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POLYMORPHISMS IN SSR-LOCI ASSOCIATED WITH *E* GENES IN SOYBEAN MUTANT LINES OFFER PERSPECTIVE FOR BREEDING

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Aim. To analyse genetic diversity in 10 new soybean lines created by using the chemical mutagens D-6, DMSO-11, DMSSO-12, DMSNPIR-11, DUDMS12, D12DMC-11B obtained from four cultivars Femida, Oksana, Podils'ka 416, Zolotysta. The microsatellite (MS) markers *Satt100*, *Satt229*, *Satt319*, *Satt354*, *Satt365*, *Sat_038* were used. These markers are linked with genes, which determine sensitivity of soybean plants to photoperiod and time to maturation. **Methods** of DNA extraction, PCR, MS-analysis, field trial, one-way analysis of variance (ANOVA) have been applied. **Results**. Parental cultivars, mutant lines and control genotypes were characterized by alleles of microsatellite loci, 25 alleles of 6 microsatellite loci were detected. Significant differences between investigated lines were detected in three year field trials for traits − days to maturation (DTM) and length of the vegetative period (LV). We have revealed effects of the factor «Alleles of MS-locus», so alleles of *Satt100* locus affected all traits except DTF (days to flowering); alleles of *Satt319* and *Satt354* affected DTM and LV; *Sat_038* affected DTF and S-F (duration of the period shoots-flowering). Lines with alleles 167 bp at *Satt100* and 175 bp at *Satt319* loci (that marks dominant *E7*) were shown to have a longer vegetation period and later maturity, than other. The lines with allele 247 bp at *Sat_038* flowered earlier, than lines with a 245 bp allele, and the lines with allele 232 bp at *Satt354* reached maturity later, than lines with other alleles at this locus. **Conclusions.** We have found that applied mutagens induce changes in the soybean genome and by using these mutagens it is possible to effectively increase genetic diversity in loci associated with genes/loci that determine time of maturity and/or photoperiod sensitivity of soybean, enabling to obtain soybean cultivars with different terms of maturity and yield. The microsatellite markers, particularly *Sat_038, Satt100, Satt319* and *Satt354* that were applied in our study are considered to be useful tools for marker assisted breeding of soybean cultivars with programmed time of development. We did not observe significant effects of «Alleles of MS-locus *Satt229*» that is known to be linked with *E3* on the investigated agronomical traits. For soybean genotypes with the *E7* allele the DTF was longer for 3-9 days and LV for 10-11 days. In lines with an allele of 175 bp at locus *Satt319* the S-F period was 6-9 days shorter.

Keywords: soybean, mutant lines, polymorphism, SSR-loci, microsatellite markers, *E* genes, photoperiod, maturity.

DOI:

INTRODUCTION

Flowering time is critical for successful seed production of plants, and with time to maturity it determines geographic adaptation, seed quality and yield. In contrast to most cereals, soybean (*Glycine max* (L.) Merr.) cultivars are confined to comparatively narrow ranges of geographical latitudes [1]. For each degree of changes in latitude (corresponding to 100 – 150 km), it is better to develop a new (better adapted to environmental conditions) cultivar [2].

Across the world, cultivars of soybean can grow in a wide range of latitudes from 55° N to 35° S [3, 4]. Northern than 55 ° N latitude most genotypes do not mature [4]. In Ukraine breeders identify the area most favorable for growing this crop, the so-called «Soybean's Belt», which is located on irrigated soils and is between 46° N and 51° N. According to [4] in 5 regions of the Forest-Steppe Zone: Vinnitsa, Kyiv, Poltava, Cherkasy and Khmelnitsky and Steppe- Kropyvnytsky, more than 60 % of all soybean in Ukraine is grown. For all regions of Ukrainian «Soybean's Belt» extra-early and early maturity groups of soybeans allow to expand the area of this crop considerably, to get dry grain without drying, to use soybean as intermediate and repeated crops are especially important [5].

The development of early-ripeness (short season) soybeans for different regions of Ukraine requires effective use of early maturity genes. Detection of alleles of *E* genes that are involved in the control of plant response to photoperiod and determination of the days to flowering (DFT) and days to maturation (DTM) with microsatellite markers could help in the evaluation of adaptive capacity of soybean cultivars under different growth conditions [2].

Soybean production in Ukraine increases every year since its wide introduction in 2007, and now Ukraine is one of the leaders in the production of soybean in Europe [6]. There are few investigations of molecular genetic polymorphism in the genome and genes that affect time to flowering (involved in mechanism of photoperiodic sensitivity of plants) with molecular markers for Ukrainian soybean cultivars [7–11]. But optimization of soybean breeding and development of new cultivars with good adaptability to the conditions of Ukraine are important.

Breeders are interested in developing new material with effective alleles of *E* genes (early maturity genes) for Ukraine [4, 5, 12]. By using experimental mutagenesis, it is possible to get a high level of genetic variability. But it is impossible to predict to which changes chemical mutagenesis will lead to. The mutagenic factors in soybean selection are most often used for production of new forms that differ from the original cultivars according to individual characteristics: seed coloration, plant height, seed size, leaf shape, duration of the vegetative period, content and quality of protein and seed oil, resistance to pathogens and increasing productivity elements, especially: main stem nodes, pods per plant, seeds per plant, weight of thousand seeds [13, 14]. The main aim of modern soybean breeding is improving the productivity, technological qualities of the seeds, increasing resistance to biotic and abiotic factors, yield, fertility, optimizing the growing season [15].

The purpose of our work was to analyze genetic diversity in mutant lines of soybean by using the microsatellite (MS) markers *Satt100*, *Satt229*, *Satt319*, *Satt354*, *Satt365*, *Sat_038* linked with genes that determine sensitivity of soybean to photoperiod and time to maturation. These markers are recommended for *E1, E3, E4* and *E7*-genes detection by Molnar et al. [16]. The markers *Satt100*, *Satt319*, we used as recommended by Rosenzweig et al. [17].

MATERIALS AND METHODS

A set of new soybean lines created with the help of chemical mutagenesis from cultivars Femida, Oksana, Podils'ka 416, Zolotysta, that belong to different groups of maturation [18–20], was used as a material for investigation.

As mutagens were used: D-6, DMSSO-11, DMSSO-12, DMSNPIR-11, DUDMS12 and D12DMC-11B, that are derivatives of tetrahydrothiophene-N-dioxide-3,4-diamine and tetrahydrothiophene-N-dioxide-3,4-pyridine, provided by P.G. Dul'nev from V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine (Kiev, Ukraine).

The mutagens were applied on seeds in aqueous solution at concentrations of 0.05 g/l, 0.5 g/l, 5 g/l, and 10 g/l. Seeds were exposed to the mutagens for 2, 4, 8, 16 hours [21]. After mutagenic treatment lines were grown in an experimental field during 5–7 agronomical seasons in the Institute of Feeds and Agriculture of Podillia of NAAS (IFAP; Vinnitsa, Ukraine). Then, mutant lines perspective for breeding with internodes less than 5 cm were selected in order to reduce the length of internodes, to decrease the vegetative mass of plants and to increase the generative mass of seeds and beans (Dr. S.V. Ivanyuk, personal communication (IFAP)).

DNA was isolated from soybean seeds of 10 mutant lines and parental cultivars using the DNA-NeoPrep100 kit (Neogen Laboratory, Kiev, Ukraine). Five randomly chosen seeds from each of the parents and mutant lines were used for DNA isolation. PCR with primers specific for the microsatellite loci *Satt100*, *Satt229*, *Satt319*, *Satt354*, *Satt365*, *Sat_038* was performed according to the method of Monlar et al. [16]. For fragment analysis and detection of the alleles of MS-loci the ABI PRISM® Genetic Analyzer

3500 (Applied Biosystems) was used in the Institute of Genetics and Cytology (Minsk, Belarus). The results were analyzed with GeneMapper® Software Version 4.1. As a standard of molecular weight, the Orange DNA size standard (MCLABs http://www.mclab.com/DNA-SizeStandard) was used. As controls for PCR analysis served DNA of accession Harosoy isolanes from OT 89-5 (here in after Harosoy OT 89-5) and DNA of soybean cultivars Vilana, Ros', Cormoran AC, Maple Arrow.

Accession Harosoy OT 89-5 and cultivar Vilana carry of the dominant *E7* allele, Maple Arrow – the dominant *E3* allele, Cormoran AC has the dominant *E1* allele and Ros' carries the dominant *E2* allele [22].

Agronomical traits, such as days to flowering (DTF), days to maturation (DTM), length of the vegetative period (LV – days), duration of the period shoots-flowering (S-F – days) and yield (t/ha) were investigated in three year trials (2016-2018) under field conditions of IFAP, 49°13`N (Vinnitsa, Ukraine). Parental cultivars, mutant lines, and the controls, cultivars Vilana and Maple Arrow were sown all at the same day.

Parental cultivars and control isolines belong to different maturity groups: (00 − early maturity (91–110 days)) − Harosoy OT 89-5, Ros', Zolotysta; (0 − middle maturity (111–130 days)) − Maple Arrow, Cormoran AC, Podilska 416; (I − middle late maturity (131–150 days)) − Vilana [18, 23]. According to the Catalog of varieties of forage and field crops of IFAP, cultivar Femida, which has LV − 116–124 days, belongs to the middle maturity group I and cultivar Oksana, which LV is 125–132 days, belongs to the middle-late maturity group II [24]. So, cultivars in the same group of maturity could have different alleles of *E*-genes. Classification of maturity groups (MGs) for soybean cultivars have been developed in the 1940s, it was revised and improved from these times and now includes 13 MGs (000, 00, 0, I – X), according to Liu et al. [25]. In different countries scientists have adopted this system to local conditions, for example, in Japan local soybeans differentiate in 8 MGs (0–VII), in India local cultivars are mainly in V–VIII MGs, in Italy soybean cultivars are from 0 to II MGs, in France – from 000 to II MGs. At the same time the system of MGs is a major approach in characterizing ecological properties and possible growing areas of cultivars and lines [25], there is a mind that the difference in maturity date between two adjacent groups is approximately 10 to 15 days in adapted area [25].

Table 1. Allelic characteristic of parental and mutant lines by microsatellite loci

Parental and control cultivars/mutant lines	Microsatellite loci (bp)					
	Satt100	*Satt229*	*Satt319*	*Satt354*	*Satt365*	*Satt038*
Oksana	167	230	175	232	301	247
Oksana M2	**141**	230	**180**	232	301	247
Oksana M12	167	**234**	175	**249**	301	**245**
Oksana M13	**141**	**234**	**180**	**249**	301	**245**
Zolotysta	141	234	180	178	301	247
Zolotysta M16	**131**	**230**	**178**	178	**270**	247
Zolotysta M20	141	**230**	180	**230**	301	247
Femida	110	212	175	230	301	247
Femida M29	**167**	212	175	178	301	245
Femida M32	113	212	175	230	301	245
Podils'ka 416	110	215	175	230	301	245
Podils'ka 416 M33	**141**	**234**	**180**	230	301	**247**
Podils'ka 416 M38	**141**	**234**	**180**	230	301	245
Podils'ka 416 M40	**113**	**212**	175	230	301	247
Ros'	145	215	178	178	270	243
Vilana	167	234	175	249	301	247
Harosoy OT 89-5	167	183	175	216	301	247
Cormoran AC	131	183	178	178	270	247
Maple Arrow	131	215	178	178	215	247

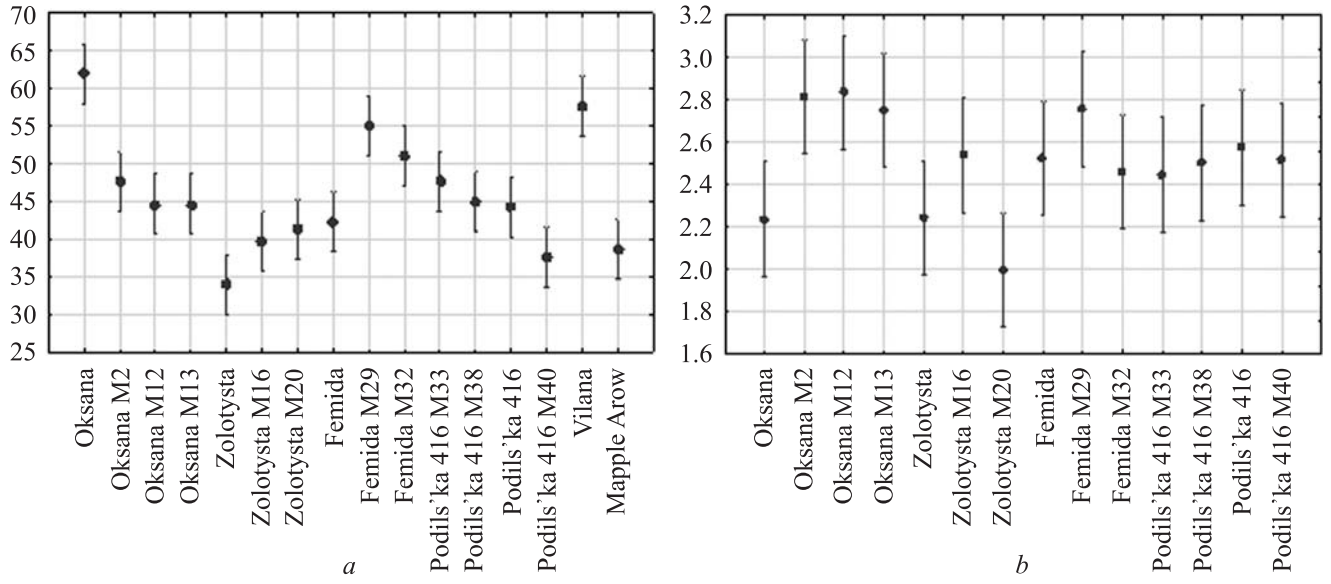

Fig. 1. Mean values ± std. err. of DTM (*a*) **y** − in days from the beginning of August; (*b*) yield in t/ha of mutant lines and parental cultivars of soybean over a period of three growing seasons (2016 to 2018). Bar = std. err.

We used the one-way analysis of variance (ANOVA) and the Statistica 10 software for analysis of the results of the field experiments. The significance of interline variations for each trait was determined using Fisher's exact test (F-test) and LSD of the corresponding level of significance for the investigated factors.

RESULTS AND DISCUSSION

In total 25 alleles of 6 MS-loci for the investigated parental, mutant and control genotypes were detected (Table 1). The number of alleles per locus ranged from 3 to 6 with an average of 4.2. In the genotypes of 10 mutant lines, which were selected as promising for breeding, we have revealed new alleles of microsatellite loci that were not present in parental cultivars (bold in the Table 1). The changes of allele size in mutant lines were detected in 52 % of the cases of comparisons with the alleles of the same loci in the parental cultivars.

These changes can be explained by the action of mutagenic substances applied at different concentrations on soybean by inducing instability and mutations in «hot spots» of the genome, among which microsatellite loci can act. We supposed that mutagenic treatment, however, could have influence on generative apparatus of soybean [26], that leads to open flowering and possible pollination by pollen of other cultivars and soybean lines grown adjacent to the experimental field. In general, soybean is self-pollinating plant, but crosspollination may still occur at a low percentage (average 1.8 %) [27] under natural conditions, it is still not enough investigations to say whether the percentage of crosspollination can increase after mutagenic treatment, but we can assume that it can be possible. Whatever the cause of the observed variation is, breeders found and selected lines, which varied at MS-loci, linked with genes that control photoperiod sensitivity.

Five alleles were detected at the *Satt100* locus in the mutant lines and parental cultivars and an additional allele for cultivar Ros' that was used as control. In comparison with the control varieties we identified dominant and recessive alleles of *E7* gene according to alleles of *Satt100* locus. Three alleles were revealed for the *Satt319* locus. According to our results cultivar Oksana, mutant lines Oksana M12 and Femida M29, which have a 167 bp amplification fragment at locus *Satt100* and a 175 bp amplification fragment at locus *Satt319*, have the dominant allele of *E7* gene. Genotypes Vilana and Harosoy OT 89-5 were used as the control, they have the same alleles (167 bp at *Satt100* and 175 bp at *Satt319* loci). We should mention that according to Molnar et al. [16], alleles 167 bp at *Satt100* and 175 bp at *Satt319* are associated with the dominant allele of *E7* gene.

In our investigation an allele of 175 bp at *Satt319* locus was detected in Femida, Femida M32, Podils'ka 416, Podilska 416 M40, but there was no confirmation of the presence of the dominant *E7* al-

lele, when we analyzed the *Satt100* locus for these lines.

The presence of *E3* dominant allele was established for Podils'ka 416, because for this cultivar we identified an amplification fragment of 215 bp at locus *Satt229*, the same as we showed to be present in the control cultivar Maple Arrow – a carrier of the dominant *E3* gene. We did not detect any dominant alleles for *E2* among investigated parental and mutant genotypes. The control allele for *E2* − fragment of 243 bp at locus *Sat_038* was found only in cultivar Ros', which is the carrier of *E2*.

One of the purposes of soybean breeding in Ukraine is creation of ultra-early cultivars with high yield potential in order to originate a cost-effective cultivar, which produce high harvest as early as possible and permit, to prepare a field for sowing winter crops. For the creation of such genotypes, according to Dr. S.V. Ivanyuk, it is necessary to combine the recessive alleles *e1, e2, e3, e4, e5*, *e7* in one genotype of new cultivar by crossing different donor sources of soybean. Golovenko et al. [28] stated on the basis of their research with the 7 alleles, that a recessive genotype for all these genes contributed to higher soybean yields in Belarus. Miladinovich et al. [29] have shown that the combina-

Table 2. Mean values of traits for different lines for three years of studies

Parental cultivars/ mutant lines	DTF (days) *	DTM (days) **	LV (days)	S-F(days)	Yield (t/ha)
Oksana	37.3	62.0	149.0	44.0	2.24
Oksana M2	44.0	47.7	134.0	52.3	2.82
Oksana M12	48.3	44.7	131.0	56.7	2.84
Oksana M13	48.0	44.7	131.0	56.3	2.75
Zolotysta	27.7	34.0	121.0	34.3	2.24
Zolotysta M16	28.7	39.7	126.0	37.0	2.54
Zolotysta M20	39.0	41.3	128.0	47.3	2.00
Femida	26.7	42.3	129.0	33.3	2.52
Femida M29	42.3	55.0	141.0	50.7	2.76
Femida M32	36.3	51.0	137.0	44.7	2.46
Podils'ka 416	35.3	44.3	131.0	42.0	2.58
Podils'ka 416 M33	43.0	47.7	134.0	51.3	2.45
Podils'ka 416 M38	43.7	45.0	131.0	52.0	2.50
Podils'ka 416 M40	35.7	37.7	124.0	44.0	2.52
Vilana	39.3	57.7	144.0	46.0	–
Maple Arrow	35.3	38.7	125.0	42.0	–
$LSD_{0,05}$	–	11.31	12.52	–	–
$LSD_{0,01}$	–	15.2	16.74	–	–

Notes: *– for DTF days from the beginning of June, ** – for DTM days from the beginning of August.

Table 3. Data of dispersion analysis of trait variations

MS-loci/Trait	Variation source, MS							
	DTF	Rd^{DTF}	DTM	Rd^{DTM}	LV	Rd^{LV}	S-F	Rd^{S-F}
Satt100 (df =4)	214.81	103.15	349.3 ***	62.0	350.1 **	70.2	243.9 *	93.6
Satt229 (df =3)	126.94	111.68	53.1	88.8	48.6	97.1	137.8	104.3
Satt319 (df =2)	183.78	109.49	334.1 *	75.5	336.6 *	83.2	212.7	101.7
Satt354 (df=3)	256.99	102.81	275.0 *	73.6	274.5 *	81.7	263.8	95.7
Satt365 (df =2)	163.71	110.38	153.1	83.5	151.8	91.4	158.1	104.1
Sat_038 (df=1)	500.00 *	104.23	74.8	86.7	66.6	94.6	586.8 *	96.0

Notes: Rd – Remaining dispersion; * Significant at p = 0.05, ** p = 0.01 and *** p = 0.001. df – degree of freedom.

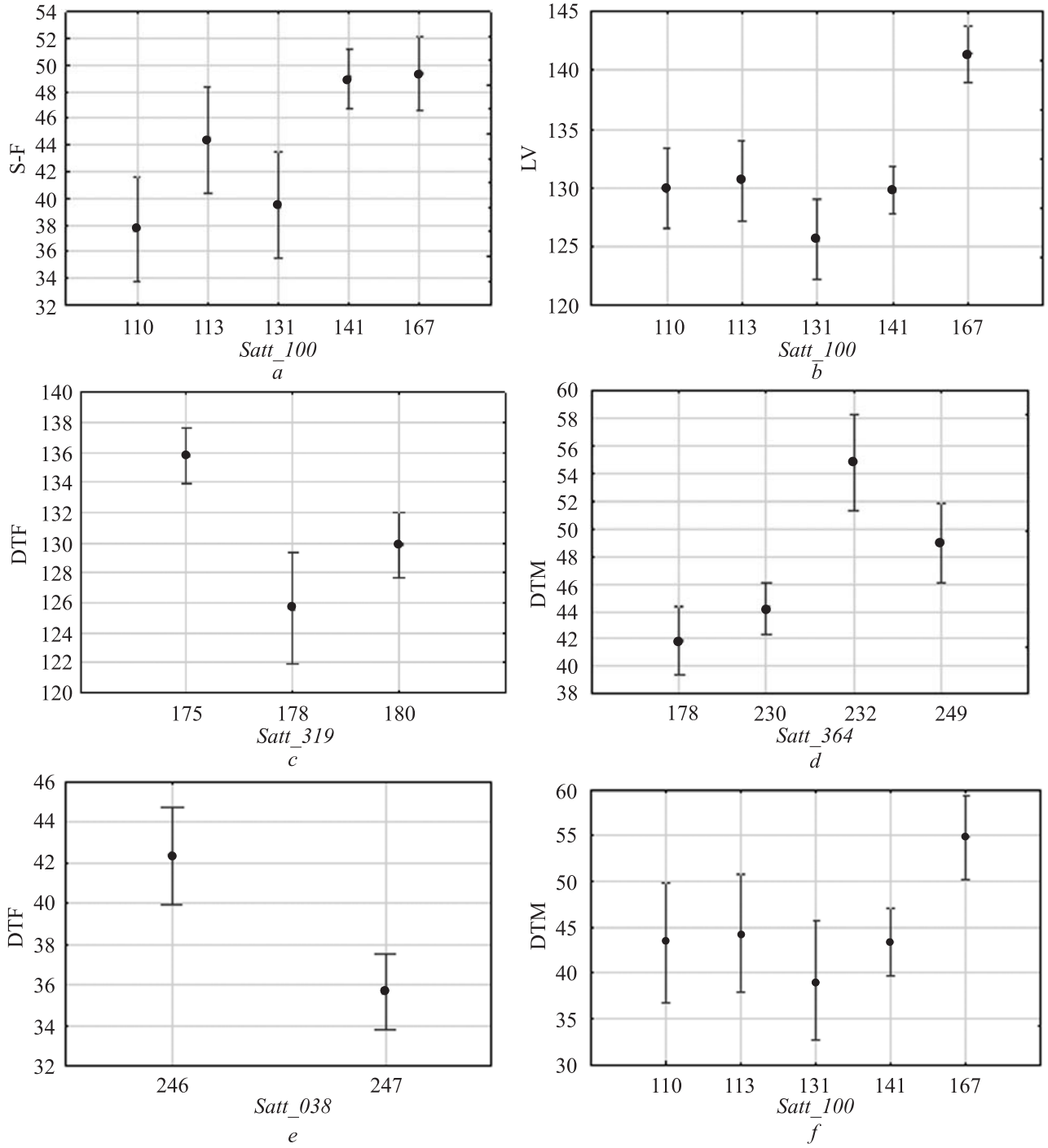

Fig. 2. Mean values of traits (vertical axis), depending on the presence of alleles of MS-loci. Bar = std. error

tion of alleles *e1-as/e2/E3/E4 was* the most common for high yielding soybean genotypes in Novi Sad. They stated that this specific allele combination possibly is the optimal one for the climatic zone of Central-Eastern Europe.

In our experiment *E1* dominant allele has been detected in Zolotysta M 16, because this line has a 270 bp allele at locus *Satt365*, the same allele was present in the control cultivars Cormoran AC and Ros' in which dominant *E1* is present.

Significant differences between investigated lines were detected in three years field trials for traits DTM (Fig. 1, *a*) and LV (Table 2).

There were no significant differences in the date of flowering, period shoots − flowering and yield (Table 2, Fig. 1, *b*) between all investigated genotypes.

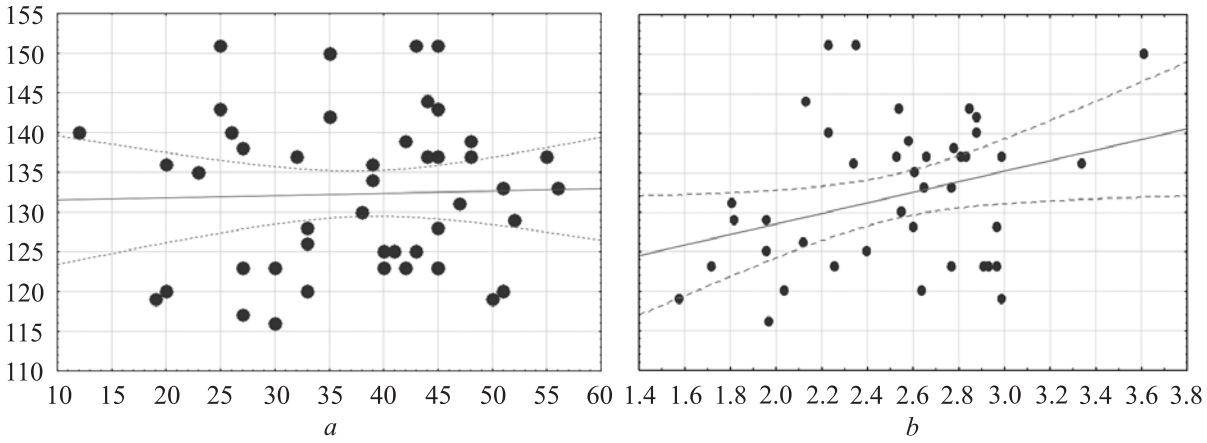

Fig. 3. Correlation between traits: (*a*) DTF (horizontal axis) and LV (vertical axis) r = 0,03; (*b*) Yield (horizontal axis) and LV (vertical axis) (r = 0,32; p = 0,05)

According to the one-way dispersion analysis, alleles of MS-loci *Satt229* and *Satt365* have not affected investigated traits (Table 3).

On the other hand, alleles of MS-locus *Satt100* affected all traits except DTF; alleles of *Satt319* and *Satt354* MS-loci affected DTM and LV; and alleles of *Sat_038* MS-locus affected DTF and S-F.

Lines with alleles 167 bp at *Satt100* and 175 bp at *Satt319* loci (that marks *E7*) were shown to have a longer vegetation period and later maturity than other ones. There were no significant differences in DTF for these lines.

There was no significant correlation between traits DTF and LV for all material studied (r = 0,03) (Fig. 3), between yield and DTM (r = 0,03), and yield and DTF (r = –0,22). Between yield and LV (r = 0,32) was significant correlation (p = 0,05). Thus, a longer growing season leads to a higher yield under Ukrainian growing conditions. Under Belarus growing conditions dominant allele *E7* delayed flowering of soybean plants for 4–5 days [22].

According to Molnar et al. [16] MS-marker *Satt354* can be used to detect alleles of *E4;* the dominant allele of *E4* is marked by a 251 bp amplification fragment. According to personal communication of Dr. E.A. Aksyonova (Institute of Genetics and Cytology, Minsk) the amplification fragment for Vilana has smaller molecular size, than that of the control line L64-4830 with the dominant allele. In our investigation all amplification fragments sizes were smaller than those of cultivar Vilana. Thus, we can conclude that lines with dominant allele *E4* were not present in our experiment. The mutant lines with allele 232 bp at *Satt354* locus reached maturity later than lines with other alleles at this locus (Fig. 2). In literature, there is, however, no information that a 232 bp fragment marks the dominant allele of *E4*.

The lines with allele 247 bp at the *Sat_038* locus flowered earlier than the lines with a 245 bp allele in our field experiment.

Genotypes with the same alleles for each of the mentioned *E*-genes were grouped together. Dominant *E1* allele, which is present in Zolotysta M16, could be involved in earlier flowering (about 10 days), than the other lines and cultivars demonstrated and the S-F period was 10 days shorter for this line in comparison with all investigated material. But of course, these shorter periods could also be caused/influenced additionally by interaction of the genotype by *E*-genes with environmental conditions and genetic background of cultivars.

We did not observe significant *E3* effects on the investigated agronomical traits.

Alleles of the *E7*-gene significantly influenced on the DTF, DTM, LV and S-F. Genotypes with the dominant allele were characterized by a longer DTF, DTM and LV. Genotypes with *E7* and *e7* alleles had not significant differences in the duration of S-F period, but for the lines with an allele 175 bp at locus *Satt319* the characteristics S-F was shorter for 9 or 6 days for *E7* dominant and *e7* recessive alleles respectively. Furthermore, for lines with *e7* and an allele of 175 bp at locus *Satt319* there were no differences in the traits for LV and DTM and for soybean genotypes with the *E7* allele the maturity was later and LV was longer for 10 days.

Both groups with *E1* and *E3* dominant alleles contained only one genotype each. According to our results of MS analysis we predicted *E1* to be present in

Zolotysta M16 and *E3* in Podils'ka 416. Presumably, lines created on the basis of one cultivar had smaller differences than those created on the basis of another cultivar. Perhaps in this case, it will be possible to determine the influence of the genotype on the DTF, DTM, LV, S-F and yield, which is also of importance.

Inside a group created from one ancestral genotype, for example, the group created on the base of Podils'ka 416 (included Podils'ka 416, Podils'ka 416 M33, Podils'ka 416 M38, Podils'ka 416 M40) we have not detected significant effects of the *E3* on the investigated traits. The same situation was with *E1* in the group produced on the base of cultivar Zolotysta. Inside the group of genotypes created on the base of cultivar Oksana dominant *E7* allele significantly decreased yield and did not significantly affect other traits. Also we observed difference in yield between both genotypes with *E7* Oksana and Oksana M12.

It should be noted that in our three year field experiment cultivar Zolotysta, which belongs to maturity group 00, was earlier among the genotypes tested but its mutant lines have a tendency to be later. Oksana was the latest genotype, which we included to maturity group I. This cultivar has mutant derivates with a shorter LV on 18 days. For cultivar Zolotysta the length of the vegetative period was shorter for 28 days (almost 1 month) as compared to that of Oksana.

CONCLUSIONS

Our results show that the mutagens D-6, DMSSO-11, DMSSO-12, DMSNPIR-11, DUDMS12, D12DMC-11B induced changes in soybean genome. By using these mutagens, it is possible to effectively increase genetic diversity in loci associated with the genes that determined photoperiod sensitivity of soybean. The obtained mutant lines could be involved in the breeding of soybean cultivars with different levels of photoperiodic sensitivity, terms of maturity, length of vegetation period and therefore adaptation ability. For example, mutant lines created on the basis of variety Oksana have significantly shorter LV ($P = 0.05$ and $P = 0.01$) for about 15-18 days than Oksana because of the earlier maturity. Line Femida M29 reached maturity significantly later ($P = 0.05$) then parental cultivar Femida.

The three year observations of the 10 mutant lines, parental cultivars and controls obtained under field conditions of the Vinnitsa region of Ukraine permit to make the following conclusion: the duration of the shoots-flowering (S-F) period for soybean lines with *E7* and *e7* alleles differed not significantly, but the lines with an allele of 175 bp at locus *Satt319* the S-F period was 6–9 days shorter. Lines with *e7* and an allele of 175 bp at locus *Satt319*, however, did not show differences in LV and DTM. For soybean genotypes with the *E7* allele the DTF was longer for 3–9 days and LV for 10–11 days. We can conclude, that the changes in DTF and LV, which we have detected for mutant lines in comparison with parental cultivars, are on the measure of variation in MG groups and between the MGs, when we compared with the studies Liu et al. [25], but if the IFAP's soybean mutant lines is grown in other ecological condition the differences in DTM can became significant. In general, mutant lines that have been developed in the IFAP are interesting not only for breeding process, but also for investigation of molecular mechanism of changes in the soybean genome that permit to create lines with different times of maturity and LV.

These findings demonstrate again that under Ukrainian growing conditions the microsatellite loci studied, can be useful tools for marker assisted breeding of soybean cultivars with programmed terms of development in order to widen its cultivation range as much as possible. As noted by Rosenzweig et al. [30] and Miroshnichenko et al. [31], the most prominent for this target is the dominant *E7* allele, which did not affect the flowering time in comparison with recessive allele, although in our hands it had an extended reproductive stage that is preferable for soybean varieties. But in our experiments we obtained data that *E7* decrease yield inside the group of genotypes created on the basis of cultivar Oksana, but there are diffe-rences between lines with the same *E7* genotype, that is why research should be continued on more special genetic material, for example, near-isogenic lines or analogue lines.

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The authors declare that they have no conflict of interest.

The authors declare that this study complies with the current laws of the countries in which the experiments were performed.

Поліморфізм за SSR-локусами асоційованими з генами *E* у перспективних для селекції мутантних ліній сої

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Мета. Аналіз генетичної різноманітності 10 ліній сої, створених шляхом хімічного мутагенезу за допомогою мутагенів: Д-6, ДМССО-11, ДМССО-12, ДМСНПІР-11, ДУДСМ-12, Д12ДМЦ-11Б на основі сортів Феміда, Оксана, Подільська 416, Золотиста були використані мікросателітні (МС) маркери: *Satt100, Satt229, Satt319, Satt354, Satt365, Sat_038.* Ці маркери зчеплені з генами, які визначають чутливість рослин сої до фотоперіоду та час дозрівання. В роботі використовували методи − екстракції ДНК, ПЛР, МС-аналізу, проводили польовий експеримент, за допомогою дисперсійного аналізу обраховували отримані данні. **Результати**. Батьківські, мутантні лінії та контрольні генотипи охарактеризовано за алелями мікросателітних локусів, у дослідженому матеріалі виявлено 25 алелів за 6 мікросателітними локусами. Виявлені достовірні відмінності між дослідженими лініями за три роки польових випробувань за ознаками – час дозрівання та тривалість вегетаційного періоду. Виявлено вплив фактора «Алелі МС-локуса»: алелі *Satt100* мали ефекти на всі ознаки, крім DTF (кількість днів до цвітіння); алелі *Satt319* і *Satt354* впливали на DTM (кількість днів до дозрівання) та LV (тривалість вегетаційного періоду); алелі *Sat_038* мали вплив на DTF і S-F (тривалість періоду від сходів до цвітіння). Лінії з алелями 167 п.н. за *Satt100* та 175 п.н. за *Satt319* локусами (що маркують домінантний стан гену *E7*) мали більш тривалий вегетаційний період та більш пізніше дозрівання, ніж інші. Лінії з алелем 247 п.н. за *Sat_038* зацвітали раніше, ніж лінії з алелем 245 п.н., а лінії з алелем 232 п.н. за *Satt354* досягали зрілості пізніше, ніж лінії з іншими алелями цього локусу. **Висновки**. Нами виявлено, що застосовані мутагени індукували зміни в геномі сої і за допомогою цих мутагенів можна ефективно збільшувати генетичне різноманіття сої за локусами, пов'язаними з генами/ локусами, що визначають час дозрівання та/ або фотоперіодичну чутливість, що робить можливим створення сортів сої з різними термінами дозрівання та урожайністю. Мікросателітні маркери, зокрема *Sat_038, Satt100, Satt319* і *Satt354,* можуть бути корисним інструментом в маркер-опосередкованій селекції сортів сої із запрограмованими темпами розвитку. Ми не спостерігали достовірного впливу «Алелів МС-локусу *Satt229*», який, як відомо, зчеплений з *E3,* на досліджувані агрономічні ознаки. Для генотипів сої з алелем *E7* кількість днів до колосіння була більшою на 3-9 днів, а довжина вегетаційного періоду на 10–11 днів. У ліній з алелем 175 п.н. в локусі *Satt319* період сході-цвітіння був коротший на 6–9 днів.

Ключові слова: соя, мутантні лінії, SSR-локуси, мікросателітні маркери, *E* гени, фотоперіод, строки дозрівання.

Полиморфизм по SSR-локусам ассоциированным с генами *E* в перспективных для селекции мутантных линиях сои

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Цель. Анализ генетического разнообразия 10 линий сои, созданных путем химического мутагенеза с помощью мутагенов: Д-6, ДМСС-11, ДМСС-12, ДМСНПИР-11, ДУДСМ-12, Д12ДМЦ-11Б на основе сортов Фемида, Оксана, Подольская 416, Золотистая с использованием микросателлитных (МС) маркеров: *Satt100, Satt229, Satt319, Satt354, Satt365, Sat_038,* сцепленных с генами, которые определяют чувствительность растений сои к фотопериоду и определяют сроки созревания. В работе использованы **методы** − экстракции ДНК, ПЦР, МС-анализа, полевого эксперимента, дисперсионного анализа (ANOVA) для обсчета полученных данных. **Результаты**. Родительские сорта, мутантные линии и контрольные генотипы охарактеризованы по аллелям микросателлитных локусов, выявлено 25 аллелей по 6 микросателлитным локусам. Выявлены достоверные различия между исследуемыми линиями за три года полевых испытаний по признакам − время созревания и продолжительность вегетационного периода. Установлено влияние факторов «Аллели МС-локусов» на исследуемые признаки, так аллели *Satt100* оказывали влияние на все признаки, кроме DTF (дни до цветения); *Satt319* и *Satt354* влияли на DTM (на количество дней до созревания) и LV (на продолжительность вегетационного периода); аллели *Sat_038* влияли на DTF и S-F (на продолжительность периода всходы-цветение). Линии с аллелями 167 п.н. по *Satt100* и 175 п.н. по *Satt319* локусам (маркирующим доминантный ген *E7*), имели

более длительный вегетационный период и созревали позднее, чем другие. Линии с аллелем 247 п.н. по локусу *Sat_038* зацветали раньше, чем линии с аллелем 245 п.н., а линии с аллелем 232 п.н. по *Satt354* достигали зрелости позже, чем линии с другими аллелями по этому локусу. **Выводы**. Нами выявлено, что примененные мутагены индуцировали изменения в геноме сои и с помощью этих мутагенов можно эффективно увеличивать генетическое разнообразие сои по локусам, связанным с генами / локусами, определяющими сроки созревания и/или фотопериодическую чувствительность, что делает возможным создание сортов сои с разными сроками созревания и урожайностью. Микросателлитные маркеры, а именно *Sat_038, Satt100, Satt319* і *Satt354,* могут быть полезным инструментом в маркер-опосредованной селекции сортов сои с запрограммированными темпами развития. Мы не детектировали достоверного влияния «Аллелей МС-локуса *Satt229*», который, как известно, сцеплен с *E3*, на исследуемые агрономические признаки. Для генотипов, у которых детектирован *E7,* количество дней до колошения было больше на 3–9 дней, а продолжительность вегетационного периода на 10–11 дней. У линий с аллелем 175 п.н. по локусу *Satt319* период всходы-цветение был короче на 6–9 дней.

Ключевые слова: соя, мутантные линии, SSR-локусы, микросателлитные маркеры, *E* гены, фотопериод, сроки созревания.

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ABBREVIATIONS AND TERMS

IFAP	Institute of Feeds and Agriculture of Podillia of NAAS, Vinnitsa, Ukraine
SSR	Simple sequence repeat
MS	Microsatellite markers
MS-locus	Microsatellite locus
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
DTF	Days to flowering
DTM	Days to maturation
LV	Length of the vegetative period (days)
S-F	Duration of the period shoots-flowering (days)
LSD	Least significant difference
Rd	Remaining dispersion
bp	Base pair