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CREATING NEW GENETIC VARIABILITY IN SUNFLOWER USING INDUCED MUTATIONS

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SUMMARY

The objective of the study was to provide new genetic variability in important agronomic traits that can be exploited for improvement of sunflower production. Seeds of eight sunflower inbred lines from gene collection of Institute of Field and Vegetables, Novi Sad were irradiated with gamma rays (γ) and fast neutrons (Nf) and treated in ethyle-methane-sulphonate (EMS) solution. The manifestation of mutations was mostly expressed in M_2 and M_3 generation. Seven mutants were developed; one early flowering, two short stature and one high, two with higher oil content and one branching. The stable progenies were evaluated in micro-plot tests in M_6 generation for seed yield and other traits in comparison with respective original line. Further studies should be focused on testing new mutant lines in hybrid combinations, as well as the determination of inheritance of mutant traits.

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

INTRODUCTION

Genetic variability among plants in population is a basic prerequisite for successful plant breeding. Natural genetic variation *e.g.*, hybridization and spontaneous mutations have been used in plant breeding for a long time. Discovery that radiation can induce hereditary alterations in plant genome and thereby enhance the frequency of mutations allowed breeders to use induced mutagenesis to obtain more desirable mutations (Brunner, 1995).

Induced mutations have been successfully used in sunflower breeding to increase genetic variability by changing plant characteristics and productivity (Gvozdenović *et al.*, 2009). Many authors have used induced mutations in sunflower breeding (Cvetkova, 1970; Gundaev, 1971; Plotnikov, 1971; Soldatov, 1976; Ivanov, 1988; Fernandez-Martinez *et al.*, 1988; Christov, 1995; Osorio, 1995; Miller & Vick, 1999; Kalaydzhyan *et al.*, 2007; Cvejić *et al.*, 2009) and many mutants with

altered characteristics were developed. Gundaev (1971) and Voskoboinik and Soldatov (1974) have created mutants with shorter growing season, which had thinner hull and lower plant height. Mutants with short stature and larger head diameter were developed by Leclercq (1985) using gamma rays. Other sunflower mutants were also obtained: the increase of 1000 seed mass (Savin & Stepanenko, 1968), increased leaf area and decreased plant height (Cvetkova, 1970), increased oil content (Schuster & Kubler, 1983), resistance to rust (Lofgren & Ramaraje Urs, 1982) and cytoplasmic male sterility (Jan & Rutger, 1988). Encheva *et al.* (2008) produced mutants resistant to broomrape, races present in Bulgaria, by treating immature embryos with ultrasound. Application of mutagenic agents had greatly contributed to alteration of sunflower oil quality. Mutants having high concentration of palmitic (Ivanov, 1988; Osorio *et al.*, 1995), stearic (Osorio *et al.*, 1995) and oleic (Soldatov, 1976; Ivanov *et al.*, 1992; Andrich *et al.*, 1992) acid have been developed using chemical or physical mutagen treatments (Velasco *et al.*, 1999).

The main objectives of this research were to increase genetic variation within the collection of sunflower inbred lines from Institute of Field and Vegetable Crops, Novi Sad by mutagenesis. The research was directed to development of mutants with changed one or few agronomic traits and than to investigate productivity and stability of this mutants in comparative trial.

MATERIALS AND METHODS

Seed treatments

Eight different sunflower inbred lines from the genetic collection of the Institute of Field and Vegetable Crops, Novi Sad (Table 1) were used in this study. Approximately 500 seeds were treated with the following mutagens: gamma rays (γ: 70-160 Gy), fast neutrons (Nf: 3-5 Gy) and ethyl-methane-sulfonate (EMS: 0.1 and 0.25%, for 3.5 h). Treatments were carried out in Joint IAEA/FAO Laboratories in Seibersdorf, Austria. The doses/concentrations were chosen based on LD_{30} values described by Gvozdenović *et al.* (2009).

Table 1: List and characteristics of treated sunflower inbred lines

Inbred lines	Type of inbred line	Vegetation period	Plant height	Seed color	Coat type
L1	High oleic	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	Cream	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 Institute of Field and Vegetable Crops, Novi Sad. Plants were self-pollinated and M_2 seeds were harvested. The same procedure was followed for control plants. Based on observed changes of individual plants, seeds were planted in the next generation. This M_2 generation was grown in the field and, after self-pollination, the M_3 seeds were collected. In M_2 and M_3 generations the selection of individual plants 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 trail in order to test their productivity and stability, as well as morphological and biological characteristics. The trail was organized in randomized block design with three replications. Plant height and head diameter were recorded at plant maturity on 10 plants of each entry. Days to flowering were calculated as the days from plant emergence to 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.

The results were statistically analysed in Statistica 8. Differences between mutants and original lines were determined by applying t-test for the level of significance 0.05 and 0.01.

RESULTS AND DISCUSSION

Induced mutagenesis affected sunflower inbred lines by changing their characteristics. The selection of desirable mutant plants started in M_2 generation assuming that the changed characters were genetically inherited. 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. Mutants were planted in M_3 generation and seventeen were directly produced from mutant forms, among them four were early flowering, nine had short stature and high, two had higher oil content and one was branching (Table 2). Mutants were developed from all eight sunflower inbred lines. Female line L1 and restorer line R2 produced most mutants (3 each). Almost all mutants (13) regarding different plant traits were observed in the case of gamma irradiation. Less efficient agents were fast neutrons (3 mutants) and ems (only one mutant).

In the following generations (M_4, M_5, M_6), during selfing and selection, a few mutants were discarded because the trait was not completely fixed or it was not genetically inherited, since most of traits are quantitative and under the influence of the environment. Seven mutants were fixed in M_6 generation: Early-1, Shorty-5,

Table 2: Types and values of morphological and physiological mutations in M_3 generation

Type of mutations	Mutant line	Original line
Early flowering	M3-L3-Nf3 (53.10±0.16 days)	L3 (60.40±0.06 days)
	M3-L4-γ120 (55.20±0.14 days)	L4 (62.40±0.12 days)
	M3-R2-γ160 (53.10±0.10 days)	R2 (57.40±0.13 days)
	M3-R2-Nf5 (53.70±0.08 days)	
Short stature	M3-L1-γ80 (120.85±1.51 cm)	L1 (135.55±2.60 cm)
	M3-L1-ems0.25 (123.89±1.54 cm)	
	M3-L2-γ120 (166.75±1.05 cm)	L2 (181.14±1.94 cm)
	M3-L2-γ160 (172.39±1.24 cm)	
	M3-L3-γ70 (155.91±1.66 cm)	L3 (167.34±1.65cm)
	M3-R1-γ100 (111.25±1.73 cm)	R1 (124.63±2.49 cm)
	M3-R1-γ120 (114.69±1.60 cm)	
	M3-R3-γ200 (58.02±0.81 cm)	R3 (63.68±1.26 cm)
	M3-R4-γ150 (154.30±1.66 cm)	R4 (165.70±1.42 cm)
High stature	M3-R3-γ200 (130.54±0.83 cm)	R3 (63.68±1.26 cm)
Branching	M3-L4-γ120 (1 central head and 8 branches)	L4 (1 central head)
Oil content	M3-L1-Nf3 (54.11±0.10)	L1 (50.06±0.19)
	M3-R2-g120 (53.71±0.22)	R2 (49.71±0.05)

Table 3: Comparison of agronomic traits of sunflower M_6 mutant lines and their original lines

	Earliness	Plant height	Head diameter	Seed yield	Oil content	Oil yield
	(days)	(cm)	(cm)	(g)	(%)	(g)
L3	63.00 (±0.18)	122.45 (±0.34)	16.57 (±0.45)	17.90 (±0.23)	35.41 (±0.56)	6.36 (±0.17)
Early-1	57.33** (±0.38)	123.84 (±0.33)	16.28 (±0.09)	14.38 (±0.49)	37.00 (±0.26)	5.30 (±0.14)
L2	75.33 (±0.38)	160.43 (±0.14)	17.08 (±0.04)	33.48 (±0.08)	36.78 (±0.09)	12.31 (±0.05)
Shorty-5	74.67 (±28)	146.59** (±0.23)	14.49* (±0.19)	36.99** (±0.13)	37.37 (±0.27)	13.83 (±0.14)
R1	72.33 (±0.28)	109.20 (±0.56)	12.59 (±0.06)	19.47 (±0.61)	49.20 (±0.15)	9.59 (±0.32)
Shorty-9	63.67** (±0.42)	97.93* (±0.61)	11.48* (±0.07)	19.70 (±0.16)	48.19 (±0.17)	9.49 (±0.05)
R3	55.33 (±0.28)	47.20 (±0.62)	6.70 (±0.07)	15.13 (±0.06)	41.43 (±0.28)	6.27 (±0.06)
Tally-2	65.33** (±0.28)	75.51** (±0.52)	9.14** (±0.08)	20.48** (±0.27)	37.50 (±0.43)	7.68 (±0.15)
L1	72.33 (±0.38)	99.60 (±0.74)	17.18 (±0.21)	24.67 (±0.05)	44.62 (±0.36)	9.78 (±0.11)
Oily-3	69.00 (±0.48)	92.75 (±0.29)	16.49 (±0.04)	25.06 (±0.32)	49.69** (±0.20)	10.69 (±0.14)
R2	73.67 (±0.28)	126.63 (±0.15)	12.97 (±0.10)	23.41 (±0.25)	35.97 (±0.15)	8.41 (±0.06)
Oily-7	74.33 (±0.11)	102.23** (±0.62)	10.88* (±0.14)	22.55 (±0.24)	46.13** (±0.29)	10.40* (±0.14)
L4	73.33 (±0.11)	104.06 (±0.68)	19.63 (±0.06)	22.46 (±0.16)	35.49 (±0.42)	7.97 (±0.11)
Branchy-1	68.67** (±0.11)	101.64 (±0.76)	12.12** (±0.24)	22.84 (±0.36)	34.04 (±0.08)	7.78 (±0.14)

**significant at P=0.05 , *significant at P=0.01

Shorty-9, Tally-2, Oily-3, Oily-5 and Branchy-1.These mutants were developed from different original lines. Unlike their originals, mutants had improved one or few traits which were tested in comparative trails (Table 3). Significant differences were obtained between mutants and original lines for mutated traits, but in most cases differences were recorded for other traits.

Early flowering mutant

Line Early-1 was obtained using fast neutrons dose 3 Gy on line L3. Statistical analysis confirmed that mutant Early-1 flowers earlier than original line L3 for about 5 days. This mutation did not influence other traits, especially plant height, known to be in high correlation (Škorić, 1989), which indicated that mutation separated strong correlation from these two traits. Early mutants were reported by many authors (Gundaev, 1971; Plotnikov, 1971; Voskoboinik & Soldatov, 1974). Giriraj *et al*. (2004) isolated promising mutant lines by pedigree method and utilized them in heterosis breeding program for developing hybrids with different maturity groups.

Short stature mutants

Two short stature mutant lines were developed using gamma rays, doses 120 Gy and 100 Gy, respectively. Mutant line Shorty-5 had approximately 15 cm shorter stem than original line L2 which is generally tall line. Compare to original line, mutant Shorty-5 had significantly higher seed yield per plant despite having a smaller head. Other short mutant Shorty-9 was developed from high-oleic restorer line R1. Beside shorter stature, mutant showed wide range of variability of other traits. Line Shorty-9 differed significantly in days to flowering compared to the original line and had smaller head as well. Plant height is one of the most often investigated morphological characters and its reduction by induced mutations was reported by Cvetkova (1970), Christov (1995), Kalaydzhyan *et al*. (2007). Reduced plant height may lead to increase of sunflower yield due to improved stand-ability (Encheva *et al*., 2008), which was achieved in a case of Shorty-5 mutant.

High stature mutant

Mutant Tally-2 was produced by gamma irradiation; dose 200 Gy of dwarf line R3. Mutant was about 30 cm higher than the original line. Nevertheless, it had longer vegetation, bigger head and higher seed yield. Agronomically, mutant had an advantage concerning seed yield and hybrid production.

Mutants with higher oil content

Chemical and statistical analyses confirmed that mutant lines Oily-3 and Oily-7 had increased and stable oil content regarding their original lines L1 and R2, respectively. Mutant Oily-3 was developed by fast neutrons (Nf) using dose of 3 Gy. This mutant line showed stability in other examined traits while other mutant Oily-

7 had shorter stature and smaller head than their original line R2. The seed oil content was 49.69% (Oily-3) and 46.13% (Oily-7) in comparison to originals, 44.62% (L1) and 35.97% (R2). This results show that mutation induction is not conclusive, but no drastic mutation have been reported for seed oil content in sunflower (Vrânceanu, 1991). However, the data show that changes were induced and continued screening is underway. Regarding higher oil content, mutant line Oil-7 showed significantly higher oil yield.

Branching mutant

Branching mutant was obtained by treating seed of single-head female line L4 with gamma rays dose 120 Gy. As a consequence of this mutation, earliness and smaller heads were recorded. Branching mutant can be attributed to the mutations in genes involved in apical dominance (Nabipour *et al.*, 2004) and be used in hybrid production.

Obtained results created very useful genetic variability in certain characters of economic importance in different sunflower inbred lines. Sunflower lines showed a lot of phenotypic and genotypic variation when subject to mutagenesis, which supports previous findings (Luczkiewicz, 1975).

CONCLUSION

Induced mutagenesis lead to genetically inherited variability of sunflower inbred lines, which is suitable for use in breeding programs. Further studies should be focused on testing new mutant lines in hybrid combinations, as well as modes of inheritance of mutant traits. Since developed mutant lines differ in one or more traits, they can be used directly in hybrid production instead of their original lines.

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