mean values range and mean value (3-year average) for grain yield, content of protein and content of oil in grain of tested soybean elite breeding lines and standards for maturity groups 00, 0 and I with values of LSD-test for genotypes are presented in Table 1.

Table 1. The range and mean of yield, protein and oil content in tested soybean genotypes from 00, 0 and I maturity groups grown in Osijek, 2001-2003

Tablica 1. Raspon prosječnih vrijednosti i prosječna vrijednost uroda zrna, sadržaja bjelančevina i sadržaja ulja u zrnu ispitivanih genotipova soje unutar grupe zriobe 00, 0 i I; 2001.-2003.; Osijek

Maturity group *Grupa zriobe*	Elite breeding lines *Elitne oplemenjivačke linije*		Standards *Standardi*		Overall average *Sveukupni prosjek*	$LSD_{genotypes}$ $LSD_{genotipa}$	
	Range *Raspon*	Mean value *Prosjek*	Range *Raspon*	Mean value *Prosjek*		0.05	0.01
Grain yIeld - *Urod zrna* (t/ha)							
00	2.64 – 3.98	3.25*	2.43 – 3.11	2.77	3.01	0.32	0.73
0	3.45 – 4.22	3.86*	3.12 – 4.09	3.53	3.69	0.30	0.77
I	3.52 – 4.90	4.26*	3.22 – 4.16	3.73	4.00	0.26	0.68
Protein content in grain (% in ADM) – *Sadržaj bjelančevina u zrnu (% u AST)*							
00	35.2 – 38.7	37.74*	35.2 – 37.9	36.95	37.34	0.51	1.04
0	36.2 – 39.6	38.54*	35.9 – 38.2	37.61	38.07	0.86	1.25
I	38.7 - 41.2	39.93**	37.5 – 40.8	38.89	39.41	0.63	1.02
Oil content In grain (% in ADM) – *Sadržaj ulja u zrnu (% u AST)*							
00	22.1 - 23.8	22.92**	20.7 – 22.8	21.86	22.39	0.31	0.83
0	22.5 – 23.4	22.96**	21.3 – 22.9	22.13	22.54	0.33	0.81
I	21.9 – 22.7	22.53*	21.4 – 22.3	21.88	22.20	0.37	0.85

*,** significant difference between the levels of a factor at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 2. Mean values and estimated stability parameters for analyzed quantitative traits of the best elite breeding soybean lines from tested material into 00, 0 and I maturity group; 2001-2003; Osijek

Tablica 2. Prosječne vrijednosti i procijenjeni parametri stabilnosti za analizirana kvantitativna svojstva najboljih linija soje iz testiranog materijala unutar grupa zriobe 00, 0, I; 2001.-2003.; Osijek

Maturity group *Grupa zriobe*	Lines *Linije*	Grain yield *Urod zrna* (t/ha)		Protein content in grain *Sadržaj bjelančevina u zrnu* (% in ADM-*% u AST*)		Oil content in grain *Sadržaj ulja u zrnu* (% in ADM-*% u AST*)	
		Mean value *Prosjek*	S^2_{GxE} *	Mean value *Prosjek*	S^2_{GxE} *	Mean value *Prosjek*	S^2_{GxE} *
00	1. L-2202	3.78	13 773	38.36	0.321	23.10	0.302
	2. L-20	3.69	13 608	37.92	0.348	22.96	0.298
	3. L-30	3.65	14 122	38.12	0.307	23.42	0.327
Mean value of MG 00 *Prosjek grupe zriobe 00*		3.01	14 654	37.48	0.366	22.75	0.342
0	4. L-24	4.18	16 385	39.08	0.400	23.05	0.287
	5. L-25	4.12	16 226	38.76	0.392	23.12	0.253
	6. L-16	3.94	16 293	38.65	0.376	22.97	0.282
	7. L-27	3.90	16 115	38.69	0.398	22.81	0.266
	8. L-29	3.88	16 262	38.72	0.404	22.95	0.273
Mean value of MG 0 *Prosjek grupe zriobe 0*		3.69	16 478	38.07	0.435	22.79	0.296
I	9. L-12	4.68	17 988	40.24	0.602	22.52	0.364
	10. L-114	4.53	17 923	40.81	0.597	22.68	0.352
	11. L-119	4.37	18 164	40.38	0.618	22.49	0.370
	12. L-219	4.30	18 204	39.86	0.591	22.60	0.341
	13. L-226	4.23	17 994	41.02	0.583	22.57	0.366
	14. L-230	4.20	17 882	40.96	0.607	22.70	0.339
	15. L-236	4.12	18 104	40.14	0.599	22.64	0.358
	16. L-237	4.08	17 967	40.48	0.593	22.53	0.355
Mean value of MG I *Prosjek grupe zriobe I*		4.00	18 266	39.41	0.622	22.20	0.379

* S^2_{GxE} – portion of GEI variance of each genotype in total GEI variance – *udio varijance GEI svakog genotipa u ukupnoj varijanci GEI*

The analysis of variance showed significant variability in the phenotypic expression of grain yield, protein and oil content in grain in the elite breeding lines and standards across all maturity groups. It suggests genetic diversity within each maturity group. In a comparison between the elite breeding lines and standards for each maturity group, elite lines had higher values for most traits. In MG 00, elite breeding lines had statistical significant higher values of oil content in the seed ($P<0.01$), higher grain yield and protein content in grain ($P<0.05$) relative to the standards. In MG 0, elite lines showed a significant increase in oil content ($P<0.01$), grain yield and protein content in grain ($P<0.05$) compared to the standards. In MG I, elite lines had higher protein content ($P<0.01$), higher grain yield and oil content ($P<0.05$) than the standards. Therefore, new elite breeding lines showed significant improvement in the three traits in all MGs, suggesting a genetic advance in the important quantitative parameters of new domestic soybean lines.

The mean values and estimated stability parameters for grain yield, protein and oil content in the seed of the best elite breeding lines from MGs 00, 0 and I are given in Table 2.

The stability analysis of grain yield, protein and oil content in grain showed that the experimental material differed in the level and stability of the analyzed traits. Most of the tested lines had high level and stability in one trait and few had high level and good stability in all three traits herein referred to as the best elite lines. Within each maturity group, the best lines had higher mean values of all analyzed traits in relation to the overall means of the maturity group and lower stability parameter than the overall stability for the maturity group. Therefore, the elite lines were considered stable across changing environmental conditions, which is of great production value. Hence, this breeding material represent good genetic basis for further improving soybean production and future hybridizations aimed at further advancing of soybean grain yield and grain quality.

The best elite soybean lines were analyzed for triacylglycerol composition of oil and the results of TAG analysis are presented in Table 3.

Table 3. The range and mean of major triacylglycerols composition (area %) of oil from 16 domestic soybean elite breeding lines

Tablica 3. Raspon vrijednosti i prosječna vrijednost (površina%) značajnijih triacilglicerola ulja iz 16 domaćih ispitivanih elitnih linija soje

TAG	A r e a - *Površina (%)*		
	Minimum	Maximum	Mean value *Srednja vrijednost*
LnLnL	1.037	2.310	1.570
LnLL	5.163	7.998	6.681
LLL	16.780	21.232	19.190
PLnL	2.034	4.045	2.980
LLO	15.018	17.741	16.204
LLP	11.950	15.195	13.625
LOO	7.616	11.074	9.110
LOP	11.096	12.851	12.157
LPP	1.519	3.398	2.272
LOS	2.652	5.241	3.473
OOO	5.846	8.272	6.759
OOP	1.432	3.996	2.468
POP	0.499	3.566	2.021
OOS	0.718	1.863	1.232

Ln-linolenic; L-linoleic; O-oleic; P-palmitic; S-stearic acid - *Ln-linolenska;L-linolna;O-oleinska;P-palmitinska;S-stearinska kiselina*

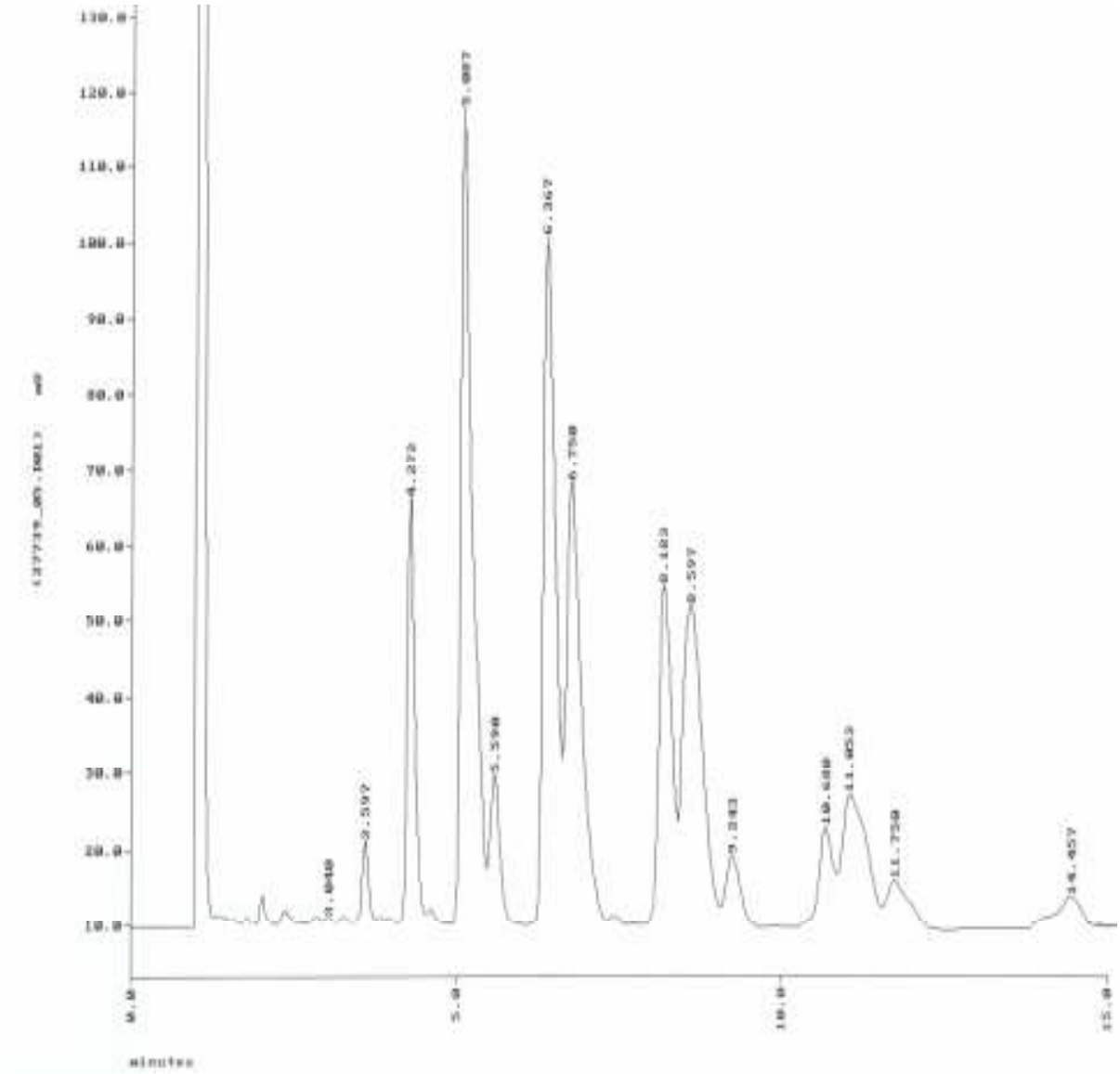

Figure 1. RP-HPLC chromatogram of triacylglycerol in soybean oil (for the soybean line L-16)
Grafikon 1. RP-HPLC kromatogram triacilglicerola sojinog ulja (za liniju soje L-16)

Soybean oil contained 19,19% trilinolein (LLL) as the major TAG, whereas LLO, LLP and LOP varied between 12,16% and 16,21% of the total TAG. The linolenodilinolein (LnLL), linoleodiolein (LOO) and triolein (OOO) varied between 6,68% and 9,11% whereas the other TAG fractions dilinolenolinolein (LnLnL), linolenolinoleopalmitin (LnLP), linoleodipalmitin (LPP), linoleoleostearin (LOS) dioleopalmitin (OOP), dipalmitolein (POP), dioleostearin (OOS), trilinolenin (LnLnLn) were minor, representing less than 4% of the total. Differences in individual triacylglycerols concentration among tested soybean lines were significant ($P \leq 0.05$) suggesting genetic variability in individual TAG concentration. These findings corresponded well to those reported elsewhere for triacylglycerol composition of soybean oil by HPLC (Sudar et al., 1997, 2003, 2004; List et al., 2000; Neff et al., 1992; 2001; Holkčapek et al., 2003). Figure 1 shows a chromatogram of triacylglycerol in the oil of tested line L-16 which is typical chromatogram of triacylglycerol in soybean oil. Individual triacylglycerols concentration was statistically different among the three MGs ($P<0.05$) (Fig.2).

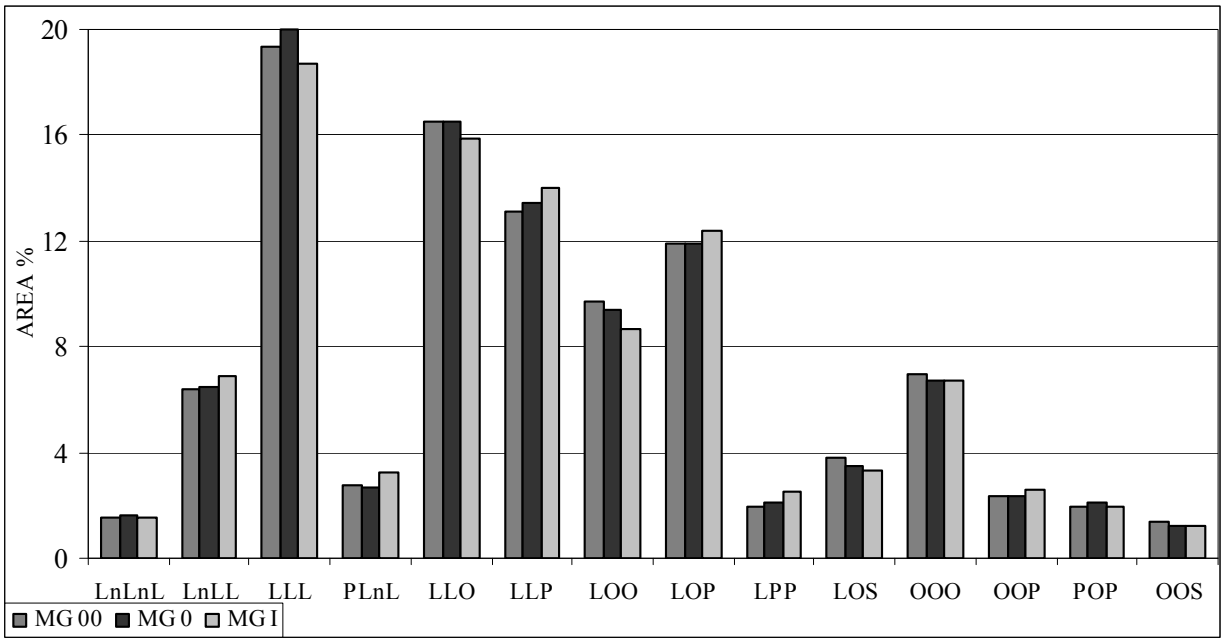

Figure 2. Mean values of triacylglycerols (area %) of soybean lines for 00, 0 and I maturity group; 2001-2003; Osijek

Grafikon 2. Prosječne vrijednosti triacilglicerola (površina %) linija soje po grupama zriobe 00,0, I; 2001.-2003.; Osijek

CONCLUSION

To summarize, data obtained in this study indicate that the new elite lines developed by the Institute were high yielding and stable with good grain quality. Further, our results indicated a genetical variability in individual TAG composition. Further efforts to define relationships between high grain yield and grain quality are in progress. Our study also suggests that choosing lines with superior agronomic and grain quality traits can improve nutritional and functional quality of the soybean oil.

REFERENCES

1. Duvnjak, T. (2004.): Phomopsis longicola Hobbs uzročnik truleži sjemena soje u Hrvatskoj. Disertacija, Poljoprivredni fakultet u Osijeku.
2. Holčapek, M., P. Jandera, P., Zderadička, P., Hruba, L. (2003): Characterization of triacylglycerol and diacylglycerol composition of plant oil using high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. A, 1010:195-215.
3. List, G.R., Neff, W.E., Holliday, J.W., King, J.W., Holster, R. (2000): Hydrogenation of Soybean Oil Triglycerides. J. Am. Oil Chem. Soc., 77:311-314.
4. Neff, W.E., Selke, E., Mounts, T.L., Rinsch, W., Frankel, E.N. (1992): Effect of Triacylglycerol Composition and Structures on Oxidative Stability of Oils from Selected Soybean Germplasm. Journal Am. Oil Chem. Soc., 69:111-118.
5. Neff, W.E., Byrdwell, W.C., List, G.R. (2001): A New Method to Analyze Triacylglcerol Composition of Vegetable Oils. Cereal Foods World, 46:6-10.

6. Plaisted, R.L., Peterson, L.C. (1959): A technique for evaluation the ability of selections to yield consistently in different locations or seasons. Am. Potato J., 36:381-385.
7. Sudar, R., Vratarić, M., Jurković, Z., Sudarić, A. (1997): Fatty acids of soybean oil of different OS cultivars. Eurosoya, 11:10-15.
8. Sudar, R., Jurković, Z., Vratarić, M., Sudarić, A., Duvnjak, T. (2003): Triacylglycerols composition of oil in OS soybean cultivars. European Food Research Technology, 217:115-119.
9. Sudar, R., Jurković, Z., Vratarić, M., Krizmanić, M., Sudarić, A., Mijić, A. (2004): Analysis of triacylglycerols of oil from OS soybean and sunflower cultivars. Proceedings of the 39th Croatian Symposium on Agriculture, Opatija, Hrvatska, 293-294.
10. Sudarić, A., Vratarić, M., Sudar, R. (2001.): Analiza stabilnosti uroda i kakvoće zrna u oplemenjivanju soje. Sjemenarstvo,18(5-6):301.-313.
11. Velasco, L., Fernandez-Martinez, J.M. (2002): Breeding Oilseed Crops for Improved Oil Quality. In: Quality Improvement in Field Crops, A.S. Basra and L.S. Randhawa (Ed.), Food Products Press, Oxford, pp. 309-344.
12. Vratarić, M., Sudarić, A. (2000.): Soja. Knjiga. Poljoprivredni institut Osijek, 1.-220.
13. Vratarić, M., Sudarić, A., Duvnjak, T. (2004): Advance in soybean lines development for conditions of the Eastern Croatia. Abstracts of contributed papers and posters of the 7th World Soybean Reserach Conference, Foz do Iguassu, Brasil, p. 307.

This study was carried out within the Project 0073005 that financed supported by the Ministry of Science, Education and Sport of Republic of Croatia

GENETSKI NAPREDAK U KVANTITATIVNIM SVOJSTVIMA LINIJA SOJE UNUTAR RAZLIČITIH GRUPA ZRIOBE

SAŽETAK

Oplemenjivački rad na soji u Poljoprivrednom institutu Osijek prvenstveno je usmjeren na kontinuirano stvaranje novih boljih kultivara soje višeg potencijala rodnosti u okvirima grupa zriobe 00 do II. Genetsko unapređenje potencijala rodnosti soje sastoji se u unapređenju genetske osnove svojstava koja određuju njegovu količinu, primjenjujući konvencionalne oplemenjivačke metode za samooplodne kulture, kao što je soja. Cilj ovog istraživanja bio je procijeniti genetski napredak u kvantitativnim svojstvima – urodu zrna, sadržaju bjelančevina i ulja u zrnu te kvaliteti ulja novih elitnih oplemenjivačkih linija soje u usporedbi sa standardima kroz komparativne poljske pokuse u trogodišnjem razdoblju (2001-2003) u Osijeku. Ispitivane elitne oplemenjivačke linije stvorene su iz različitih križanja unutar oplemenjivačkog programa soje Poljoprivrednog instituta Osijek, a izdvojene su na osnovu njihovih osobina. Vrijednost ispitivanih genotipova soje s obzirom na urod zrna i kakvoću zrna, procijenjena je analizom visine i stabilnosti uroda zrna, sadržaja bjelančevina i sadržaja ulja u zrnu. Nakon analize stabilnosti, na dijelu ispitivanog materijala koji je imao visoku razinu i dobru stabilnost sva tri svojstva, provedena je analiza triacilglicerola. Provedena biometrijska analiza pokazala je da većina ispitivanih linija ima značajno veći urod zrna, sadržaj bjelančevina i ulja u zrnu u odnosu na standarde unutar grupa zriobe 00, 0 i I, kao i dobru stabilnost analiziranih svojstava. Analizom triacilglicerola utvrđena je značajna genetska varijabilnost u ovom svojstvu. Općenito, rezultati ovog istraživanja ukazuju na ostvareni genetski napredak u urodu zrna i kakvoći zrna domaćih linija soje unutar grupe zriobe 00, 0 i I.

Ključne riječi: soja (Glycine max* (L.) Merrill*), urod zrna, sadržaj bjelančevina, sadržaj ulja, triacilgliceroli, stabilnost svojstva, RP-HPLC, genetski napredak, grupe zriobe

*(*Received on 2 December 2004; accepted on 7 February 2005 - *Primljeno 02. prosinca 2004.; prihvaćeno 07. veljače 2005.)*