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Original scientific paper

A MULTIVARIATE APPROACH TO THE SELECTION OF PEA (*Pisum sativum* L.) LINES OBTAINED BY THE SINGLE SEED DESCENT TECHNIQUE

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Grain legumes, especially pea and lupine, are important agricultural crops in Central and Northern Europe. Because of the high level of protein in their seeds, these species constitute an alternative to soya meal imported from South America. The breeding of new cultivars in a short time is a major problem in pea breeding, particularly since the production of homozygous lines in the DH (doubled haploid) system, as used for cereals, for example, is very difficult. An alternative approach may be the single seed descent (SSD) method. The materials for this study were lines of pea obtained by that method combined with *in vitro* culture of embryos. This approach enabled 9 generations to be obtained in 2.5 years. First, SSD lines (F_{10}) were investigated in a field experiment conducted in a complete randomized design with two replications, in which the dates of flowering and ripening and seed yield per plant were observed. Seventy seven lines out of 120 (64%) were selected for a subsequent experiment in which F_4 generations of each cross combination were also included. In the second field experiment SSD lines were evaluated for earliness, plant height, seed yield per plot, and 1000-seed weight. The data were processed by multivariate analysis of variance and related methods, leading to the

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selection of 16 lines for further breeding experiments. Multivariate analysis can be helpful for breeders in seeking plants with a new favorable complex of yield-forming traits.

Key words: embryo *in vitro* culture, homozygous lines, pea breeding, pre-selection

INTRODUCTION

Grain legumes, especially pea and lupine, are important agricultural crops in Central and Northern Europe. Because of the high level of protein in their seeds, these species constitute an alternative to soya meal. Currently, the main source of protein in Poland is soya meal, which is imported in a quantity of 2 million tonnes per year (equivalent to 1 million tonnes of protein).

Pea is a self-pollinated crop. Breeding new varieties of pea is based on homozygous lines that are selected from heterozygous offspring. In conventional breeding, homozygosity can be attained by selfing successive generations, and several generations are needed to develop homozygous lines. In the conditions of Central and Northern European climates only one generation per year is feasible in the field, and so the breeding cycle persists for several years. The creation of new cultivars in a shorter time is an important problem in pea breeding, particularly since the production of homozygous lines in the DH (doubled haploid) system, as used for cereals, for example, is very difficult (WĘDZONY *et al.*, 2009) To shorten the breeding cycle, the single seed descent technique (SSD) in combination with *in vitro* culture of embryos dissected from immature seeds may be applied (SURMA *et al.*, 2013). The SSD technique involves the random selection of one seed from each individual plant in each generation, starting from F_2. In F_6 and more advanced generations, all seeds from individual plants are harvested; the progeny of a single plant are treated as an SSD line (GOULDEN, 1941). In the next steps of breeding, SSD lines are assessed in experiments conducted under field conditions. From the viewpoint of shortening the breeding cycle and for narrowing breeding materials, pre-selection of lines should be performed as quickly as possible, i.e. in the first field experiment aimed at the multiplication of seeds.

In Poland, a national, multi-year program (2011–2015) focused on the improvement of domestic sources of plant protein was established by the Polish government and the Polish Ministry of Agriculture and Rural Development. The aim of the project was the replacement of ca. 50% of imported protein by domestic sources, mainly from grain legumes. The present study was conducted as part of a task dealing with the development of new methods to reduce the time needed to obtain new pea varieties.

The aim of the present work was to assess SSD pea lines observed in field experiments during the first two years after *in vitro* culture, and to select lines for future breeding experiments.

MATERIAL AND METHODS

Plant materials

The material for the study included SSD lines of pea (*Pisum sativum* L.) derived from 8 cross combinations: Medal x Cysterski (MC), Muza x Tarchalska (MT), Medal x Model (MM), POA2481/99 x WTD5409 (PW), WTD 5409 x POA 2388/99 (WP1), id 961(DND) x WTD 5409 (IW), WTD550 x POA2810 (WP2) and Cysterski x POA 2481/99 (CP). The SSD technique was combined with *in vitro* culture of embryos dissected from immature seeds (SURMA *et al.* 2013). Embryos were cultured on full-strength MS medium (MURASHIGE and SKOOG, 1962) + 6 g l^{-1} agar

(pH 6.0) at 22/20^{0}C day/night temperature with a 16/8 h photoperiod. After 3 weeks of culture, pea plantlets were transferred *ex vitro* into pots and continued their growth in a greenhouse. Plants grown in the greenhouse flowered approximately 40–45 days after the beginning of *in vitro* culture.

The production of SSD lines began in 2011, when seeds obtained as a result of crossing parental genotypes were sown in a greenhouse. In the first step, from F_1 plants in each cross combination immature seeds were randomly collected, from which embryos were dissected and cultured *in vitro* to obtain at least 50 plants of generation F_2. In the next step, from each F_2 plant one immature seed was randomly collected to dissect the embryo. Dissected embryos were cultured *in vitro* for 18–21 days, and then the developed plants were potted and grown in a greenhouse. This procedure was repeated in each generation up to F_9. The average duration of one generation was 80 days. In F_9 all seeds from each plant were collected – the seed sample of an individual plant constituted an SSD line.

Lines for which at least 20 seeds were obtained were sown in spring 2014 in field conditions for multiplication and to make a preliminary assessment of SSD lines.

Experiment I

In the first year of field experiments (2014), 120 SSD lines were examined. The experiment was carried out in a completely randomized design with 2 replications and 20 seeds in individual plots. During the growing season the dates of full flowering and maturity were noted, and on the basis of those observations the earliness and duration of vegetation were assessed, as the number of days from germination to full flowering and maturity respectively. After harvesting, the seed yield per plant was recorded as the ratio of seed weight per plot to the number of plants in the plot.

Experiment II

Out of the 120 lines examined in 2014, 77 were chosen for the next experiment, which was carried out in 2015. In that experiment, besides SSD lines, the standard cultivars Tarchalska and Ezop were included. Additionally, because in the same period (2011–2015) plant materials from the same cross combinations were subjected to conventional selection conducted under field conditions, which led to F_4 in 2015, plants of that generation from each cross combination (treated as ramsh) were also included in the experiment. The experiment was established in a completely randomized design with 3 replications. Plants were grown on 1 m^2 plots. In each plot, 80 seeds were sown in four 1 m rows with a 25 cm space between rows and 5 cm between plants within rows. The following traits were observed: number of days from sowing to full flowering, plant height (measured on 5 plants randomly selected from each plot), seed yield per plot, and 1000-seed weight.

Both experiments were conducted at Wiatrowo breeding station (a branch of Poznań Plant Breeders Ltd.) located 50 km north of Poznań (Poland).

Molecular analysis

SSD lines in generations F_7 and F_{10} were subjected to molecular analysis to check their homozygosity. Lines were analyzed with the use of chromosome-specific microsatellite (SSR) molecular markers, providing polymorphic products for parental cultivars. In F_7, samples for DNA analysis were collected from 20 individual plants of each cross combination developed *in vitro*,

just before potting, whereas in F_{10} bulk samples of leaves collected from ten 10-day seedlings of each line were analyzed.

Genomic DNA from each of the plants was extracted using a Wizard Genomic DNA Promega Kit according to the manufacturer's instructions, starting with 2-week-old seedling leaves. DNA was quantified using a NanoDrop device (Thermo Scientific). SSR markers were dispersed across the genome and evaluated based on the parental cultivars' polymorphism. Among the 50 SSRs used in genetic analysis according to the latest reports (GONG *et al.*, 2010; SUN *et al.*, 2014; YANG *et al.*, 2015; RANA *et al.*, 2017), 10 were polymorphic between parents: PSP40SG, PSBLOX13.1, PSBLOX13.2, PSCAB66, PSJ000640A, PSLEGJL, PSLEGKL, P248, P251, P314.All markers were labeled (forward primer with VIC, NED, 6-FAM or PET dyes) and multiplex PCR (GeneAmp PCR System 9700 thermocycler) amplifications were performed in a total reaction volume of 6 µl, consisting of (approximately) 50 ng template genomic DNA. All PCR analyses were performed using PCR KIT (Qiagen). The cycling parameters were: 1 cycle of 95° C for 5 min; 30 cycles of 30 s, denaturing step at 94 °C, 30 s annealing at temperatures between 52 °C and 60 °C depending on the different primer combinations, and 30 s extension at 72°C with a final extension for 30 min at 60 °C. Separation of the PCR product was achieved using capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems). The fragment sizes were visualized and scored using the GeneMapper software from the same manufacturer, by comparison with the internal size standard Liz 600.

Microsatellite alleles were detected on an Applied Biosystems 3130 Genetic Analyzer.

Statistical analysis

Univariate (ANOVA) and multivariate analysis of variance (MANOVA) and related methods were applied for the evaluation of the studied breeding materials (CALIŃSKI and KACZMAREK, 1973; MORRISON, 1976). These methods enable the verification of hypotheses of lack of differences between genotype means both for individual traits and for all traits treated simultaneously. Canonical variable analysis was used to show the mutual position of the lines on a plane, considering all of the studied traits as a complex. Also, in relation to seed yield, the studied genotypes were divided into homogeneous groups using the sum of squares of deviations within the group as a natural criterion. It was assumed that the division is best when that sum attains its minimum.

RESULTS

Homozygosity of SSD lines

The homozygosity of SSD lines was analyzed by SSR markers, the alleles of which were different in the parental genotypes. Ten SSR primers utilized in this study amplified unambiguous and reliable alleles. The repeat unit numbers of SSR loci were from 5 to 36. All of them were found to have products with the expected size range. Allele size ranged from 153 to 367 bp. Maximum (6) alleles was amplified by primer pair PSBLOX13.1 and minimum (2 alleles) by the primers PSJ000640A, PSLEGJL and P251.

The results presented in Table 1 show that 6 F_7 plants out of 160 analyzed were heterozygous, a rate of 3.75%. In generation F_{10}, 2 lines among 120 analyzed were heterozygous, a rate of 0.8%. Both heterozygous lines were identified in the POA2481/99/2 x WTD5409 (PW) cross combination.

Table. 1. Size of amplification products of SSR markers obtained in PCR for SSDF$_7$ pea plants (in brackets – number of heterozygous plants)

Cross combination	POP40SG	PSBLOX13.2	PSBLOX13.1	PSCAB66	P251	PSLEGJL	PSLEGKL	P248	P251	P314
IW	342. 367	271	224. 236	244	238. 252	168. 196	153	178. 204	242. 254	254
	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
WP2	342. 369	271	224. 236	244. 248	238. 252	168	153. 179	178. 196	242. 254	254. 258
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
WP1	338	273. 283	224. 236	244. 246	238. 252	168	153. 177	178. 204	242. 254	254. 274
	(0)	(0)	(0)	(0)	**(1)**	(0)	(0)	**(1)**	(0)	(0)
CP	342	271. 283	224. 236	244. 246	238. 252	168	153. 177	178. 204	242. 254	254. 274
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
MC	338	273	226. 236	244. 246	238. 252	168. 196	153. 177	178. 204	242. 254	254
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
MM	338. 367	273. 283	226. 236	244. 246	238	168. 196	153. 177	178. 204	242. 254	254
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
MT	338. 367	273. 283	224. 236	244. 246	238. 252	168. 196	153. 177	178. 204	242. 254	254
	(0)	(0)	**(1)**	(0)	(0)	(0)	(0)	(0)	(0)	(0)
PW	342. 367	271. 283	224. 236	244. 246	238	168	153. 177	178. 204	242. 254	254. 274
	(0)	(0)	**(1)**	(0)	(0)	(0)	(0)	(0)	(0)	**(1)**

Experiment I

SSD lines were significantly differentiated in all three observed traits (Table 2).

Figure 1 shows the relative position of the lines in the coordinates of the first two canonical variables. It is easily seen that, in general, the lines constitute one group stretched along the first canonical variable. Two lines, 63 and 114, are located at different positions from the remainder. Line 63 was characterized by early flowering and ripening and medium seed yield, whereas line no. 114 exhibited late flowering, early ripening and high seed yield. Pre-selection of lines with respect to seed yield as the main trait was performed by dividing genotypes into homogeneous groups. In our case two groups were distinguished, with respective mean seed yields amounting to 28.12 and 15.09 g per plant (Table 3). 39 lines were classified in the low-yielding group, and these lines were excluded from the further breeding program. Among the remaining 81 lines, four were excluded because of their unfavorable agronomic traits observed by breeders but not taken into account in statistical calculations. Finally, 77 lines were selected for the next step of the breeding experiment.

Table 2. Analysis of variance for traits observed – experiment I

		Mean square		
Source	DF	Seed yield per plant	No. of days to flowering	No. of days to maturity
Genotype	119	116.051	21.907	23.782
Error	119	32.532	5.701	3.283
F cal.		3.57	3.84	7.24

$F_{0.05} = 1.36$; $F_{0.01} = 1.54$

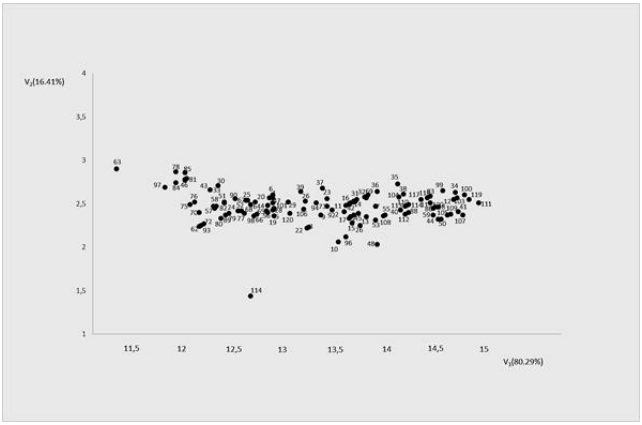

Fig. 1. Position of $SSDF_{10}$ pea lines in the coordinates of the first two canonical variables – experiment I

Table 3. Homogeneous groups of pea SSD lines for seed yield – experiment I

Group	Group mean (g)	Line number#
I	28.12	26, 59, 101, 63, 8, 62, 47, 41, 3, 67, 102, 72, 76, 24, 70, 73, 118, 89, 12, 115, 81, 25, 108, 105, 88, 112, 61, 85, 98, 90, 106, 84, 55, 82, 29, 119, 95, 68, 79, 45, 44, 35, 33, 86, 97, 1, 30, 21, 116, 65, 37, 32, 2, 91, 51, 74, 109, 92, 38, 107, 100, 34, 23, 9, 94, 83, 36, 14, 103, 110, 114, 99, 117, 77, 66, 69, 27, 96, 31, 64, 120
II	15.09	54, 56, 52, 17, 53, 57, 49, 50, 80, 78, 39, 15, 75, 4, 58, 18, 11, 40, 87, 43, 71, 19, 6, 10, 93, 48, 13, 42, 113, 60, 111, 7, 22, 5, 28, 46, 104, 20, 16

lines ordered from the lowest to the highest mean values

Experiment II

Significant differentiation of the studied lines in all observed traits was revealed by analysis of variance (Table 4). The highest variability was recorded for seed yield (17.88%), while for plant height and 1000-seed weight it was about 11–12%, and low variability (slightly above 3%) was found for days to flowering. Mean values for traits observed in generation F_4 hybrids were, generally, similar to those for SSD lines, considered regardless of their origin, although in all cases the ranges in SSD lines were wider than in F_4 (Table 5). Fig. 2 presents relative positions of $SSDF_{11}$ lines in the coordinates of the first two canonical variables V_1 and V_2. Correlations between the analyzed traits and V_1 and V_2 revealed that the V_1 variable was strongly positively correlated with days to flowering ($r=0.962$, $P<0.01$) and plant height (0.391, $P<0.01$), and significantly negatively correlated with 1000-seed weight (–0.614, $P<0.01$), whereas in the case of V_2 a highly positive correlation was found with 1000-seed weight (0.678, $P<0.01$) (Table 6). It may be seen that, taking into account all traits simultaneously, most of the lines constitute a relatively homogeneous group; only a few lines are positioned separately from this group.

Noteworthy are lines nos. 26 and 42, which were early flowering and had a high 1000-seed weight, while lines 36 and 38, located on the right side of Fig. 2, were characterized by late flowering, tall plants and low 1000-seed weight. The most interesting is line no. 25, located at the top middle of the graph, which was classified in the group of high-yielding genotypes and had the highest 1000-seed weight. Close to line no. 25 is line no. 74, which was characterized by tall plants, relatively high seed yield and 1000-seed weight, and late flowering.

Table 4. Analysis of variance for yield and yield-forming traits of pea SSD lines – experiment II

Source	DF	Mean square			
		Plant height	Seed yield per plot	1000-seed weight	No. of days from sowing to flowering
Genotype	84	150.15	0.0229	1487.12	17.95
Error	170	53.85	0.0081	143.56	0.89
$F_{cal.}$		2.788	2.83	10.359	20.257

$F_{0.05} = 1.37$; $F_{0.01} = 1.5$

Table 5. Characteristics of pea SSD lines and F_4 generations– experiment II

	Plant height (cm)	Seed yield per plot (kg)	1000-seed weight (g)	No. of days from sowing to flowering
SSD lines				
Mean	65.04	0.477	207.71	73.24
Min.	47.67	0.310	155.00	67.00
Max.	78.00	0.703	263.4	78.00
		F4 generations		
Mean	65.78	0.469	212.82	71.52
Min	58.33	0.283	194.5	68.0
Max	75.00	0.633	232.6	73.3
		Standard cultivars		
Ezop	63.67	0.490	202.5	72.3
Tarchalska	71.33	0.493	221.3	75.3
CV (%)#				

Calculated for all genotypes

Table 6. Correlation coefficients between analyzed traits and V_1 and V_2 canonical variables – experiment II

Trait	V1	V2
Plant height	0.391**	0.157
Seed yield per plot	0.242*	0.123
1000-seed weight	-0.614**	0.678**
No. of days from sowing to flowering	0.962**	0.262*

**P<0.05; ** P<0.01

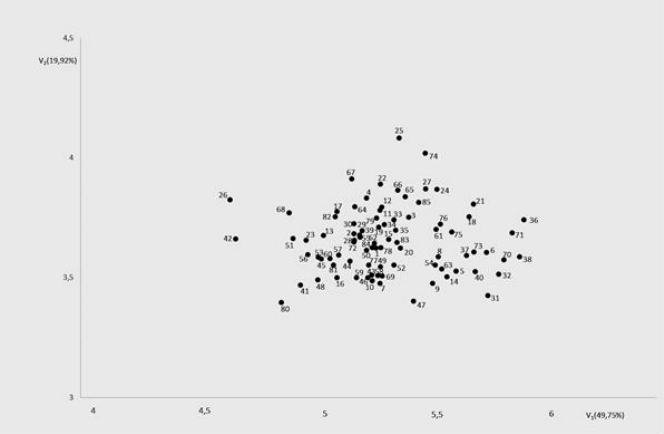

Fig. 2. Position of $SSDF_{11}$ pea lines in the coordinates of the first two canonical variables – experiment II: 1–77 – SSD lines; 78–83 – F_4 hybrids; 84 – cv. Tarchalska; 85 – cv. Ezop

The partitioning of the studied genotypes into homogeneous groups with respect to seed yield per plot is presented in Table 7. Three groups were distinguished. The first group contained only 18 genotypes, for which the average yield per plot amounted to 0.621 kg; among them were lines 25 and 74, and two hybrids: nos. 79 and 83. The standard cultivars Tarchalska and Ezop (84 and 85) as well as 51 other genotypes had medium seed yield, and 14 genotypes were classed as low-yielding.

Table 7. Homogeneous groups of pea SSD lines for seed yield per plot – experiment II

Group	Group mean	Line number#
I	0.621	5, 57, 73, 75, 79, 46, 11, 6, 3, 62, 65, 74, 51, 83, 58, 25, 8, 18,
II	0.487	34, 22, 29, 41, 55, 72, 59, 1, 39, 40, 13, 30, 23, 28, 78, 48, 36, 42, 24, 50, 77, 49, 76, 38, 10, 84, 7, 19, 33, 44, 47, 85, 27, 37, 31, 61, 69, 14, 26, 71, 2, 9, 81, 53, 20, 70, 60, 12, 15, 35, 43, 21, 56
III	0.371	80, 64, 54, 67, 82, 63, 45, 32, 68, 17, 66, 4, 16, 52

lines ordered from the lowest to the highest mean values; numbers 1–77 – SSD lines, 78–83 – F_4 hybrids, 84 – Tarchalska, 85 – Ezop (for details see Suppl. 2)

Comparisons of lines belonging to the high-yielding group with the mean for the standards Tarchalska and Ezop, for individual traits and all traits treated simultaneously, are presented in Table 8. Out of 18 lines, only 4did not differ significantly from the standards in a complex of analyzed traits ($F_{cal}<F_{0.01}$). Considering particular traits separately, it can be seen that plant height was generally similar to that for the standards; only in the case of line no. 5 it was significantly lower, and in the case of line no. 25 significantly higher, than the mean plant height of Ezop and Tarchalska. Four lines were characterized by significantly higher (nos. 11, 25, 51, 74) and four by lower (nos. 5, 6, 46, 73) 1000-seed weight. Among high-yielding lines, late flowering

genotypes were prevalent – the number of days from sowing to flowering was significantly higher for lines no. 6, 18, 25, 73, 74, 75, and only four (nos. 46, 51, 57, 58) flowered earlier than the standards.

Table 8. Estimates (Est.) and F statistics for contrasts between mean high-yielding genotypes and average mean of standard cultivars with respect to individual traits and all traits treated simultaneously – experiment II

Line no.	Plant height (cm)		Seed yield (kg)		1000-seed weight (g)		No. of days from sowing to flowering		F statistic for all traits simultaneously
	Est.	F	Est.	F	Est.	F	Est.	F	
5	-11.50	4.91	0.085	3.74	-20.57	5.89	3.50	3.07	7.19
57	-2.50	0.23	0.088	3.80	0.53	0.00	-2.83	18.12	3.34
73	5.83	1.26	0.083	3.80	-31.07	13.45	2.17	10.59	4.59
75	1.17	0.01	0.092	3.87	-8.27	0.95	1.83	7.58	2.93
79	2.50	0.23	0.092	3.87	8.17	0.93	-1.17	3.07	4.58
46	2.50	0.23	0.095	3.93	-23.23	7.52	-2.17	10.59	2.86
11	4.17	0.64	0.102	4.07	17.10	4.07	-0.50	0.56	2.63
6	-5.50	0.05	0.108	4.21	-32.17	14.41	2.83	18.12	5.91
3	-0.17	0.00	0.112	4.29	8.10	0.91	0.50	0.56	2.06
62	-1.50	0.08	0.115	4.36	1.20	0.02	-1.17	3.07	2.42
65	-0.83	0.03	0.125	4.61	15.50	3.35	0.83	1.57	2.14
74	8.17	2.48	0.125	4.61	29.57	12.18	2.17	10.59	10.31
51	5.17	0.99	0.138	4.97	20.80	6.03	-4.50	45.70	8.07
83	7.50	2.09	0.142	5.07	-2.43	0.08	-0.50	0.56	2.56
58	-3.833	0.55	0.188	6.66	-16.23	3.67	-1.83	7.58	3.22
25	10.50	4.01	0.208	7.47	51.50	39.95	1.83	7.58	6.81
8	7.83	2.28	0.212	7.62	-12.93	2.33	0.83	1.57	4.46
18	7.83	2.28	0.212	7.62	0.70	0.01	2.83	18.12	4.87
$F_{0.05}$				3.90					2.00
$F_{0.01}$				6.79					2.62

DISCUSSION

This study has shown that the SSD technique used in pea breeding can shorten the breeding cycle. That method, supplemented with *in vitro* culture of embryos, enabled us to obtain nine generations in the course of 2.5 years, while in conventional breeding conducted simultaneously under field conditions only generation F_3 was achieved. Molecular analysis

revealed the presence in F_{10} of residual heterozygosity. This is in agreement with theoretical considerations, because in self-pollinated plants homozygosity in all segregating loci occurs only in F_∞ (MATHER and JINKS, 1982).

The SSD technique requires breeders to take a different approach to selection. In conventional breeding individual plants are selected, usually from generation F_2 onward, on the basis of phenotypic evaluations, which can be biased by the influence of the environment and the effects of heterozygosity. Thus, positive effects observed in early generations may be reduced in subsequent generations with increasing homozygosity. In the case of SSD, selection is based on near-homozygous genotypes observed in plot experiments, and this type of selection is more effective.

In the SSD technique, any number of SSD lines can be derived from each cross combination, in contrast to the doubled haploid (DH) system, the effectiveness of which is dependent on genotype (PICKERING and DEVAUX, 1992; THOMAS *et al.*, 2003; CROSER *et al.*, 2006; WĘDZONY *et al.*, 2009). The proposed procedure combines the SSD method with *in vitro* culture of embryos, similarly to the approach presented by OCHATT *et al.* (2002). In our case the process of line development (up to F_9) was carried out *in vitro* and in a greenhouse. The next step was the multiplication of seeds in a field experiment, in which lines were represented by a limited number of plants, e.g. 20–50. We showed that multiplication of lines in the field may be combined with pre-selection based on easily observed traits, such as date of flowering and maturity, and, more time-consumingly, seed yield per plant as the ratio of seed yield per plot to the number of plants in a plot. Using uni- and multivariate statistical methods, lines of low seed yield as well as lines representing a new complex of traits can be distinguished and, based on these results, selected for the subsequent stages of breeding. Such preliminary selection permits the reduction of plant materials used for experiments in the subsequent year(s), when more precise observations of seed yield and its structure can be made. In our studies the second field experiment was conducted in three replications, and the results of statistical calculations showed that 16 out of 77 (21%) yielded significantly higher than standard cultivars. It should be noted that this experiment enabled a more precise evaluation of the yield than that conducted in the previous year.

Thus, the proposed approach enabled the development over 5 years of pea lines with a high degree of homozygosity, and the assessment of those lines in field experiments.

CONCLUSIONS

The combination of the SSD technique with *in vitro* culture of embryos enables a reduction in the time needed to obtain pea lines of high homozygosity by approximately 7 years. Evaluation of a large number of lines and selection of the most valuable lines require the use of appropriate statistical methods. A proposed first step is one-dimensional assessment with respect to seed yield and the division of lines into homogeneous groups. This allows breeders to distinguish high-yielding lines.

The second step may be multivariate analysis, taking account of other characteristics in addition to seed yield, such as earliness, plant height and 1000-seed weight. Canonical variable analysis made it possible to distinguish pea lines that are characterized by a complex of observed traits different from the remaining genotypes.

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MULTIVARIACIONI PRISTUP ZA SELEKCIJU LINIJA GRAŠKA (*Pisum sativum* L.) DOBIJENIH SSD TEHNIKOM

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Izvod

Zrnene mahunarke, posebno grašak i lupine su veoma značajni poljoprivredni usevi u Centralnoj i Severnoj Evropi. Zbog visokog sadržaja proteina u zrnu, predstavljaju alternativu sojinom brašnu koje se uvozi iz Severne Amerike. Stvaranje novih sorata za kratko vreme je glavni problem u oplemenjivenju graška, posebno zato što je proizvodnja homozigotnih linija sistemom duplih haploida (DH), koji se koristi kod žitarica, veoma zahtevna. Alternativni pristup može biti metod SSD. Materijal za ovo istraživanje bile su linije graška dobijene tom metodom u kombinaciji sa *in vitro* kulturom embriona. Ovaj pristup omogućava dobijanje 9 generacija za 2,5 godine. Prvo, SSD linije (F_{10}) su ispitivane u polju u kompletno randomiziranom blok dizajnu sa dva ponavljanja, gde su evidentirani datumi cvetanja, zrenja i meren prinos zrna po biljci. Od 120 linija, odabrano je 77 (64%) za naredne eksperimente u koje su uključene i F_4 generacije svakog ukrštanja. U drugom eksperimentu u polju SSD linije su ocenjivane za ranostasnost, visinu biljke, prinos zrna po parcelici i masu 1000 zrna. Podaci su obrađeni multivarijacionom analizom varijanse i odgovarajućim metodama, tako da je odabrano 16 linija za dalja testiranja. Multivarijaciona analiza može biti od koristi oplemenjivačima u odabiru biljaka sa poželjnim osobinama značajnim za prinos.

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