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NEW GENETIC VARIABILITY IN SUNFLOWER INBRED LINES CREATED BY MUTAGENESIS

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ABSTRACT

The successful use of plant breeding for improving desirable traits requires the existence of genetic variability for these traits. Induced mutations are often used to create new genetic variability within a plant species. The objective of this study was to provide new genetic variability that can be exploited for improvement of important agronomic traits in sunflower production. The seeds of 8 sunflower inbred lines from the gene collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia were irradiated with gamma rays (*γ*) and fast neutrons (*Nf*) and treated in an ethyle-methane-sulphonate (*ems*) solution. The manifestation of mutations was mostly expressed in the M_2 and M_3 generations. Seven mutants were selected: 1 early flowering (L3ME), 2 short (L2MS and R1MS) and 1 high stature (R3MT), 2 with higher oil content (L1MO and R2MO) and 1 with branching (L4MBr). The stable progenies were evaluated in micro-plot tests in M_6 and M_7 generations for seed yield and other agronomic traits in comparison with their respective original lines. Further studies should be focused on testing new mutant lines in hybrid combinations, as well as determining the inheritance of mutant traits.

Key words: induced mutations, agronomic traits, inbred lines, sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops in the world. The main objective of the sunflower breeding programme is to develop high seed- and oil-yielding hybrids. In addition, the objectives of breeding oilseed-type sunflowers include the content and quality of oil in seed, earlier maturity, shorter stems, resistance to disease and broomrape, adaptability, stability and uniformity of plants (Kaya et al., 2012). During the creation of an ideal model plant, great attention is paid to the architecture of plants, plant height, size, shape and position of the head on the stem, number of leaves and their size, duration and position on the plant (Skoric, 1989). The advantage of hybrids over varieties lies in the exploitation of the heterosis phenomenon and the uniformity of crops, higher genetic potential for seed yield, ease of introduction of genetic disease resistance; allowing easier harvesting and providing uniform seed moisture and storage suitability (Miklic et al., 2008).

The improvement of agronomic traits in hybrids is mostly based on crossing between genetically divergent inbred lines. If new combinations have limited improvement, breeders have to find a way to increase genetic variability within the collection. However, genetic variability within the sunflower is often limited, as its genetic base of available inbred lines is narrow. Genetic variability can be broadened by interspecies hybridisation with wild species and mutation breeding. Mutation breeding has been successfully used in sunflower breeding by changing plant characteristics and productivity (Cvejic et al., 2011). The most commonly used mutagens in sunflowers are X-, gamma and beta rays, thermal and fast neutrons, ultraviolet and infrared radiation (Skoric, 2012). Researchers have used induced mutations in sunflower breeding programmes (Voskoboinik and Soldatov, 1974; Schuster and Kubler, 1983; Jan and Rutger, 1988; Girigaj et al., 2004; Encheva et al., 2008) and created numerous mutants with altered agronomic traits (early maturity, dwarf growth, thinner husk, oil

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content and composition etc.), which can be used for cultivation purposes. Miller and Fick (1997), Schuster (1993) and Cvejic (2009) compiled lists of induced mutations for these traits. The present study is focused on broadening genetic variability within the collection of sunflower inbred lines from the Institute of Field and Vegetable Crops, Novi Sad, Serbia (IFVCNS) using chemical and physical mutagens and finding mutants with altered characteristics, as well as to investigate whether and how the mutant trait influences other traits.

MATERIAL AND METHODS

Plant material

8 different sunflower inbred lines from the gene bank of the IFVCNS were used in this study (Table 1). Approximately 500 seeds of each inbred line were treated with three different mutagens. The doses/concentrations were chosen based on LD_{30} values described by Gvozdenovic et al. (2009). Treatment with gamma rays (*γ*: 70-160 Gy) was done using a Cobalt-60 gamma source. Prior to mutagenic treatment, the seeds were kept in a desiccator over a 60% glycerol/water mixture for 7 days at room temperature for seed moisture equilibration. For fast neutron treatment (*Nf*: 3-5 Gy), the seed samples were bombarded inside a cadmium (Cd) capsule with a wall thickness of 2 mm. Chemical treatment was carried out by treating the seeds of each line with ethyl-methane-sulphonate (*ems*) solution, with a concentration of 0.25%, for 3.5 hours. The seeds were previously soaked in distilled water for 24 hours. All treatments were carried out in Joint IAEA/FAO Laboratories in Seibersdorf, Austria.

Table 1. List and characteristics of treated sunflower inbred lines

Inbred lines	Type of inbred line	Vegetation period	Plant height	Seed colour	Seed-coat type
L1	High oleic female	Medium late	Medium	Black	Thin
L2	Standard female	Late	Tall	Black	Thick
L3	Standard female	Medium early	Medium	Black	Thick
L4	Standard female	Medium early	Medium	Black	Thick
R1	High oleic restorer	Medium early	Short	Light brown	Medium
R2	Standard restorer	Medium late	Tall	Black	Medium
R3	Standard restorer	Early	Very short	Black	Thin
R4	Standard restorer	Medium early	Medium	Brown	Medium

Selection procedure

The treated (M_1) and untreated (control) seeds were planted in the experimental field of the IFVCNS. Approximately 10000 M_1 plants were self-pollinated and M_2 seeds were harvested. The pedigree method of selection was used. Based on observed changes of individual plants, seeds were planted in the next generation. The M_2 generation was grown in the field and, after self-pollination, the M_3 seeds were collected. The selection of individual plants in M_2 and M_3 generations was made based on changes in plant height, flowering time, branching and oil content. The stability of new characteristics was verified in the following generations (M_4, M_5 and M_6).

Agronomic evaluation

Selected mutants (M_6) and original lines were planted in comparative trial. In order to test their productivity and stability, comparative trial was repeated in the next (M_7) generation. The trials were organized in randomised block design with three replicates. Plant height and head diameter were recorded at plant maturity on 10 plants of each entry. Days to flowering were calculated as days of plant emergence to days of full flowering (UPOV - stage F3.2). After harvesting, seed yield was determined for each plant separately. Oil content in seed was analysed by NMR for each plant separately.

Statistics

The results were statistically analysed in *Statistica 12*. Differences between mutants and original lines were determined by t-test for level of significance 0.05 and 0.01. Broad sense heritability (H^2) and genetic advance (GA) were calculated according to Bozokalfa et al. (2010).

RESULTS AND DISCUSSION

Mutation breeding has been successfully used to alter sunflower characteristics. Both chemical and physical mutagens produced mutant lines. The most efficient agents were treatment with gamma rays in interval from 100-200 Gy, followed by fast neutrons. Similarly, Saadat et al. (1974) and Sarafi (1976) reported accomplishments in developing sunflower mutants using gamma irradiation. Gamma rays and other physical mutagens are often used in sunflower mutation breeding because of their easy application and high mutation rate (Skoric, 2012). This indicates that gamma irradiation could be an efficient tool for morphologically generating diverse sunflower germplasm. In contrast, Osorio et al. (1995), Velasco et al. (2004), Girigaj et al. (2004) and Kumar et al. (2013) developed mutant populations using chemical mutagens, mostly ethyl-methane-sulphonate (*ems*) and N-nitroso-N-methylurea (*nmu*).

Induced mutagenesis affected sunflower inbred lines by changing their characteristics. The selection of desirable mutant plants started in the M_2 generation, with the assumption that the changed characters were genetically inherited. In the M_3 generation, different mutations were observed in the field and promising mutants were selected for early flowering, short and high stature, appearance of branches and oil content. In the following generations (M_4, M_5, M_6), during self-pollination and selection, a few mutants were discarded because the observed traits were not completely fixed or not genetically inherited. Only seven mutants were selected from over 10000 M_1 plants over 7 years of observation in the field. Selected mutants were L3ME (early flowering), L2MS and R1MS (shorter), R3MT (taller), L1MO and R2MO (high oil content) and L4MBr (branching). In M_6 generation mutants were evaluated and showed significant differences in one or more characteristics in regards to their original lines. In the next generation the fixation of the mutant traits were improved and mutant plants showed stability regarding all examined traits (Table 2).

Early flowering mutant

Mutant line L3ME had a significantly earlier flowering time, by five and eight days, respectively, in both the M_6 and M_7 generations. Early mutation showed high heritability rate and did not influence other traits, except significantly higher seed and oil yield in the M_7 generation. This mutant was developed by treating the L3 line with fast neutrons dose 3 Gy.

Short stature mutants

Short stature mutant lines were developed using gamma rays, dose 120 Gy. Mutant line L2MS was approximately 15 cm shorter than the original L2 line, which is generally a tall line. Compared to the original line, mutant L2MS had highly significant higher seed and oil yield per plant in both generations.

Another short mutant R1MS was developed from high-oleic restorer line R1 using gamma rays, 100 Gy. Beside shorter stature, this mutant showed a wide range of variability of other traits. Line R1MS differed significantly in days to flowering compared to the original line and had a smaller head than the original line.

High stature mutant

Mutant R3MT was produced by gamma irradiation; dose 200 Gy from dwarf line R3. This mutant was about 30 cm taller than the original line. The mutant matured later, had a bigger head and higher seed yield. This mutant had an advantage in seed and oil yield. All examined traits showed high heritability and GA values.

Mutants with higher oil content

Chemical and statistical analyses confirmed that mutant line L1MO had increased and stable oil content compared to the original line L1. This mutant was developed by fast neutrons (*Nf*) using dose of 3 Gy. This mutant line showed stability in other examined traits.

Mutant R2MO was developed by gamma irradiation dose of 120 Gy and had significantly shorter stature and a smaller head, but higher oil yield than the original R2 line. This mutant had high heritability rate for oil content.

Branching mutant

Branching mutant was obtained by treating seed of single-head female line L4 with gamma rays dose of 120 Gy. As a consequence of this mutation, earliness and smaller heads were recorded. Mutant had high heritability for earliness, plant height and head diameter.

The seed and oil yield per unit area are the most important traits in sunflower production. Sunflower oil yield is determined as the product of seed yield per unit area and the oil percentage in the seed (Leon et al., 1995). In the present study, three mutant lines (L2MS, R3MT and L4MBr) exhibited highly significant higher seed and oil yield than their originals, besides changes in other traits. Yield in sunflower depends on many characteristics, especially yield components which are controlled by many genes, and their effects are being modified by the environment (Miller and Fick, 1997). Due to maturity reduction, there is a possibility of influencing yield. However, newly developed early-flowering mutant L3ME sunflowers had even significantly higher seed and oil yield in fixed M_7 generation. Early maturing genotypes are more favourable in sunflower production, due to a possible unstable environmental condition during the harvesting period. Development of early maturing genotypes in any crops depends on the reduction of days to 50% flowering (Chatterjee et al., 2012). This mutation had no influence on other traits, especially plant height, known to be in high correlation (Skoric, 1989), which indicated that mutation separated strong correlation of these two traits. Early mutants were reported by many authors (Plotnikov, 1971; Voskoboinik and Soldatov, 1974; Giriraj et al., 2004). Giriraj et al. (2004) isolated promising mutant lines by pedigree method and utilised them in a heterosis breeding programme for developing hybrids with different maturity groups. Heritability of the sowing to flowering date ranges from 0.62 to 0.95 (Jan, 1986), which is in agreement with our results.

Plant height is one of the most investigated morphological characteristics and short stature mutants are the most common products of induced mutations (Christov, 1995; Jambhulkar, 2002). Height reducing is a result either of reducing the number of internodes or the length of internodes. Results of Ramos et al. (2013) indicated that reduced height in the lines is controlled by a semidominant allele, *Rht1*, and phenotypic effects of this allele included shorter height and internode length, insensibility to exogenous GA application, normal morphogenetic response. Reduced plant height may lead to increase of sunflower yield due to improved stand-ability (Encheva et al., 2008), which was achieved in the case of L2MS mutant. Other short stature mutant R1MS, was early-flowering compared to the original line. From an agronomic point of view, taller plants are less desirable as a negative effect on the logging of the plant. Taller mutants are desirable only in case of specific agronomic requirements, such as biomass or for animal feed. Mutant line R3MT, derived from dwarf and very early line R3, exhibited better yield performance, medium maturity and medium height, which is considered favourable in practical use as a hybrid component.

The oil content in the seed and oil yield are closely linked to seed yield, which is the main purpose of sunflower growing (Skoric, 1989). Significant increases in oil content were observed in two mutant lines L1MO and R2MO resulting in higher oil yield per unit area. These oil content increases are notable results, since no drastic mutation has been reported for seed oil content in sunflower (Vranceanu and Iuoras, 1991).

Table 2. Comparison between original and mutant lines in M_6 and M_7 generation for earliness, plant height, head diameter, seed yield, oil content and oil yield

	Earliness (days)		Plant height (cm)		Head diameter (cm)		Seed yield (g)		Oil content (%)		Oil yield (g)	
	M_6	M_7	M_6	M_7	M_6	M_7	M_6	M_7	M_6	M_7	M_6	M_7
Original (L3)	63.00 (±0.18)	66.33 (±0.28)	122.45 (±0.34)	118.26 (±0.22)	16.57 (±0.45)	14.73 (±0.01)	17.70 (±0.23)	17.26 (±0.04)	35.41 (±0.55)	38.20 (±0.17)	6.36 (±0.17)	6.59 (±0.05)
Mutant (L3ME)	57.33 (±0.38)	58.33 (±011)	123.84 (±0.33)	119.69 (±0.15)	16.28 (±0.09)	14.5 (±0.14)	14.38 (±0.49)	18.67 (±0.10)	37.00 (±0.26)	38.33 (±0.16)	5.30 (±0.14)	7.15 (±0.01)
t-test	3.10**	4.90**	-0.54	-0.98	0.12	0.25	1.19	-2.38*	-0.47	-0.10	0.88	-2.22*
H^2 (%)	98.97	98.45	33.02	38.02	47.91	38.14	58.38	87.31	37.01	48.51	46.55	83.99
GA (%)	14.30	19.77	0.73	0.87	10.41	3.19	24.44	10.07	2.06	2.42	7.00	10.13
Original (L2)	75.67 (±0.28)	74.67 (±0.21)	160.43 (±0.14)	153.73 (±0.25)	17.08 (±0.04)	12.17 (±0.18)	33.48 (±0.08)	27.34 (±0.06)	36.78 (±0.09)	39.85 (±0.22)	12.31 (±0.05)	10.89 (±0.04)
Mutant (L2MS)	74.67 (±0.28)	71.33 (±0.21)	146.59 (±0.23)	142.31 (±0.43)	14.49 (±0.19)	13.72 (±0.06)	36.99 (±0.13)	33.53 (±0.14)	37.37 (±0.27)	42.68 (±0.55)	13.83 (±0.14)	14.30 (±0.14)
t-test	0.26	2.04	9.51**	4.22**	2.42*	1.52	-4.18**	-7.44**	-0.47	-0.84	-2.99**	-4.41**
H^2 (%)	40.00	88.89	90.59	98.83	82.54	90.37	92.42	98.67	18.33	46.10	83.19	93.07
GA (%)	1.01	6.29	10.95	13.65	4.20	24.34	13.10	26.66	2.06	2.65	14.05	33.04
Original (R1)	72.33 (±0.28)	63.33 (±0.21)	109.20 (±0.56)	112.63 (±0.36)	12.59 (±0.06)	12.91 (±0.16)	19.47 (±0.61)	24.76 (±0.22)	49.20 (±0.15)	50.71 (±0.08)	9.59 (±0.32)	12.55 (±0.09)
Mutant (R1MS)	63.67 (±0.42)	60.33 (±0.11)	97.93 (±0.61)	97.49 (±0.37)	11.48 (±0.07)	10.80 (±0.04)	19.70 (±0.16)	23.25 (±0.17)	48.19 (±0.17)	48.89 (±0.17)	9.49 (±0.05)	11.37 (±0.12)
t-test	3.13**	2.32*	2.48*	5.31**	2.22*	2.33*	-0.07	0.99	0.81	1.76	0.06	1.43
H^2 (%)	92.01	89.65	78.99	93.35	93.26	84.51	48.59	79.18	43.70	77.05	49.20	86.24
GA (%)	18.75	6.73	14.28	21.59	13.92	25.31	8.78	8.05	2.31	4.55	10.05	13.68
Original (R3)	55.33 (±0.28)	57.67 (±0.21)	47.20 (±0.62)	44.95 (±0.22)	6.70 (±0.07)	6.93 (±0.08)	15.13 (±0.06)	14.14 (±0.11)	41.43 (±0.28)	41.05 (±0.06)	6.27 (±0.06)	5.81 (±0.05)
Mutant (R3MT)	65.33 (±0.28)	63.67 (±0.28)	75.51 (±0.52)	76.29 (±0.17)	9.14 (±0.08)	9.90 (±0.14)	20.48 (±0.27)	22.6 (±0.28)	37.5 (±0.43)	41.91 (±0.05)	7.68 (±0.15)	9.47 (±0.11)
t-test	-4.63**	-3.13**	-6.32**	-20.40**	-4.32**	-3.37**	-3.49**	-5.03**	1.40	-1.98	-1.62	-5.67**
H^2 (%)	91.51	92.11	98.97	99.72	94.37	98.77	93.17	97.24	83.88	97.39	75.49	97.46
GA (%)	21.01	12.99	54.25	59.72	37.41	43.35	36.29	53.50	13.57	2.94	22.10	55.39
Original (L1)	72.33 (±0.38)	65.33 (±0.10)	99.60 (±0.74)	103.11 (±0.41)	17.18 (±0.21)	16.77 (±0.46)	24.67 (±0.05)	21.51 (±0.19)	44.62 (±0.36)	44.08 (±0.16)	11.0 (±0.03)	9.48 (±0.11)
Mutant (L1MO)	69.00 (±0.48)	64.67 (±0.10)	92.75 (±0.29)	99.49 (±0.16)	16.49 (±0.04)	16.08 (±0.44)	24.06 (±0.33)	22.68 (±0.72)	49.69 (±0.20)	49.87 (±0.02)	12.45 (±0.17)	10.81 (±0.07)
t-test	0.99	0.82	1.58	1.51	0.59	1.97	0.22	-0.24	-4.53**	-6.40**	-1.54	-0.68
H^2 (%)	36.21	50.00	83.72	68.68	17.34	76.65	41.97	42.74	93.15	97.97	74.32	47.50
GA (%)	3.36	0.92	9.54	4.09	1.57	5.24	4.11	5.52	6.93	16.68	13.83	7.17
Original (R2)	73.67 (±0.28)	71.33 (±0.21)	126.6 3(±0.15)	126.66 (±0.57)	12.97 (±0.10)	13.67 (±0.14)	23.41 (±0.25)	25.01 (±0.31)	35.97 (±0.14)	39.23 (±0.13)	8.41 (±0.06)	9.8 (±0.09)
Mutant (R2MO)	74.33 (±0.11)	65.33 (±0.46)	102.23 (±0.62)	96.41 (±0.28)	10.88 (±0.14)	11.30 (±0.12)	22.55 (±0.24)	25.88 (±0.26)	46.13 (±0.29)	45.68 (±0.02)	10.4 (±0.14)	11.82 (±0.12)
t-test	-0.41	2.17*	7.01**	8.59**	2.22*	2.34*	0.45	-0.39	-5.68**	-9.18**	-2.42*	-2.39*
H^2 (%)	30.00	82.79	97.09	98.36	99.01	79.06	29.41	33.91	95.58	99.21	77.82	72.17
GA (%)	1.07	11.77	34.09	45.21	27.80	26.08	4.41	5.09	31.12	20.48	23.48	19.92
Original (L4)	55.33 (±0.28)	57.67 (±0.21)	47.20 (±0.62)	44.95 (±0.22)	6.70 (±0.07)	6.93 (±0.08)	15.13 (±0.06)	14.14 (±0.11)	41.43 (±0.28)	41.05 (±0.06)	6.27 (±0.06)	5.81 (±0.05)
Mutant (L4MBr)	65.33 (±0.28)	63.67 (±0.28)	75.51 (±0.52)	76.29 (±0.17)	9.14 (±0.08)	9.90 (±0.14)	20.48 (±0.27)	22.6 (±0.28)	37.5 (±0.43)	41.91 (±0.05)	7.68 (±0.15)	9.47 (±0.11)
t-test	-4.63**	-3.13**	-6.32**	-20.40**	-4.32**	-3.37**	-3.49**	-5.03**	1.40	-1.98	-1.62	-5.67**
H^2 (%)	91.51	92.11	98.97	99.72	94.37	98.77	93.17	97.24	83.88	97.39	75.49	97.46
GA (%)	21.01	12.99	54.25	59.72	37.41	43.35	36.29	53.50	13.57	2.94	22.10	55.39

* and ** – mutant significantly different from original mean at the 5% and 1% level according to a Student's t-test;

H^2 – broad sense heritability; GA – genetic advance.

Branching mutant can be attributed to the mutations in genes involved in apical dominance (Nabipour et al., 2004) and can be used in hybrid production. From data in the literature it is known that branchiness in sunflower may be of different types, either dominant or recessive, the first controlled by the *Br* gene system and the second by the *b* gene (Sharypina et al., 2008). Inheritance of branching in mutant line L4MBr remains to be tested.

Broad sense heritability percentage is estimated as a ratio between the genotypic variance and the total phenotypic variance. Estimates of heritability in broad sense were the highest for earliness, plant height and head diameter, while yield and oil content showed moderate values at some mutant lines (Table 2). In general, the higher heritability estimates for traits in mutant lines indicate that environmental factors did not greatly affect phenotypic variation of such characteristics. Genetic advance refers to the expected gain in the mean of a population for a particular quantitative character by a generation of selection of a specified proportion of the highest-ranking plants. Genetic advance as a percent of the mean was the highest for head diameter (88.95%) at mutant L4MBr, plant height (59.72%), oil yield (55.39%) and seed yield (53.50%) at R3MT and the remaining traits showed moderate to very low values of genetic advance (Table 2).

Induced mutations created very useful genetic variability in certain characteristics of economic importance in different sunflower inbred lines. These induced variations might be due to genetic changes, such as chromosomal aberrations, or even structural mutations of some genes. Sunflower lines showed a lot of phenotypic and genotypic variation when subject to mutagenesis, which support previous findings (Luczkiewicz, 1975). In the breeding programme high heritability alone is not enough to make sufficient improvements through selection and genetic advance should be accompanying (Shukla et al., 2006). Stainfield (1971) (*cit.* Bozokalfa et al., 2010) classified heritability in three groups: traits having heritability values higher than 0.50 describe high, between 0.50 to 0.20 are referred to as medium and lower than 0.20 is defined as low heritability. Regarding heritability accompanied by the genetic advance together for traits significantly different between mutants and originals showed high heritability and high genetic advance. Moreover, broad sense heritability increased from M_6 to M_7 generation, indicating that improvement or selection could be made based on these characters. Heritability for yield is relatively low compared to other agronomic traits (Fick, 1978), whereas seed oil content heritability is rather high and was estimated to vary from 0.65 to 0.70 (Fick, 1975). Our results showed that traits that occurred through mutation had high values of heritability and genetic advance. Wani and Anis (2008) estimated fairly high heritability for almost all polygenic traits among the mutants, in comparison to the control.

CONCLUSIONS

The limited genetic variability of cultivated sunflower has been partly overcome by using induced mutation in the breeding programme. Induced mutagenesis lead to genetically inherited variability of sunflower inbred lines, which is suitable for use in breeding programmes. Further studies should be focused on testing new mutant lines in hybrid combinations, as well as on modes of inheritance of mutant traits. Since developed mutant lines differ in one or several traits, they can be used directly in hybrid production instead of their original lines.

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