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CHARACTERIZATION OF VALUABLE SOYBEAN MUTANT LINES BASED ON THE RESISTANCE TO *FUSARIUM* DISEASE AND MICROSATELLITE MARKERS

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ABSTRACT

The paper reports the results of the studies on twenty two soybean lines developed *via gamma* radiation of two soybean varieties, Alina and Zodiac. The new genotypes and parental types were studied for the resistance to the fungi *Fusarium* spp. attack and manifestation of some biological indices including germination capacity, plant height, shoot and node number, weight of 1000 beans. The valuable lines were also analyzed at a molecular level using microsatellite markers. A distribution phenogram for the mutant lines based on the Jaccard/Tanimoto comparison coefficient was constructed using the UPGMA method.

Key words: *Glycine max*, *mutant lines, SSR markers, resistance, Fusarium*.

INTRODUCTION

Along with the abiotic stress factors that have been more frequent and severe during the last years, fungal diseases contribute directly to a considerable reduction in the farm crops productivity, including soybean (*Glycine max* (L.) Merrill). Because the *plant* x *pathogen* relations are, essentially, trophic (Hammond-Kosack et al., 2004), this crop is one of the most disadvantaged due to the rich protein content that is a biochemical substrate especially attractive for numerous fungi and bacteria. Soybean is attacked by about 120 of pathogens. In the pedoclimatic conditions of the Republic of Moldova, the *Fusarium* spp. fungi are considered the most devastating pathogen species for soybean, that cause severe diseases during plant ontogenesis – rot of roots and cotyledons, tracheomycotic wilt, damage of beans and pods, their pollution with mycotoxins, deterioration of germination capacity etc. (Hartman et al., 2004).

Soybean genetic diversity remains quite restricted despite a rich collection of germplasm, which diminishes a successful implementation of traditional breeding programs. Induced mutagenesis is a secure way to improve genetic diversity, including in soybean, through development of mutant lines based on the utilization of *gamma* radiation *in vivo* and *in vitro* followed by their utilization in breeding programs (Mendi-Younessi Hamyekhanlu et al., 2011).

Evaluation and involvement of mutant types in hybridological analyses play an important role in assessment of their performance. Newly developed material may be appreciated using morphological/ quantitative analyses and efficient techniques including genetic/molecular methods (Arulbalachandran et al., 2010; Shiran et al., 2007).

A number of protocols are currently known, including those based on PCR analyses, characterization of genetic diversity of different crops including soybean. Detection of polymorphism between mutant forms of Sesbania rostrata Bremek & Oberm and the parental genotype was possible by using comparative methods based on the utilization of three molecular marker systems - RAPD (random amplified polymorphic DNA), ISSR (inter simple sequence repeat), and AFLP (amplified fragment length polymorphism).

Out of 200 RAPD primers used only 3% produced polymorphism, while AFLP and ISSR ones provided 12.5% and 15.7% respectively. Hence, hypervariable regions based on the ISSR markers were more

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informative in view of detection of genetic variation in the genotypes under study (Josni-Saha, and Gopalakrisma, 2007). This research indicated that along with deletions and aberrations that are quite frequent in mutagenesis, radiation induced mutations may be also derived from point mutations/ small insertions or deletions of the repetitive regions or those adjacent to them. Molecular mechanisms that ensure formation of the mutations involving tandem repeat are little known (Josni-Saha and Gopalakrisma, 2007).

The genome of eukaryotic organisms contains a high number of repeat sequences, i.e. minisatellites, microsatellites, simple tandem repeat named tandem repeat DNA loci (TRDLs) with an unstable heritability (Kovalchuk et al., 2000; Bridjes, 2001). Radiation induced genomic instability is often manifested as „delayed mutations", i.e. the mutation occurs much later after radiation exposure (Niva, 2006).

The mechanism of such delayed effects is not well known, but it is evident that radiation induces effects that cause genetic instability of repeat sequences (Bouffler et al., 2006). Use of repeat sequences as mutant markers has been demonstrated (Armour, 2006).

The goal of the study was characterization of twenty two soybean mutant lines, according to the *Fusarium* diseases resistance and microsatellite markers.

Therefore our objectives were:

- characterization of the resistance to *Fusarium* diseases in field conditions at the stage of plantlets;
- characterization of biological indices – germination capacity, plant height, shoot and node number, weight of 1000 beans;
- molecular analysis based on SSR markers;
- cluster analysis through the UPGMA method according to the resistance indices and
- calculation of Jacard/Tanimoto comparison coefficient.

MATERIAL AND METHODS

Twenty two lines of soybean produced through induced mutagenesis from two soybean varieties, Alina and Zodiac developed in the Institute of Genetics, Plant Physiology and Protection and registered in the Republic of Moldova in 2002 and 2004, respectively (Plant Variety Patent MD 54 2009.12.31, Plant Variety Patent MD 55 2009.12.31) were used as study material.

The Alina variety is characterized by drought resistance, tolerance to *Fusarium* disease, phomopsis, bacteriosis, peronosporosis, and white rot. The bean production is about 2.05-2.63 t ha^{-1}. The weight of 1000 beans is 127.1 g. Beans contain 35.9-40.4% proteins, and 19.8-21.1% oil. The vegetation period lasts 119-129 days.

The Zodiac variety is resistant to drought, tolerant to *Fusarium* disease, phomopsis, bacteriosis, phytophthrosis, peronosporosis, and white rot. The bean production is about 1.83-2.34 t ha^{-1}. The weight of 1000 beans is 167.1 g. Beans contain 35.2-39.8% proteins, and 18.9-21.9% oil. The vegetation period is 108-111 days. Thus, the varieties differ in producing capacity and vegetation period.

Analytical methods

Gamma radiation

Soybean variety seeds were radiated with three rate levels: 100, 150, and 200 Gy and were sown in field conditions.

Fusarium disease tests in field conditions

The samples were sown in the field in randomized blocks. The number of replicates was three. The row length – 1.0 m, the distance between rows – 30 cm. Plant resistance to *Fusarium* disease was assessed at the plantlet stage based on the necrotized area of roots (root *Fusarium* disease) and cotyledons (cotyledon *Fusarium* disease) at a 5 ranking scale: 0 – immune (I); 1 – highly resistant (HR); 2 – resistant (R); 3 – medium sensibility/medium resistance (MS/MR); 4 – sensible (S); 5 – highly sensible (HS). The assessment scale of the disease intensity was as follows (R, %) 0% – immune (I); 1-25% – highly resistant (HR); 26-50% – resistant (R); 51-75% – medium sensibility/medium resistance (MS/MR); 76-100% – highly sensible (HS) (Figure 1).

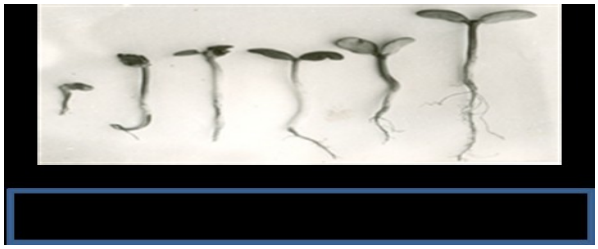

Figure 1. Evaluation of root *Fusarium* disease and cotyledon *Fusarium* disease attack degree in soybean at the cotyledon formation stage

Figure 2. *Fusariums* wilt in soybean plants

At the blossoming stage, rot of root *Fusarium* infection causes wilt and drying/yellowing of leaves/plants (Figure 2). Due to the pronounced drought in the study year of 2012, the majority of plants were wilting/yellowed during the whole ontogenetic development period; therefore it was difficult to assess the *Fusarium* intensity at the advanced stages.

Molecular analysis of soybean genotypes

The soybean genotypes were analyzed at the molecular level through the SSR (Simple Sequence Repeat) that allows estimation of microsatellite region variation and is characterized by multiallelism and high reproducing capacity. Table 1 presents the nucleotide succession of the primers developed by Cregan et al. (1999).

Table 1. Nucleotide succession of SSR primers

No.	Primer	Forward Nucleotide Succession	Reverse Nucleotide Succession
1	Satt147*	CCA TCC CTT CCT CCA AAT AGA T	CTT CCACACCCTAGTTTAGTGACAA
2	Satt002	TGT GGG TAA AAT AGA TAA AAA T	TCA TTT TGA ATC GTT GAA
3	Satt479*	GCGCTTTCAAAAAGTAACAATTAATGAAA	GCG GGA ATT GGT TAA TCT CAT CGT GAC
4	Satt184	GCGCTATGTAGATTATCCAAATTACGC	GCCACTTACTGTTACTCAT
5	Satt420*	GCGTATTCAGCAAAAAAATATCAA	TTATCGCACGTGTAAGGAGACAAAT
6	Satt570*	CTCATGTGGTCCTACCCAGACTCA	CGCTATCCCTTTGTATTTTCTTTTGC
7	Satt309	GCG CCT TCA AAT TGG CGT CTT	GCG CCT TAA ATA AAA CCC GAA ACT
8	Satt317*	GCGAACAAACTTTCTATACATGATAACA	GCGGGTATATTTTTGTACATAAGTTGGAA
9	Satt183	TAGGTCCCAGAATTTCATTG	CACCAACCAGCACAAAA
10	Satt346*	GGAGGGAGGAAAGTGTTGTGG	GCGCATGCTTTTCATAAGTTT
11	Satt237	GCGTGATTTCAATCCTTTTTC	GCGGTTGTCCTGTTAGAACCT
12	Satt005	ATG TAA GTC AGC AAG TGG CG	AGA CCC AGA ATC TCC ACC G
13	Satt477	GTTGGGAAAAGGTTACTACCATATC	GGTCCGTATGCAATTCTTGACTAATA
14	Satt212	CCAATCCAAACAAATCCACT	CAGCAATGATGATAATGAATGA
15	Satt213	CCGCTTATTTCTGTCATC	AGCCAAAACCCACAA
16	Satt335	CAAGCTCAAGCCTCACACAT	TGACCAGAGTCCAAAGTTCATC
17	Satt277	GGTGGTGGCGGGTTACTATTACT	CCACGCTTCAGTTGATTCTTACA
18	Satt251	CCTCCACCCCCTTCCCACCCAAAA	GGTGATATCGCGCTAAAATTA
19	Satt042	CCAAAGAATGGTTTGACTTGG	CACCTGTTTTAATGGTGGTGG

* Primers that generated bands selected afterwards for variation studies

The results of the molecular analysis were compared through calculation of the Jaccard/Tanimoto coefficient (UPGMA method) (DendroUPGMA: A dendrogram construction utility. http://genomes.urv.cat/UPGMA/).

The primers comprise 19 of the 20 linkage groups known for soybean. The main criteria for primer selection were based on the tri-nucleotide nature, each of them having a motive (ATT)n, and on their abundance in soybean and simple interpretation of the

allelic parent. The preference was given to those presenting polymorphism in earlier studies (Narvel et al., 2000; Priolli et al., 2002; Tantasawat et al., 2011) and that were associated with important QTLs, such as yielding ability and resistance.

The SSR analysis was performed at the Saraykoy *Nuclear Research and Training Center*, Ankara, Republic of Turkey, within the regional projected supported financially by the International Atomic Energy Agency.

DNA Isolation. DNA was isolated from young leaves of fifteen soybean genotypes according to the CTAB method (*hexadecyltrimethylammonium bromide*) (Doyle and Doyle, 1990) modified by Sağel et al. (2009). At least five individual greenhouse grown plants were used for DNA pooling. DNA was dissolved in distilled DEPC (*diethylpyrocarbonate*) treated water or in Tris.HCl (pH 8; 1 M) + EDTA (ethylenediaminetetraacetic acid, 0.5 M). The DNA quantity and quality was measured using spectrometry (using *Eppendorf BioPhotometer*) and 1% agarose gel electrophoresis in 1xTBE ((*Tris borate-EDTA*).

DNA Amplification. The PCR reactions were conducted in the *MultiBlock PCR System* (Thermo Electron Corporation). The reactive mixture included 0.5 U/µl Taq-polymerase (Sigma, Germany); 1 x buffer for PCR (containing Mg^{2+}); dNTP (0.2 mM each); primers (reactive concentration equal to 1 M); matrix; deionised water added to the final volume of 20 µl.

The program of optimal amplification included temperature succession as follows: I – 94°C 2 min.; II – 94°C 30 sec., 47°C 1 min., 72°C 1 min. x 50 cycles; III – 72°C 5 min. The analyses were repeated twice to check reproducibility of the results.

Visualization of the amplification products was accomplished using 4% agarose gel electrophoresis (Invitrogen) in the buffer solution 1 x TBE at a voltage of 10 V/cm in the ultraviolet rays with a wave length of 305 nm at the transilluminator. The bands were analyzed and photographed using *Vilber Lourmat Gel Documentation System*. 6x *Gel Loading Solution* (Sigma) was used to insert samples into the gel. The polymorphic indices (PIC) were calculated according to [http: //www.genomics.liv.ac.uk/animal/Pic1.html].

RESULTS AND DISCUSSION

The degree of the resistance to the attack of root *Fusarium* disease (RFD) and cotyledon *Fusarium* disease (CFD) was assessed in the field conditions in twenty two M5 lines of soybean derived through *gamma* radiation from var. Alina and Zodiac.

The indices of the root fusarium disease intensity in the lines developed through *gamma* radiation of the var. Alina seeds varied at a wide range between 0.3±0.1 (line M8) and 72.0±1.3 (line M9), while those developed from the var. Zodiac between 1.5±0.1 (line M20) and 44.0±2.6 (line M14), the index variability being higher for the genotypes developed from the var. Alina. Thus, their variation constituted 659.0 in comparison with the lines produced from the var. Zodiac (205.0). The mean value of the index for the var. Alina and Zodiac were 16.5±1.5 and 29.5±0.9, respectively (Table 2).

Table 2. The intensity of the root *Fusarium* disease development of soybean lines

Genotype	RFD ($X \pm m_x$)	Genotype	RFD ($X \pm m_x$)
Alina control	16.5±1.5	Zodiac control	29.5±0,9
M1	0.8±0.06	M13	5.6±0,8
M2	0.9±0.1	M14	44.0±2.6
M3	0.5±0.2	M15	30.0±3.3
M4	0.5±0.1	M16	25.0±3.3
M5	36.0±4.0	M17	8.0±0.6
M6	19.3±0.8	M18	1.5±0.2
M7	15.0±2.0	M19	15.6±1.7
M8	0.3±0.1	M20	1.5±0.1
M9	72.0±1.3	M21	40.0±3.3
M10	53.0±2.6	M22	21.3±1.1
M11	56.3±0.8	M23	28.0±0.6
M12	31.3±0.8	M24	14.0±1.3
Mean	23.8		
Variance	656.9	19.5	205.0

A similar trend was observed for the intensity of cotyledon *Fusarium* disease, the indices ranging between 2.3±1.1 (M2) and 74.0±0.6 (M9), and 9.3±1.1(M1) and 58.0±0.6 (M9), their variability in the first case being also higher (655.8) in comparison with case two (363.3).

The general pattern of the soybean mutant line manifestation according to the index of resistance demonstrated that the Zodiac lines recorded 63.6% (M12, M15, M16, M17, M18, M19, and M21) HR reaction, while 63.6% (M12, M15, M16, M17, M18, M19, and M21) recorded R reaction to RFD. As for the resistance to CFD, 63.6% (M12, M14, M15, M16, M17, M18, and M19), 18.2% (M13 and M22) and 18.2% (M20 and M21) recorded HR, R, and MS reaction, respectively.

The following classification was attested for the var. Alina lines according to the index of the resistance to RF/CF: HR reaction – 54.5% (M1, M2, M3, M4, M7, and M8), R – 18.2% (M5 and M6), MS – 18.2% (M10 and M11), S – 9.1% (M9). The findings prove that the majority of the lines displayed HR reaction to the *Fusarium* disease attack (Table 3).

Table 3. The intensity of the cotyledon *Fusarium* disease development of soybean lines

Genotype	CFD ($X\pm m_x$)	Genotype	CFD ($X\pm m_x$)
Alina (control)	25.2±1.2	Zodiac (control)	19.0±1.3
M1	7.0±0.6	M13	9.3±1.1
M2	2.3±1.1	M14	38.0±1.3
M3	4.3±1.1	M15	22.3±1.5
M4	8.0±0.6	M16	19.0±0.6
M5	15.6±1.1	M17	12.0±1.3
M6	29.0±0.6	M18	5.0±0.6
M7	16.0±0.6	M19	14.0±1.3
M8	12.3±1.5	M20	3.0±0.6
M9	740±0.6	M21	58.0±0.6
M10	72.0±1.3	M22	54.0±2.0
M11	26.6±1.1	M23	42.3±1.5
M12	52.0±1.3	M24	37.3±1.7
Mean	26.5		26.1
Variance	655.8		363.3

It was demonstrated, that mutation produced both more resistance and more susceptible lines, in comparation with the original cultivars. As for the intensity of the root *Fusarium* disease development, the resistant (*R*) and high resistant (*RR*) lines consists respectively about 75% – cultivar Alina, and 100% – cultivar Zodiac. The susceptible and high susceptible lines consist about 25% – cultivar Alina and 0% – cultivar Zodiac. As for the intensity of the cotyledon *Fusarium* disease development, the *R* and *RR* lines consists 83.3% for both cultivars. The susceptible, high susceptible lines consist 16.7% for both cultivars.

Analysis of biological indices such as germination capacity, plant height, shoot number, node number, weight of beans per plant, weigh of 1000 beans demonstrated their differentiated variability, the capacity of germination being the highest. The phenomenon was more pronounced in the lines derived from the var. Zodiac lines in which the variation was 201.0 in comparison with that of the variety Alina (75.4).

The effect of mutation was positive for the germination capacity parameter in three lines from the cultivar Alina and in four lines from the cultivar Zodiac. Regarding the weight of 1000 beans a significant difference in comparison with the control was not observed in most cases. It was very interesting. The results obtained for line's M5, which had a lower plant height but higher weight of 1000 beans in comparison with the original cultivar, are particularly interesting (Table 4).

The soybean genotypes were analyzed at the molecular level through the SSR method (*Simple Sequence Repeat*). The results of the molecular analyses showed that of the nineteen molecular markers tested (Table 1, Cregan, 1999), six (marked with "*" in Table 1) produced bands. They were involved in further studies (Table 5). The total number of the bands produced for the var. Alina and Zodiac lines varied depending on the locus analyzed and were 19 and 22 for Satt147; 31, and 22 for Satt 479; 9 and 20 for Satt420; 24 and 20 for Satt317; 15 and 20 for Satt 346, respectively. The Satt570 locus was monomorphic.

Table 4. Characterization of some biological indices of the soybean M5 lines

Genotype	Germination capacity (%)		Plant height (cm)		Shoot no.		Node no.		Weight of 1000 beans	
	$X\pm m_x$	S	$X\pm m_x$	S	$X\pm m_x$	S	$X\pm m_x$	S	$X\pm m_x$	S
Alina (control)	**75.0±1.5**	**4,3**	**56.3±2.7**	**14,1**	**3±0,6**	**1,1**	**11±1,6**	**21,3**	**122,6±2,6**	**10,7**
M1	70.0±**0.6**	1.0	50.6±**2.0**	5.0	2±**0.6**	0,6	11±**1,4**	23,7	126,5±**1,7**	4,7
M2	70.0±**0**	0	54.6±**1.2**	2.5	3±**0.8**	1,3	12±**1,8**	25,4	120,9±**0,9**	1,5
M3	60.0±**2.0**	7.0	50.2±**1.1**	2.2	3±**0.4**	0,4	11±**1,2**	20,3	123,0±**1,6**	4,6
M4	70.0±**1.3**	4.0	58.4±**1.4**	3.2	3±**0.6**	0,8	12±**1,2**	24,4	124,4±**1,5**	3,9
M5	**80.0±2.6**	13.0	49.8±**1.2**	2.6	2±**0.6**	0,6	9±**0,8**	14,7	123,2±**1,7**	4,2
M6	**80.0±0.6**	1.0	52.2±**0.7**	0.8	2±**0.4**	0,4	11±**1,2**	24,1	125,4±**0,7**	1,1
M7	78.0±**2.0**	7.0	54.6±**1.0**	1.7	3±**0.6**	0,8	13±**1,2**	29,8	110,8±**1,5**	4,1
M8	**86.0±1.3**	4.0	46.4±**0.4**	0.2	2±**0.8**	1,1	9±**1,2**	15,0	108,2±**2,5**	10,8
M9	64.0±**2.6**	16.0	48.2±**0.5**	0.5	3±**1.0**	1,7	11±**1,6**	20,6	120,4±**1,9**	5,3
M10	66.0±**3.3**	19.0	48.4±**1.2**	2.4	2±**1.2**	2,0	11±**1,2**	23,2	105,6±**0,4**	10,7
M11	60.0±**2.0**	7.0	49.6±**0.5**	0.5	2±**0.8**	0,8	10±**1,2**	18,8	120,9±**2,6**	8,7
Average	72.2±7.1	75.4	51.1±2.7	12.2	2.5±0.4	0,3	10,9±0,8	1,5	119,3±5,6	49,5
Zodiac (control)	**70.0±3.3**	**19**	**42.6±1,2**	**2,3**	**3±0.8**	**1,8**	**11±0,8**	**1,8**	**126,6±1,9**	**9,3**
M12	**90**.0±2.6	13	40.2±1.3	2,9	3±0.8	1,8	11±0,8	1,8	128,8±0,9	1,1
M13	**80.**0±2.4	10.3	38.6±0.9	1,4	3±1.2	2,7	11±1,2	2,6	130,2±1,9	8,9
M14	75.0±1.3	4.0	40.2±0.9	1,1	2±0.8	0,9	11±0,8	0,9	**135,5**±1,7	3,8
M15	50.0±0.6	1.0	38.2±0.9	1,5	3±0.4	0,9	9±0,4	0,9	125,6±0,9	1,3
M16	55.0±0.8	1.3	38.6±0.9	1,0	2±1.0	1,8	9±1,0	1,8	123,6±2,3	7,2
M19	**82**.0±1.3	4.0	40.4±0.8	1,2	3±0.8	1,8	11±0,8	1,8	129,2±1,1	1,7
M20	66.0±0.6	1.0	38.8±0.7	1,0	2±0.8	0,9	9±0,8	0,9	127,0±1,6	5,2
M21	**80**.0±4.0	2.8	40.4±0.9	1,1	3±0	0	12±0	0	132,0±1,3	3,1
M22	50.0±2.6	13.0	36.2±0.5	0,6	2±0.6	0,7	11±0,6	0,6	130,4±1,9	5,6
Average	63.8±11.6		38.7±1.3		2.5±0.4		10.1±0.9		128.89±2.6	
Variance	201.0		2.9		0.2		1.1		11.7	

Table 5. Characterization of SSR primers and amplification results

SSR loci	Reference	*Linkage groups* (LG)	Association with QTL	Total band No		Allele No		PIC	
				Alina Mutation	Zodiac Mutation	Alina Mutation	Zodiac Mutation	Alina Mutation	Zodiac Mutation
Satt147	14969862	D1a	Resistance to *Sclerotinia sclerotiorum (Sclero)*, bean weight	19	22	2	2	**0.358**	**0.373**
Satt479	14970154	O	*Oil*	31	44	6	5	**0.625**	**0.745**
Satt420	14970102	O	*Oil*	9	20	2	4	**0.371**	**0.562**
Satt570	14970239	G	Resistance to *Fusarium* disease, Proteins (*Prot*)	monomorphic				**0**	**0**
Satt317	14970011	H	*Oil*	24	20	2	2	**0**	**0.375**
Satt346	14970039	M		15	20	3	3	**0.483**	**0.466**

The allele number varied between 0.358 (Satt147) and 0.625 (Satt479) for the var. Alina mutants, while its number was 0.375 (Satt317) and 0.745 (Satt479) for Zodiac. An advanced polymorphism was detected for the locus Satt479 in the case of the mutants of both var. Alina and var. Zodiac, which proves the efficiency of the utilization of this locus for testing genotypic variability.

A distribution dendrogram was constructed for the genotypes (UPGMA method) based on the molecular data through calculating the Jaccard/Tanimoto coefficient. It should be mentioned that the value of the cophenetic correlation coefficient was high and reached 0.78, which proves that the data presented in the distribution phenogram of the lines under study based on this coefficient are veridical.

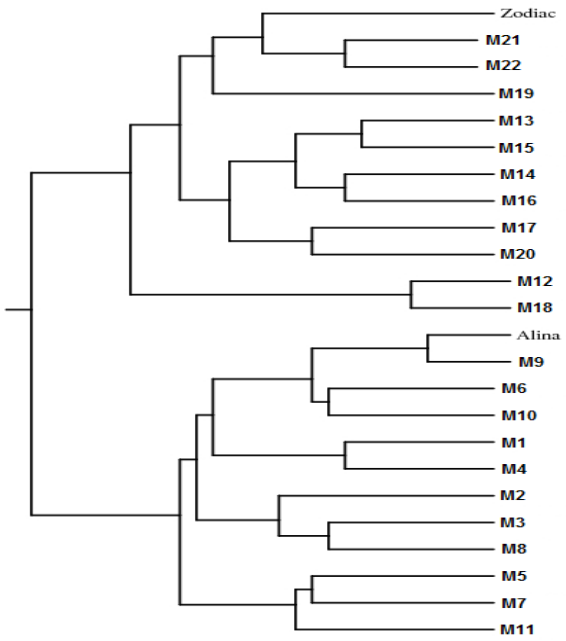

Figure 3. Distribution phenogram of the mutant lines analyzed according to the Jaccard/Tanimoto comparison coefficient

A distribution phenogram of the mutant lines of the var. Zodiac according to the Jaccard/Tanimoto coefficient demonstrates that 71.4% of the lines presented a cluster formed from two subclusters. Thus, the lines M12 and M18 were distributed in subcluster 1 with a close genetic distance between them, while the lines M15, M16, and M17 – in subcluster 2 with a more remote genetic distance. The lines M20 and M21 were distributed in different subclusters. Thus, the first formed a subcluster with the line M17, while the second – with M22 (Figure 3).

The distribution phenogram of the mutant lines of the var. Alina analyzed according to the Jaccard/Tanimoto comparison coefficient reveals that 87.5% of the genotypes presented a cluster. A closer genetic distance is attested for M1 and M4; M3 and M8. The lines M11, M10, and M9 were distributed as follows: M11 formed a separate cluster, while M10 together with M6 and M9 formed a subcluster.

CONCLUSIONS

The genetic/molecular study of a number of soybean mutant lines and parental types – var. Zodiac and var. Alina according to the index of the resistance to *Fusarium* disease and SSR microsatellite markers was performed. A positive effect of mutation was demonstrated. The percentage of the resistant to root *Fusarium* disease new genotypes obtained from cultivars Alina, and Zodiac was 75% and 100% respectively. As for the resistance to cotyledon *Fusarium* disease, new resistant lines represented 83.3% for both cultivars. The clustering of the genotypes in the phenogram constructed according to the Jaccard/Tanimoto comparison coefficient through the UPGMA method was analyzed. The cophenetic correlation coefficient was 0.78. The total number of the bands produced for the var. Alina and Zodiac lines varied depending on the locus analyzed and were 19 and 22 for the Satt147; 31 and 44 for Satt479; 9 and 20 for Satt420; 24 and 20 for Satt317; 15 and 20 for Satt346, respectively. The allele number varied between 2 and 6 for the var. Alina mutants, while it ranged between 2 and 5 for the mutant lines of var. Zodiac.

The polymorphic index for the first group of lines varied between 0.358 (Satt147) and 0.625 (Satt479), while it constituted 0.375 (Satt317) and 0.745 (Satt479) for the second group. Thus, advanced polymorphism was detected for the locus Satt479 in the case of both var. Alina and Zodiac, which proves feasibility of this locus in testing mutagenic variability.

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REFERENCES

Armour, J.A.L., 2006. *Tandemly repeat DNA: Why should anyone care*? Mutat. Res., 598: 6-14.

Arulbalachandran, D., Mullainathan, L., Karthigayan, S., Somasundaram, S.T. and Velu, S., 2010. *Genetic Variation in Mutants of Black Gram (Vigna mungo (L.) Hepper) evaluated by RAPD Markers*. J. Crop Sci. Biotech., 1: 1-6.

Budac,, A., Coreţchi, L., Chirtoacă, I., 2003. *Plant Variety Patent MD 54 2009.12.31. Cultura Soia (Glycine max (L.) Merill), SOIUL ALINA* / deposit date: 2003.11.11, BOPI, no.12/2009.

Budac, A., Celac, V., Coreţchi, L., Chirtoacă, I., 2009. *Plant Variety Patent MD 55 2009.12.31. Cultura Soia (Glycine max (L.) Merill), SOIUL ZODIAC* / deposit date: 2003.11.11, BOPI, no.12/2009.

Coreţchi, L., Lupascu, G., Budac, A., 2011. *Phytopathological assessment and indices of the producing capacity of promising genetically soybean types*. Crop plants for resistant agriculture in the 21st century (Immunity, breeding, introduction). Scientific works dedicated to the 110th Anniversary of Academician M.S. Dunin. RAA, M., IV, 1: 240-245. ISBN 978-5-902178-50-7 ISBN 978-5-901695-48-7. (In Russian)

Bridjes, B.A., 2001. *Radiation and germline mutation at repeat sequences: are we in the middle of a paradigm shift*? Radiation Res., 156: 631-641.

Cregan, P.B., Jarvik, T., Bush, A.L., Shoemaker, R.C., Lark, K.G., 1999. *An Integrated Genetic Linkage Map of the Soybean Genome*. Crop Sci., 39: 1464-1490.

DendroUPGMA: *A dendrogram construction utility*. http://genomes.urv.cat/UPGMA/

Doyle, J.J., Doyle, J.L., 1990. *Isolation of plant DNA from fresh tissue*. Focus, 2: 13-15.

Hammond-Kosack, K., Urban, M., Baldwin, Th., Daudi, A., Rudd, J., Keon, J., Lucas, J., Maguire, K., Kornyukhin, D., Jing Hai-Chun, Bass, Ch. and Antoniw, J., 2004. *Plant pathogens: how can molecular genetic information on plant pathogens assist in breeding disease resistant crops*. Proceedings of the 4th International Crop Sciences Congress, Brisbane, Australia: 1-18.

Hartman, G.L., Huang, Y.H., Li, S., 2004. *Phytotoxicity of Fusarium solani culture filtrates from soybeans and other hosts assayed by stem cuttings.* Australasian Plant Pathology, 33: 9-15.

Josni-Saha, A., Gopalakrisma, T., 2007. *Inter simple sequence repeat (ISSR) markers for detecting radiation induced polymorphism and its application as genetic marker system in Sesbania rostrata (Bremek „Obrem"*). Plant mutation Reports, 3: 41-45.

Samal, S., Rout, G. R., Nayak, S., Nanda, R.M., Lenka, P.C. and Das, P., 2003. *Primer screening and optimization for RAPD analysis of cashew.* Biol. Plant, 46: 301-304.

Kovalchuk, O., Arkhipov, A., Dubrova, Yu., Hohn, B. and Kovalchuk, I., 2000. *Wheat mutation rate after Chernobyl*. Nature, 407: 583-584.

Mehdi-Younessi Hamzekhanlu, Ali Izadi-Darbandi, Nejat Pirvali-Beiranvand, Mohammad Taher-Hallajian, and Abbas Majdabadi, 2011. *Phenotypic and molecular analysis of M7 generation of soybean mutant lines through random amplified polymorphic DNA (RAPD) marker and some morphological traits*. African Journal of Agricultural Research, 6(7): 1779-1785.

Narvel, J.M. Jakkuda, L.R., Phillips, D.V., Wang, T., Lee, S.-H and Boerma, H.R., 2001. *Molecular mapping of Rxp conditioning reaction to bacterial pustule in soybean*. J. Hered., 92, 3: 267-270.

Niva, O., 2006. *Indirect mechanism of genomic instability and the biological significance of mutations at tandem repeat loci*. Mutat Res., 598: 61-72.

Priolli, R.H., Mendes-Junior, C.T., Arantes, N.E., Contel, E.P.B., 2002. *Characterization of Brazilian soybean cultivars using microsatellite markers*. Genetics and Molecular Biology, 25, 2: 185-193.

Sağel, Z., Tutluer, M.İ., Peşkircioğlu, H., Kantoğlu, Y., Tümbilen, Y., Kunter, B., 2009. *Mutant Nohut Çeşitinin Verim, Kalite Özellikleri Ve Moleküler Tanımlaması Türkiye VIII. Tarla Bitkileri Kongresi*, Hatay. Cilt I: 913-918s. (In Turkish)

Shiran, B., Amirbakhtiar, N., Kiani, S., Mohammadi, Sh., Sayed-Tabatabaei, B.E., Moradi, H., 2007. *Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers*. Sci. Hortic., 111: 280-290.

Tantasawat, P., Trongchuen, J., Prajongjai, T., Jenweerawat, S. and Chaowiset, W., 2011. *SSR analysis of soybean (Glycine max (L.) Merr.). Genetic relationship and variety identification in Thailand*. AJCS, 5(3): 283-290.

http://www.genomics.liv.ac.uk/animal/Pic1.html