PROGRESS, ADAPTABILITY AND STABILITY OF SOYBEAN GRAIN YIELD AND GRAIN QUALITY IN CONVENTIONALLY CREATED ELITE LINES

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The objective of this study was to determine the progress in grain yield and grain quality accomplished with conventional breeding methods, as well as to identify stable, widely or specifically adapted genotypes under central European growing conditions. Recently developed soybean elite lines of maturity groups (MGs) 00, 0 and I were compared with commercial cultivars (standards) in comparative field tests during three consecutive years (2018-2020) in Osijek, Croatia. The ANOVA results showed significant genotype, environment, and genotype-by-environment interaction effects. There was a significant improvement in productivity and quality in comparison to standards, while stability parameters for tested traits indicated there are stable and mostly specifically adaptable elite lines. Improvement of the domestic gene pool and high agronomic performances of elite lines stable in most important economic traits will considerably contribute to increasing and improving soybean production in central Europe.

Keywords: eco-valence, grain yield, grain quality, soybean, stability

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INTRODUCTION

The production of soybean (*Glycine max* L. Merr.) significantly increased in the last 20 years, becoming one of the most important and highly profitable crops worldwide. Over the last few years, soybean production has had a positive trend in Croatia as well, both in area and yield (FAOSTAT, 2021). The increase in area and production together with the increased frequency of adverse weather events (JUG et al., 2018) is followed by continuous breeding efforts, aiming to create high grain yield and high grain quality cultivars, stable in phenotypic expression and adaptable to variable climate, which would become an integral part of conservation agriculture. Although Croatian soybean producers are still not as concerned with soybean grain quality, the trends are changing. Furthermore, as European countries import 95% of the annual demand for soybean grains, meal and oil from overseas, causing an enormous trade deficit (DIMA, 2016; KURASCH et al., 2017a), breeding non-genetically modified soybean with increased protein or oil content which will increase the profitability of processing, becomes a matter of high importance. In creating new cultivars, breeders often look for good average performance over a wide range of environments, and the concept of stability is overlooked, which is justified only if there is no genotype-by-environment interaction (GEI). Nevertheless, quantitative traits such as grain yield, grain protein and oil content are usually under the significant influence of the environment (E) and GEI. For example, maximal soybean yield potential is 7 t/ha in theory (SPECHT et al., 1999; SINCLAIR et al., 2004), but heritability estimates range from low (3%) to middle (58%) because it is a trait with polygenic inheritance under the significant environmental influence (BRIM, 1973; BURTON, 1987), which lowers the possibility of achieving a genetic gain. The heritability estimates for protein and oil content are usually higher than for grain yield, but the effect of the environmental conditions is still significant (JAUREGUY et al., 2013; RODRIGUES et al., 2014). Protein content in soybean grain can vary from 30 to 50% of the absolute dry seed weight (ADW), while oil content ranges from 12% to 24% of ADW (VRATARIĆ and SUDARIĆ, 2008). As most of the agronomic traits are under the influence of GEI, stability analysis is necessary for understanding genotype responses to different environmental conditions. As a result, stable and widely adaptable or unstable but specifically adaptable genotypes can be identified. The stability in the phenotypic expression of economically important traits over a range of production environments is important for creating new cultivars specifically adapted to target environments and their constraints and a criterion crucial in cultivar recommendation. The GEI can be evaluated by many methods. Among the commonly used linear models are WRICKE's (1962) ecovalence (W_i^2) , FINLAY and WILKINSON's (1963) regression coefficient (b_i), EBERHART and RUSSELL'S (1966) regression analysis model or KANG'S (1988) rank-sum (K_R), all used in this study. W_i^2 represents the contribution of each genotype to the GEI sum of squares. Eberhart and Russell's model integrates b_i as a measure of adaptability and variance of deviations from the regression (s_{di}^2) as a measure of stability. K_R uses both yield and SHUKLA's (1972) stability variance (σ^2_i) as selection criteria.

For mentioned agronomic traits, breeding efforts are hindered not only by the significant influence of the E and GEI but also by the negative correlation between grain yield and grain protein content and the negative correlation between protein and oil content (KURASCH *et al.*, 2017b; PANNECOUCQUE *et al.*, 2018), where every 2% protein content increase results in a 1% oil content decrease (CLEMENTE and CAHOON, 2009). Therefore, breeding objectives must be

clearly defined according to the market requirements to satisfy the needs of the food processing industry and end-users alike.

The objectives of this study were (i) to evaluate the progress made in soybean grain yield and grain quality with the conventional breeding methods, and (ii) to evaluate the stability of the newly developed germplasm in tested agronomic traits. Estimating the agronomic value of elite soybean lines in multi-year comparative field trials is essential for the decision-making process, enabling the selection of only the best genetic material.

MATERIALS AND METHODS

Plant materials and trial design

The three-year (2018-2020) study included 11 MG 0-I, 6 MG 0 and 11 MG 00 soybean elite breeding lines and commercial cultivars (3) of respective MGs as standards. Tested elite lines were developed from crossings within the soybean breeding programme of the Agricultural Institute Osijek (AIO) and singled out from previous selection cycles based on their superior field performance. Comparative trials were set up in the experimental field of the AIO. Individual plots arranged in a randomised complete block design with three replicates were 10 m², i.e. they consisted of 4 rows, each 5 m long. The inter and intra-row spacings were 50 cm and 3 cm, respectively. Sowing was done at the optimal time for each MG and standard agricultural measures were applied each year. In the growing conditions of the trial location, optimal sowing times are the middle of April for MG 0-I, the end of April for MG 0 and the beginning of May for MG 00, allowing for deviations depending on the weather conditions. Harvest was at full maturity.

Environmental conditions

The soil at the experimental site is classified as Anthropogenic Eutric Cambisol (WRB, 2014), silty clay loamy texture (MARKOVIĆ *et al.*, 2021). Monthly average air temperatures and monthly total precipitation for Osijek for the soybean growing season (April – September) in the investigated period (2018-2020) are presented in Figure 1.

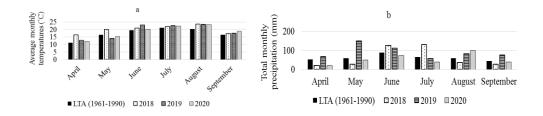


Figure 1. The long-term average (LTA) monthly temperatures and average monthly temperatures (°C) (a), total monthly precipitation LTA, and total monthly precipitation (mm) (b) in the years 2018–2020 for a soybean growing season (April – September) in Osijek, Croatia (Croatian Meteorological and Hydrological Service)

Trait determination

Grain yield was measured for each plot, converted to 13% grain moisture and expressed in t ha⁻¹. Grain protein and oil contents (% ADW) were determined with Infratec 1241 Analyzer (FOSS, Denmark) on grain samples taken from the three middle rows after grain yield was measured.

Statistical analyses

Collected experimental data were processed by the analysis of variance (ANOVA) followed by Fisher's post hoc test in Statistica 12.0 (STATSOFT Inc., USA, 2013) software. The parameters used for GEI evaluation, i.e. W_i^2 , b_i , s_{di}^2 and K_R , were all calculated in Stabilitysoft online programme (POUR-ABOUGHADAREH *et al.*, 2019). According to WRICKE (1962), genotypes with the lowest W_i^2 values are considered more stable. According to FINLAY and WILKINSON (1963), genotypes are considered widely adaptable if their b_i did not significantly differ from 1. If $b_i > 1$, a genotype is better adapted to high-yielding environments, while a $b_i < 1$ indicates better adaptability to low-yielding environments. According to EBERHART and RUSSELL's regression analysis model (1966), a genotype can be considered stable if b_i does not significantly differ from zero. According to KANG (1988), genotypes with the lowest rank-sum are the most desirable. Pearson's correlation coefficients for grain yield, grain protein and oil content, and their stability parameters were calculated in Microsoft Excel. The strength of the correlation was determined based on the scale reported by EVANS (1996).

RESULTS AND DISCUSSION

The analysis of variance showed a highly significant influence (p ≤ 0.01) of genotype (G), different environments, i.e. E as well as GEI, across all maturity groups and for all tested traits (Table 1). The same was earlier reported by many researchers (KURASCH et al., 2017a,b; PANNECOUCQUE et al., 2017; LI et al., 2020; PERIĆ et al., 2021). The G effect indicates the existence of variability among tested material, which is a prerequisite of all selection processes. The E and GEI effect, expected in quantitative traits, necessitate the evaluation of stability and adaptability. Although the use of multivariate stability analyses techniques is a common practice nowadays, a positive correlation of univariate analyses, such as the ones used in this study, with multivariate techniques is reported by many authors (TEMESGEN et al., 2015; BASSA et al., 2019; MOHAMMADI et al., 2020; HASHIM et al., 2021). This enables the use of simple, free and userfriendly programmes for stability analyses, such as Stabilitysoft, capable of calculating all parametric and non-parametric statistics in one package (POUR-ABOUGHADAREH et al., 2019). Significant progress in tested agronomic traits and trait stability was achieved in all MGs. In MG 0-I, the highest average grain yields were achieved by OS-L-28 (4.84 t ha⁻¹) and OS-L-23. All elite lines except OS-L-13, OS-L-15, and OS-L-26 have had a higher average grain yield than the standard cultivar (ST-0I; 3.8 t ha⁻¹), but none could be considered widely adaptable ($b_i \approx 1$) for grain yield or stable according to Eberhart and Russell's model (Table 2). Four of these lines with superior grain yield (OS-L-17, OS-L-21, OS-L-23, OS-L-28) have had higher yield stability compared to ST-0I according to both W_i^2 and K_R , with one (OS-L-28) being better adapted to high-yielding (bi > 1) and three (OS-L-17, OS-L-21, OS-L-23) to low-yielding environments (bi < 1).

Table 1. ANOVA (mean squares and significance) for analysed traits in 31 soybean genotype tested in a three-year field trial (2018-2020) in Osijek, Croatia.

Source of variation	Gra	Grain yield (t ha ⁻¹)			Protein content (% ADW)			Oil content (% ADW)		
	0-I	0	00	0-I	0	00	0-I	0	00	
Genotype (G)	2.29*	1.62*	0.85*	23.47*	16.23*	10.81*	6.44*	4.34*	3.38*	
Environment (E)	8.37*	0.43*	5.57*	15.43*	1.33*	3.06*	19.55*	2.58*	5.31*	
G x E Interaction (GEI)	1.53*	1.99*	0.96*	4.09*	0.56*	1.63*	0.81*	0.77*	0.34*	

* - significant differences at $p \le 0.01$

According to FINLAY and WILKINSON (1963), a year with the highest average year grain yield $(\bar{x}_{\rm Y})$ in a trial can be considered favourable for the investigated trait, while a year with the lowest average year grain yield (\bar{x}_{Y}) can be considered unfavourable. The elite line considered better adapted to high-yielding environments (OS-L-28) had the highest average grain yields in 2018. As the highest average year grain yield (4.42 t ha^{-1}) was achieved in 2019, it could be argued that it had environmental conditions better suited for accumulating yield than the other two years (2018, 2020). Although a significantly lower average year grain yield was achieved in 2018 (4.34 t ha⁻¹), it is higher than the yield grand average (YGA; 4.1 t ha⁻¹), meaning environmental conditions have been favourable as well. The lowest average year grain yield value was registered in 2020 (3.55 t ha⁻¹), so it can be considered as having environmental conditions less favourable for accumulating yield. One possible reason why the yield was lower in 2020 is that the average temperature for the soybean vegetation period has been above the LTA while the precipitation total has been well below the LTA and the precipitation in the other two years (Figure 1). Such warm and dry conditions were previously considered unfavourable for yield accumulation (SUZUKI et al., 2014; MATOŠA KOČAR et al., 2017; SCHAUBERGER et al., 2017; SIEBERT et al., 2017; HAMED et al., 2021). Elite lines with superior yields compared to ST-0I considered better adapted to low-yielding environments (OS-L-17, OS-L-21, OS-L-23) have had an above-average yield in 2020 (Table 2).

All elite lines except OS-L-15 had average protein content higher than ST-0I, with OS-L-21 having the highest (47.33% ADW) in the trial (Table 3), but none could be considered protein-stable according to Eberhart and Russell's model. Four of these lines (OS-L-11, OS-L-12, OS-L-16, OS-L-17) have had higher protein stability than ST-0I, according to both W_i^2 and K_R . OS-L-12 was better adapted to high-protein ($b_i > 1$) and the other three to low-protein environments ($b_i < 1$). OS-L-12 had higher average protein content in 2020 than in 2018 and 2019. The highest average year protein content (44.32% ADW) was reported in 2020, meaning it had environmental conditions more suitable for protein accumulation compared to 2018 and 2019.

		Grain yi	eld (t ha ⁻¹)		0	Grain yield sta	bility parame	ters
MG 0-I	2018	2019	2020	\bar{x}_{G}	W_i^2	b_i	sdi ²	$K_{\rm R}$
OS-L-11	4.67	4.31	2.89	3.96^{gh}	0.49	1.88^{*}	0.02	15
OS-L-12	5.68	4.27	2.96	4.30^{d}	2.04	2.29^{*}	0.18^{*}	15
OS-L-13	4.10	3.64	2.98	3.57^{k}	0.15	1.03	0.02	12
OS-L-15	3.37	2.98	3.05	3.13^{l}	0.44	0.12^{*}	0.01	16
OS-L-16	3.36	5.89	3.39	4.21^{ef}	3.12	1.68^{*}	0.42^{*}	18
OS-L-17	3.96	4.52	3.62	4.03^{g}	0.15	0.79^*	0.02	9
OS-L-18	3.71	4.97	4.87	4.52°	1.94	-0.53*	0.12^{*}	13
OS-L-21	4.31	4.26	4.26	4.28^{de}	0.44	-0.10^{*}	≈ 0	10
OS-L-23	4.69	4.98	4.82	4.83^{ab}	0.47	0.04^*	0.01	8
OS-L-26	3.79	4.56	2.83	3.73^{ij}	0.42	1.67^{*}	0.03	13
OS-L-28	5.69	5.08	3.74	4.84^{a}	0.68	1.91^{*}	0.04	9
ST-0I	4.75	3.52	3.13	3.80^{i}	0.87	1.10^{*}	0.12^{*}	18
\bar{x}_{y}	4.34^{b}	4.42^{a}	3.55 ^c	4.10				
LSD _{0.01(G)}				0.086				
LSD _{0.01(Y)}				0.043				

Table 2. Three-year average grain yield (t ha⁻¹) and grain yield stability parameters for 11 maturity group(MG) 0-I newly created elite breeding lines (OS-L) and one standard cultivar (ST-0I) tested in athree-year field trial (2018-2020) in Osijek, Croatia

Genotype averages (\mathbf{x}_{G}) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\mathbf{x}_{Y}) with the same letter in superscript are not significantly different $p \le 0.01$; *Significant at $p \le 0.05$, for $b_i \ne 1$, $Sd_i^2 \ne 0$.

Although temperatures during the grain filling (August) were above the LTA and similar in all three years, the amount of precipitation was much higher in 2020 than in the other two years and the LTA (Figure 1), which can be the reason for higher protein contents (KIRNAK *et al.*, 2010; MATOŠA KOČAR *et al.*, 2017; GHASSEMI-GOLEZANI and FARSHBAF-JAFARI, 2012). Low-protein environment adapted OS-L-11, OS-L-16 and OS-L-17 are supposed to perform well in unfavourable environmental conditions, which is confirmed by the high individual protein contents in 2018 and 2019, both considered less favourable for protein accumulation (Table 3). The only widely adaptable elite line is OS-L-23 ($b_i \approx 1$). OS-L-23 had lower average protein content than the protein grand average (PGA, 43.56% ADW), so it could be considered poorly adapted (Table 3).

OS-L-11, OS-L-13 and OS-L-15 are superior to ST-0I (25.28% ADW) and the oil grand average (OGA; 24.54% ADW) in average oil content (Table 4), but none were considered more stable than ST-0I. The highest average oil content in the trial was achieved by OS-L-13 (25.72% ADW).

01) tested in a three-year field trial (2018-2020) in Osijek, Croatia. Protein content (% ADW) Protein content stability parameters MG 0-I 2018 2019 2020 \bar{x}_{G} W_i^2 sd_i^2 bi $K_{\rm R}$ OS-L-11 43.11^{gh} 43.00 43.19 43.14 0.82 0.03* ≈0 11 ≈ 0 OS-L-12 42.83 42.63 45.02 43.49^{f} 0.90 2.02^{*} 11 OS-L-13 41.01 42.36^{ij} 1.95* 0.09 18 42.26 43.80 1.43 OS-L-15 40.19 38.96 44.47 41.21^{1} 10.13 4.35* 0.07 24 44.22^d OS-L-16 43.99 44.59 0.22 0.50^{*} ≈ 0 5 44.07 OS-L-17 44.0044.11 44.09 44.07^{de} 0.82 0.03* ≈ 0 7 12 OS-L-18 44.58 45.51 44.00 44.69^b 3.65 -0.95* 0.06 -0.78^{*} OS-L-21 47.12 48.10 46.76 47.33^a 3.15 0.06 10 OS-L-23 42.80 41.39 43.13 42.44ⁱ 0.93 0.96 0.13* 15 OS-L-26 44.34 44.90 44.62 44.62^{bc} 1.04 -0.02^{*} 0.02 10 OS-L-28 41.12 43.28 45.64 43.35^{fg} 5.99 2.97^{*} 0.38* 18 ST-0I 40.82 42.54 41.86^k 0.90 0.95 0.13* 42.21 15 \bar{x}_{Y} 43.21^b 43.16^{b} 44.32^{a} 43.56 LSD_{0.01(G)} 0.299 0.149 LSD_{0.01(Y)}

Table 3. Three-year average protein content (% ADW) and protein content stability parameters for 11 maturity group (MG) 0-I newly created elite breeding lines (OS-L) and one standard cultivar (ST-0I) tested in a three-year field trial (2018-2020) in Osijek, Croatia.

Genotype averages (\vec{x}_G) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\vec{x}_Y) with the same letter in superscript are not significantly different $p \le 0.01$; *Significant at $p \le 0.05$, for $b_i \ne 1$, $Sd_i^2 \ne 0$.

Table 4. Three-year average oil content (% ADW) and oil content stability parameters for 11 maturity group (MG) 0-I newly created elite breeding lines (OS-L) and one standard cultivar (ST-0I) tested in a three-year field trial (2018-2020) in Osijek, Croatia

MG 0-I		Oil conten	t (% ADW)		Pro	otein content s	stability paran	neters
MG 0-1	2018	2019	2020	\bar{x}_{G}	W_i^2	b_i	sd _i ²	$K_{\rm R}$
OS-L-11	25.63	26.24	24.32	25.39 ^b	0.17	1.31*	0.01	7
OS-L-12	24.28	25.78	23.14	24.40^{g}	0.71	1.79^{*}	0.01	15
OS-L-13	25.48	26.79	24.90	25.72^{a}	0.19	1.28^{*}	0.02	7
OS-L-15	25.18	26.93	23.80	25.30^{bc}	1.40	2.12^{*}	0.01	15
OS-L-16	24.10	24.72	23.33	24.05^{hij}	0.01	0.94	≈ 0	11
OS-L-17	24.15	24.55	23.77	24.16^{h}	0.24	0.53^{*}	≈ 0	15
OS-L-18	23.96	24.25	24.18	24.13^{hi}	1.04	0.04^{*}	0.01	19
OS-L-21	23.60	23.99	23.74	23.78^{k}	0.81	0.17^{*}	0.01	20
OS-L-23	24.78	25.16	24.15	24.69 ^{ef}	0.11	0.69^{*}	≈ 0	9
OS-L-26	22.70	23.18	22.28	22.72^{l}	0.17	0.61^{*}	≈ 0	16
OS-L-28	25.58	25.67	23.34	24.86^{e}	1.09	1.60^{*}	0.1^{*}	16
ST-0I	25.30	25.96	24.59	25.28^{bcd}	0.01	0.93	≈ 0	6
\bar{x}_{y}	24.56^{b}	25.27^{a}	23.79 ^c	24.54				
LSD _{0.01(G)}				0.179				
LSD _{0.01(Y)}				0.089				

In MG-0I, the line that stands out for having improved grain yield, protein contents and both traits' stabilities according to W_i^2 and K_R compared to standard cultivar is OS-L-17 (Table 1-2). As its oil content and oil stability were lower than ST-0I's (Table 3), this genotype is better suited for food and feed industries than for oil production.

In MG 0, all the elite lines had a higher average grain yield than ST-0, and OS-L-1 had the highest (4.63 t ha⁻¹; Table 5). All of these lines could be considered more stable than ST-0I according to W_i^2 and K_R , but none could be described as stable according to Eberhart and Russell's model or widely adaptable. Three elite lines (OS-L-6, OS-L-7, OS-L-14) were better adapted to high-yielding environments (b_i > 1). They had higher than average grain yields in 2019, which is considered more favourable for yield accumulation than the other two trial years (Table 5). The other three elite lines (OS-L-1, OS-L-2, OS-L-19) were better adapted to lowyielding environments (b_i < 1). OS-L-6 and OS-L-14 had the highest yield in 2018 considered more favourable for yield accumulation, but OS-L-7 did not (Table 5).

Table 5. Three-year average grain yield (t ha⁻¹) and grain yield stability parameters for six maturity group (MG) 0 newly created elite breeding lines (OS-L) and one standard cultivar (ST-0) a three-year Cult wird (2018 2000) in Orbital Courting

		Grain yie	ld (t ha ⁻¹)			Grain yield sta	bility paramete	rs
MG 0	2018	2019	2020	\bar{x}_{G}	W_i^2	bi	sd _i ²	$K_{\rm R}$
OS-L-1	3.67	5.02	5.19	4.63^{a}	1.68	-3.09*	0.14^{*}	7
OS-L-2	3.86	4.83	4.53	4.41^{b}	0.58	-0.51*	0.07	4
OS-L-6	5.38	4.23	3.37	4.33^{bc}	1.63	5.53*	0.11^{*}	8
OS-L-7	3.63	4.44	3.56	3.88^{ef}	0.35	2.12^{*}	0.04	7
OS-L-14	4.20	4.44	3.06	3.90^{e}	0.71	5.16*	≈0	8
OS-L-19	3.92	3.92	4.78	4.21^{d}	0.82	-3.48*	≈0	8
ST-0	4.54	2.46	3.06	3.35 ^g	2.23	1.27^{*}	0.32^{*}	14
\bar{x}_{y}	4.17^{a}	4.19^{a}	3.94^{b}	4.09				
LSD _{0.01(G)}				0.034				
LSD _{0.01(Y)}				0.022				

Genotype averages (\vec{x}_G) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\vec{x}_Y) with the same letter in superscript are not significantly different $p \le 0.01$, * Significant at $p \le 0.05$, for $b_i \ne 1$, $Sd_i^2 \ne 0$.

Among the elite lines, only OS-L-19 (45.73% ADW) had a higher average protein content (Table 6) than ST-0 (45.15% ADW) and PGA (43.93% ADW). It can be considered better adapted to low-protein environments ($b_i < 1$), which is confirmed by higher than average protein content (45.62% ADW) in 2018, the year considered less favourable for protein accumulation. Furthermore, it had very similar protein contents in all three years, which may be why it can be considered stable according to, W_i^2 and K_R (Table 6).

OS-L-1, OS-L-2, OS-L-6 and OS-L-14 had higher average oil content (Table 7) than ST-0 (23.86% ADW). All of them can be considered more oil-stable than ST-0 according to W_i^2 and K_R , but only OS-L-6 could be considered widely adaptable (bi \approx 1) and oil-stable according to EBERHART and RUSSELL (1966). OS-L-1 and OS-L-2 can be considered better adapted to low-oil environments (b_i < 1), both having had higher than average trait values in 2020, which is

considered the least favourable for oil accumulation (Table 7). OS-L-14 can be considered better adapted to high-oil environments ($b_i > 1$) (Table 7).

	F	Protein conte	nt (% ADW)	Protein content stability parameters			
MG 0	2018	2019	2020	\bar{x}_{G}	W_i^2	b_i	sd _i ²	$K_{\rm R}$
OS-L-1	42.78	43.46	42.50	42.91 ^f	0.40	0.84^*	0.06	12
OS-L-2	41.64	42.06	41.50	41.73^{g}	0.16	0.54^{*}	0.02	9
OS-L-6	43.87	44.22	44.38	44.16^{cd}	0.04	0.89^{*}	0.01	5
OS-L-7	43.92	44.10	44.70	44.24°	0.25	0.83^{*}	0.04	8
OS-L-14	43.65	43.60	43.57	43.61 ^e	0.16	-0.13*	≈0	8
OS-L-19	45.62	45.56	46.00	45.73^{a}	0.19	0.18^{*}	0.02	5
ST-0	44.05	45.88	45.51	45.15^{b}	1.03	3.84*	≈0	9
\bar{x}_{Y}	43.65^{b}	44.13^{a}	44.02^{a}	43.93				
LSD _{0.01(G)}				0.338				
LSD _{0.01(Y)}				0.221				

Table 6. Three-year average protein content (% ADW) and protein content stability parameters for six maturity group (MG) 0 newly created elite breeding lines (OS-L) and one standard cultivar (ST-0) a three-year field trial (2018-2020) in Osijek. Croatia

Genotype averages (\vec{x}_G) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\vec{x}_Y) with the same letter in superscript are not significantly different $p \le 0.01$, * Significant at $p \le 0.05$, for $b_i \ne 1$, $Sd_i^2 \ne 0$.

Table 7. Three-year average oil content (% ADW) and oil content stability parameters for six maturity group (MG) 0 newly created elite breeding lines (OS-L) and one standard cultivar (ST-0) a three-year field trial (2018-2020) in Osijek, Croatia.

MCO		Oil content	(% ADW)		Oil content stability parameters			
MG 0	2018	2019	2020	\bar{x}_{G}	W_i^2	b_i	sdi ²	K _R
OS-L-1	24.58	24.61	24.60	24.59°	0.24	0.02^{*}	≈0	7
OS-L-2	25.28	25.42	25.03	25.24^{a}	0.06	0.54^{*}	≈ 0	3
OS-L-6	24.11	24.55	23.91	24.19^{d}	0.00	0.93	≈0	5
OS-L-7	23.30	22.99	23.78	23.36 ^f	1.10	-1.10*	≈0	13
OS-L-14	24.72	25.92	24.35	24.99^{b}	0.46	2.31*	0.01	7
OS-L-19	23.52	24.28	23.36	23.72 ^{ef}	0.06	1.36^{*}	≈0	9
ST-0	24.11	24.80	22.67	23.86^{de}	1.16	2.94^{*}	0.03	12
\bar{x}_{y}	24.23^{b}	24.65^{a}	23.96 ^c	24.28				
LSD _{0.01(G)}				0.387				
LSD _{0.01(Y)}				0.253				

Genotype averages (\vec{x}_G) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\vec{x}_Y) with the same letter in superscript are not significantly different $p \le 0.01$, *Significant at $p \le 0.05$, for $b_i \ne 1$, Sd_i² $\ne 0$.

Among the elite lines in which progress has been achieved, low-yielding environment adapted OS-L-19 stands out for having improved grain yield, protein content, and both traits' stabilities according to W_i^2 and K_R compared to standard cultivar (Table 5-6). Its average oil

content is lower than ST-0, but it has higher oil stability than ST-0, according to W_i^2 and K_R (Table 7). Although it is inferior to ST-0, its average oil content is relatively high (23.72% ADW), so it is suitable for the food, feed and oil industries. OS-L-1, OS-L-2 and OS-L-6 stand out for having improved grain yields, oil contents and both traits' stabilities according to W_i^2 and K_R compared to standard cultivar (Table 5 and 7). Low-oil environment adapted OS-L-1 had the highest average yield in the trial (Table 5), but its protein content was much lower than ST-0's. Its protein stability is higher than ST-0's according to W_i^2 or lower according to K_R (Table 6). Low-oil environment adapted OS-L-2 had the highest average oil content among the elite lines (Table 7) and a second highest average yield (Table 5). Its protein content was below ST-0's, but protein stability is higher than ST-0's according to W_i^2 or at the same level according to K_R (Table 6). OS-L-6 is the only elite line that could be considered widely adaptable and oil-stable according to EBERHART and RUSSELL (1966) (Table 7). It has superior protein stability but lower protein content compared to ST-0 (Table 6). As their average protein content is low, both OS-L-2 and OS-L-6 is better suited for the oil industry.

Table 8. Three-year average grain yield (t ha⁻¹) and grain yield stability parameters for 11 maturity group (MG) 00 newly created elite breeding lines (OS-L) and one standard cultivar (ST-00) tested in a three-year field trial (2018-2020) in Osiiek. Croatia.

		Grain yie	ld (t ha ⁻¹)			Grain yield sta	bility paramete	ers
MG 00	2018	2019	2020	\overline{x}_{G}	W_i^2	b_i	sdi ²	$K_{\rm R}$
OS-L-3	2.74	3.95	3.99	3.56^{k}	1.21	0.17^{*}	0.14^{*}	20
OS-L-4	4.00	4.51	3.51	4.01^{g}	0.03	1.26^{*}	≈ 0	9
OS-L-5	3.69	4.58	3.91	4.06^{f}	0.16	0.95	0.02	12
OS-L-8	3.56	4.65	3.29	3.83^{ij}	0.24	1.79^{*}	0.01	18
OS-L-9	5.03	3.76	3.79	4.19^{cd}	1.52	-0.26*	0.15^{*}	15
OS-L-10	4.24	5.59	2.76	4.19°	2.11	3.56*	0.01	15
OS-L-20	3.83	4.41	3.56	3.93^{h}	0.01	1.10^{*}	≈ 0	9
OS-L-22	4.20	3.34	3.97	3.84^{i}	1.26	-0.89^{*}	0.02	19
OS-L-24	3.99	4.71	4.17	4.29^{b}	0.12	0.76^{*}	0.02	7
OS-L-25	3.83	4.63	3.99	4.15^{e}	0.12	0.89^*	0.02	9
OS-L-27	4.50	5.04	3.68	4.41^{a}	0.20	1.69^{*}	0.01	8
ST-00	3.53	3.65	2.83	3.34^{l}	0.09	0.98	0.01	15
\bar{x}_{Y}	3.93^{b}	4.40^{a}	3.62°	<i>3.98</i>				
LSD _{0.01(G)}				0.026				
LSD _{0.01(Y)}				0.046				

Genotype averages (\vec{x}_G) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\vec{x}_Y) with the same letter in superscript are not significantly different $p \le 0.01$, * Significant at $p \le 0.05$, for $b_i \ne 1$, $Sd_i^2 \ne 0$.

In MG 00, all elite lines had a higher average grain yield (Table 8) than ST-00 (3.34 t ha⁻¹), but only OS-L-4 and OS-L-20 are more yield-stable than ST-00 according to W_i^2 and K_R . OS-L-24 can be considered better adapted to low-yielding environments (b_i < 1), and it had a higher than average grain yield in 2020, the year considered less favourable for yield accumulation (Table 8). OS-L-20 can be considered specifically adapted to high-yielding

environments (bi > 1). It achieved the highest yields in 2019, which is considered more favourable for yield accumulation than the other two trial years. On the other hand, OS-L-5 can be considered stable based on Eberhart and Russell's model and widely adaptable ($b_i \approx 1$) (Table 8).

ST-00 had the lowest average grain yield but not the lowest grain quality. All the elite lines except OS-L-3, OS-L-5, OS-L-8 and OS-L-22 had higher protein contents than ST-00 (43.3% ADW), and six of these were more stable than ST-00 according to W_i^2 and K_R (Table 9). OS-L-27 can be considered specifically adapted to high-protein environments ($b_i > 1$), while the rest are specifically adapted to low-protein environments ($b_i < 1$). OS-L-27 had maximal protein content in 2020, one of the years considered more favourable for protein accumulation. Furthermore, all the elite lines specifically adapted to low-protein environments except OS-L-9 have had higher than average protein content in 2018, which is considered less favourable for protein accumulation (Table 9).

Table 9. Three-year average protein content (% ADW) and protein content stability parameters for 11 maturity group (MG) 00 newly created elite breeding lines (OS-L) and one standard cultivar (ST-00) tasted in a three year field trial (2018-2020) in Osiiek Croatia

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MG 00	F	Protein conte	ent (% ADW	D	Pr	otein content	stability param	eters
100 00	2018	2019	2020	\bar{x}_{G}	W_i^2	b_i	sd _i ²	K _R
OS-L-3	42.40	42.67	42.26	42.44^{k}	0.19	0.18^*	0.01	16
OS-L-4	44.28	46.87	44.40	45.18^{b}	3.42	3.01*	0.39^{*}	14
OS-L-5	43.15	42.46	43.50	43.04^{ij}	0.89	-0.48^{*}	0.08	18
OS-L-8	43.04	43.36	42.79	43.06^{hi}	0.28	0.15^{*}	0.02	16
OS-L-9	43.42	43.67	43.87	43.65^{fg}	0.05	0.66^*	≈ 0	9
OS-L-10	43.60	43.49	43.88	43.66 ^f	0.21	0.12^{*}	0.01	11
OS-L-20	44.80	44.67	44.92	44.79 ^{de}	0.22	-0.04*	≈0	11
OS-L-22	42.30	41.66	43.76	42.57^{k}	2.31	0.53^{*}	0.32^{*}	21
OS-L-24	44.90	45.02	44.99	44.97^{bcd}	0.11	0.21^{*}	≈ 0	7
OS-L-25	45.17	45.63	45.54	45.45^{a}	0.01	0.83^{*}	≈ 0	2
OS-L-27	44.12	45.10	46.08	45.10^{bc}	1.15	2.77^{*}	0.09^{*}	12
ST-00	42.02	44.92	42.98	43.31^{h}	3.15	4.07^{*}	0.22^{*}	19
$\bar{x}_{_{ m Y}}$	43.60^{b}	44.13 ^a	44.08^{a}	43.94				
LSD _{0.01(G)}				0.263				
LSD _{0.01(Y)}				0.455				

Genotype averages (\vec{x}_G) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\vec{x}_Y) with the same letter in superscript are not significantly different $p \le 0.01$, *Significant at $p \le 0.05$, for $b_i \ne 1$, Sd_i² $\ne 0$.

In MG 00, only OS-L-3 has had average oil content (25.46% ADW) higher than ST-00 (25.18% ADW) and OGA (24.55% ADW). The rest of the elite lines have had oil contents ranging from 23.64 to 25.17% ADW (Table 10), which is relatively high, considering most commercial cultivars contain 19 to 23% ADW oil (PANTHEE *et al.*, 2004). OS-L-3 can be considered better adapted to low-oil environments ($b_i < 1$) but stable according to W_i^2 . Furthermore, it has a better K_R ranking than ST-00 (Table 10).

Table 10. Three-year average oil content (% ADW) and oil content stability parameters for 11 maturity group (MG) 00 newly created elite breeding lines (OS-L) and one standard cultivar (ST-00) tested in a three-year field trial (2018-2020) in Osijek, Croatia.

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		Oil content	(% ADW)		Pı	otein content	stability param	eters
MG 00	2018	2019	2020	\bar{x}_{G}	W_i^2	b_i	sdi ²	K _R
OS-L-3	25.36	25.58	25.43	25.46^{a}	0.22	0.16^{*}	≈0	10
OS-L-4	23.64	23.99	23.3	23.64^{k}	0.01	0.89^*	≈0	14
OS-L-5	24.87	25.41	24.62	24.97^{d}	0.04	0.99	0.01	8
OS-L-8	24.94	25.11	24.73	24.93^{de}	0.08	0.49^{*}	≈0	11
OS-L-9	24.06	24.31	24.00	24.12^{hi}	0.12	0.38^{*}	≈0	17
OS-L-10	24.87	25.13	23.55	24.52^{g}	0.47	2.15^{*}	0.01	18
OS-L-20	24.77	25.00	24.29	24.69 ^f	0.00	0.94	≈0	7
OS-L-22	25.10	25.63	24.78	25.17^{bc}	0.03	1.08	≈0	6
OS-L-24	23.90	24.45	23.72	24.02^{ij}	0.05	0.91	0.01	15
OS-L-25	23.45	24.14	23.34	23.64^{k}	0.09	0.97	0.01	18
OS-L-27	24.83	24.95	22.99	24.26^{h}	1.12	2.70^{*}	0.04	20
ST-00	25.52	25.10	24.92	25.18^{b}	0.29	0.34^{*}	0.02	12
$\bar{x}_{_{ m Y}}$	24.61 ^a	24.90^{a}	24.14^{b}	24.55				
LSD _{0.01(G)}				0.163				
LSD _{0.01(Y)}				0.281				

Genotype averages (\vec{x}_G) with the same letter in superscript are not significantly different at $p \le 0.01$; year averages (\vec{x}_Y) with the same letter in superscript are not significantly different $p \le 0.01$, * Significant at $p \le 0.05$, for $b_i \ne 1$, $Sd_i^2 \ne 0$.

Among the MG 00 elite lines, high-yielding environment adapted OS-L-20 stands out as it had improved grain yield and protein content, as well as both traits' stabilities according to W_i^2 and K_R compared to standard cultivar (Table 8-9). It had a relatively high average oil content (24.69% ADW) and oil stability higher than ST-00, so it is well suited for food and feed, as well as for oil production.

Pearson's correlation coefficients (Table 11) indicated a well-known, highly significant, strong negative correlation between the average grain protein and oil contents (r = -0.68, $p \le 0.01$), which prevents simultaneous improvement of these two traits (KURASCH *et al.*, 2017a,b; PANNECOUCQUE *et al.*, 2018). Although a negative correlation between protein content and yield most often hinders breeding (KURASCH *et al.*, 2017a,b), no significant relationship was observed here or in some other earlier studies (PANNECOUCQUE *et al.*, 2018; COBER and VOLDENG, 2000). The relationship between grain yield and oil content, which is usually positive and significant (WILCOX and SHIBLES, 2001), was significant but very weak (r = 0.15, $p \le 0.05$) in this study.

The correlation between yield and W_i^2 (Table 12) was significant and positive but very weak (r = 0.14, p ≤ 0.05). A significant, positive and moderate correlation between grain yield and W_i^2 was previously determined by BELETE *et al.* (2020) in finger millet, but a non-significant correlation was determined by BALCHA (2020) in bread wheat, BASSA *et al.* (2019) in common bean, and TEMESGEN *et al.* (2015) in faba bean. A highly significant, strong and negative correlations calculated between K_R , yield (r = -0.65, p ≤ 0.01), protein (r = -0.77, p ≤ 0.01) and oil content (r = -0.63, p ≤ 0.01) are expected as genotypes with the highest trait values have the

lowest rank-sum (KANG, 1988). All other average trait values were in a non-significant relationship with other stability parameters (Table 12). The lack of correlation between tested traits and other stability parameters indicates stability estimating procedures provide information that cannot be gathered from estimating average trait values alone (DUARTE and ZIMMERMANN, 1995). Furthermore, it could indicate simultaneous breeding for important agronomic traits and trait stability is possible (BALCHA, 2020).

Table 11. Pearson's correlation coefficients (r) for yield, protein and oil content in 31 soybean genotypes form three maturity groups (MG), tested in a three-year field trial (2018-2020) in Osijek, Croatia (n = 279)

	Correlation c	Correlation coefficient (r)			
	Protein content	Oil content			
Grain yield	0.00 ^{ns}	0.15^{*}			
Protein content		-0.68**			

** - $p \le 0.01$; * - $p \le 0.05$; ^{ns} - non-significant

Table 12. Pearson's correlation coefficients (r) for yield, protein and oil content stability parameters $(W_i^2, s^2d_i, CV_i, b_i)$ in 31 soybean genotypes form three maturity groups (MG), tested in a three-year field trial (2018-2020) in Osijek, Croatia (n = 279)

	Co	rrelation coefficient (r) for g	rain yield stability parame	ters
	W_i^2	bi	sd _i ²	K _R
Grain	0.14^{*}	0.06 ^{ns}	0.06 ^{ns}	-0.65**
yield				
W_i^2		0.12 ^{ns}	0.85**	0.56**
bi			0.03 ^{ns}	-0.00 ⁿ
$s^2 d_i$				0.49**
	Correlat	tion coefficient (r) for grain	protein content stability pa	rameters
	W_i^2	bi	sdi ²	K _R
Protein	-0.35 ^{ns}	-0.32 ^{ns}	-0.29 ^{ns}	-0.77*
content				
W_i^2		0.75**	0.80^{**}	0.62**
bi			0.39*	0.43*
$s^2 d_i$				0.58^{**}
	Corre	lation coefficient (r) for grai	n oil content stability para	meters
	W_i^2	bi	sd _i ²	K _R
Oil	0.11 ^{ns}	0.28 ^{ns}	0.13 ^{ns}	-0.63*
content				
W_i^2		0.43*	0.43*	0.54**
bi			0.44^{*}	-0.02 ⁿ
s²d _i				0.26 ^{ns}

 ** - $p \leq 0.01;$ * - $p \leq 0.05;$ $^{ns}-non-significant$

Highly significant, positive and very strong correlations were found between W_i^2 and s_{di}^2 for grain yield (r = 0.85, p ≤ 0.01) and protein content (r = 0.8, p ≤ 0.01) (Table 12). A very strong or strong correlation between W_i^2 and s_{di}^2 was earlier determined by many authors for different crops (TEMESGEN *et al.*, 2015; YAGHOTIPOOR *et al.*, 2017; BALCHA, 2020; MOHAMMADI *et al.*, 2020; HASHIM *et al.*, 2021), and it is expected because W_i^2 is a function of b_i and deviation mean square (BECKER, 1981). The correlation between W_i^2 and b_i for protein content was highly significant, positive and strong (r = 0.75, p ≤ 0.01), the same as between W_i^2 and K_R (r = 0.62, p ≤ 0.01) (Table 12). Very strong and strong correlations between stability parameters indicate these are similar in ranking genotypes and can be used one instead of the other without losing data.

A highly significant, positive and moderate correlation was found between K_R , W_i^2 (r = 0.53, p ≤ 0.01) and s_{di}^2 (r = 0.49, p ≤ 0.01) for grain yield, K_R and s_{di}^2 for protein content (r = 0.58, p ≤ 0.01) and K_R and W_i^2 for oil content (r = 0.54, p ≤ 0.01) (Table 12). The correlation between K_R and b_i for protein content was significant, positive and moderate (r = 0.43, p ≤ 0.05), the same as between W_i^2 and b_i (r = 0.43, p ≤ 0.05), W_i^2 and s_{di}^2 (r = 0.43, p ≤ 0.05), and b_i and s_{di}^2 (r = 0.44, p ≤ 0.05) for oil content (Table 12). A moderate correlation could indicate an overlap in estimation of stability (BALCHA, 2020), meaning each gives a certain amount of unique information.

As for the stability parameters insignificantly correlated or in a significant but weak correlation (Table 12), each provides unique stability information, so they should be combined, i.e. used together in trait and genotype stability estimation. A non-significant correlation between b_i , s_{di}^2 and K_R found in this study (Table 12) was previously determined by HASHIM *et al.* (2021) while evaluating 40 advanced fragrant rice accessions were in different rain-fed environments, OLADOSU *et al.* (2017) in 15 rice genotypes tested in 10 environments and BUJAK *et al.* (2014) in eight maize hybrids tested at 16 locations in Poland. A non-significant correlation between W_i^2 and b_i for grain yield (Table 12) was previously found in finger millet (BELETE *et al.*, 2020), bread wheat (BALCHA, 2020), rice (OLADOSU *et al.*, 2017) and maize (BUJAK *et al.*, 2014), as well.

CONCLUSIONS

Significant progress in grain yield, protein and oil contents and trait stability was achieved with conventional breeding methods. Elite lines with superior trait expression compared to standard cultivars were found in each MG. Although many elite lines were stable according to W_i^2 and K_R , OS-L-6 is the only elite line that could be considered widely adaptable and stable according to Eberhart and Russell's model. Nevertheless, its stability and wide adaptability were determined only for oil content. Expectedly, none of the lines has improved trait expression and trait stability for all three tested traits. Elite lines with improved grain yield, protein content and both traits' stabilities according to W_i^2 and K_R are OS-L-19 and OS-L-20. Elite lines with improved grain yield and oil content as well as both traits' stabilities according to W_i^2 and K_R are OS-L-1, OS-L-2 and OS-L-6. As expected, the relationship between grain yield and protein content was highly significant, strong and negative, but other trait combinations were either in a non-significant or significant but weak correlation. Trait values were in a highly significant, strong and negative correlation with K_R , but all other stability parameters were in a non-significant relationship with grain yield, protein and oil content.

Correlation analysis indicates W_i^2 and s^2d_i can be used interchangeably for grain yield stability estimation, while there is only some overlapping between W_i^2 and K_R , as well as s^2d_i and K_R . For protein content stability estimation, W_i^2 can be used instead of b_i , s^2d_i and K_R , and vice versa, while there is only some overlapping between b_i , s^2d_i and K_R . More than one parameter should be used for oil content stability estimation, as there are only moderate correlations between them, meaning each gives a certain amount of unique information.

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PROGRES, ADAPTABILNOST I STABILNOST PRINOSA ZRNA SOJE I KVALITET ZRNA KONVECIONALNO STVORENIH ELITNIH LINIJA

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Izvod

Ova studija je imala za cilj da utvrdi napredak u prinosu zrna i kvalitetu zrna koji se postiže konvencionalnim metodama oplemenjivanja, kao i da identifikuje stabilne, široko ili specifično prilagođene genotipove u uslovima gajenja u centralnoj Evropi. Nedavno razvijene elitne linije soje grupa zrelosti (MGs) 00, 0 i I upoređene su sa komercijalnim sortama (standardima) u uporednim terenskim ispitivanjima tokom tri uzastopne godine (2018-2020) u Osijeku, Hrvatska. Rezultati ANOVA pokazali su značajne efekte interakcije genotip, okruženje i genotip po životnu sredinu. Došlo je do značajnog poboljšanja produktivnosti i kvaliteta u odnosu na standarde, dok su parametri stabilnosti za ispitivane osobine ukazivali da postoje stabilne i uglavnom specifično prilagodljive elitne linije.

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