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Induced Mutagenesis for Creation of New Starting Material In Helianthus Annuus (Sunflower) Breeding

Pooja Yadav and Neetu singh
Department of Botony
T.D.P.G .College Jaunpur U.P. India

Abstract
The study's goal was to uncover novel genetic diversity in crucial agronomic parameters that may be used to boost sunflower yield. Seeds of eight sunflower inbred lines were bombarded with gamma rays () and fast neutrons (Nf) and treated in ethyle-methane-sulphonate (EMS) solution from the Institute of Field and Vegetables, Novi Sad's gene collection. Mutations were mostly shown in the M2 and M3 generations. One early blooming mutant, two short stature mutants, one tall mutant, two with greater oil content, and one branching mutant were created. In contrast to the parent line, the stable progenies were tested in micro-plot tests in the M6 generation for seed yield and other features. Testing novel mutant lines in hybrid combinations, as well as determining the inheritance of mutant features, should be the focus of future research.
Keywords: *Induced, mutagenesis*

I. Introduction

A crucial need for effective plant breeding is genetic heterogeneity among plants in a population. Plant breeding has historically relied on natural genetic diversity, such as hybridization and spontaneous mutations. The discovery that radiation may cause hereditary changes in plant genomes, increasing mutation frequency, encouraged breeders to utilise induced mutagenesis to get more desired mutations. Exploited induced mutations in sunflower breeding to improve genetic variety by modifying plant traits and production. Various authors have utilised induced mutations in sunflower breeding, and many mutants with different traits have been established.) have created mutants with a shorter growth season, thinner hulls, and shorter plant heights. used gamma rays to create mutants with low height and greater head dimensions. Other sunflower mutants include those with a 1000 seed mass increase increased leaf area and decreased plant height (Cvetkova, 1970), increased oil content, rust resistance and cytoplasmic male sterility . By treating immature embryos with ultrasound, (2008) created mutants immune to broomrape, races seen in Bulgaria. The use of mutagenic agents had a significant impact on the quality of sunflower oil. Using chemical or physical mutagen treatments, mutants with high concentrations of and oleic acid have been generated (Velasco et al., 1999). The major goal of this study was to use mutagenesis to promote genetic variety in a collection of sunflower inbred lines from Novi Sad's Institute of Field and Vegetable Crops. The goal of the study was to create mutants with altered one or a few agronomic characteristics, then test their productivity and stability in a comparative experiment.

II. Review Of Literature

Osborne and London (2013) studied the LD50 value for survival of soybean seeds after treating with gamma rays and found that it was in the range of 10-18 kR. Koo (2010) reported that 15 kR of gamma ray was optimum for mutation induction in soybean. Constantin et al. (1976) recommended that 20 to 30 kR of gamma rays or 25 to 50 mM of EMS solution were the optimum LD50 doses in soybean.

The LD50 for survival showed slight deviation from the LD50 values for germination among gamma rays (48 kR) and EMS (0.45 %) in mutated seedlings of soybean (Balakrishnan, 1991). Geetha (1994) reported LD50 for gamma rays between 20 and 40 kR in soybean while for the LD50 for germination in case of ethidium bromide was around 30 to 35 ppm.

Addai and Kantanka (2006) reported that gamma ray dose of 250 Gy reduced both percentage of emergence and seedling height by about 50% relative to control.

Karthika and Subbalakshmi (2016) underwent a study on induced mutagenesis with gamma rays and EMS on Co 1 and Co 2 varieties of soybean. In an assessment to fix the LD50 value, the varieties responded differently and subtle difference was observed between the varieties in the degree of tolerance to the mutagens viz., Co 1 (62 KR Gamma rays, 26.4 mM EMS) and Co 2 (58.3 KR Gamma rays, 25.7 mM EMS). It was concluded that Co 1 tolerates higher doses of gamma rays and EMS.

Kumar et al. (2009) reported that LD value was found at 30mM for EMS in black gram. Khan et al. (2010) exposed the seeds of two soybean genotypes viz., Pusa-16 and PK-1042 to various doses of gamma rays, EMS and combinations of both. A linear reduction in germination, lethality and sterility was noticed. The LD50

for Pusa-16 was found to lie between 30kR + 0.2% EMS and 45 kR + 0.2% EMS, while in PK-1042, it was found to lie between 30 kR and 45 kR gamma rays.

Thilgavathi and Mullainathan (2012) mutagenised the seeds of Urdbean variety Vamban1 with gamma rays, EMS and dES. In the M1, germination percentage, plant growth and yield parameters were adversely affected in the mutagenic treatments and the effects were more pronounced in higher doses indicating almost a linear relationship. The LD50 value was observed in 15 mM of EMS, 25 mM of dES and 60 kR of gamma rays.

Hassan et al. (1985) irradiated seeds of Bragg, Hodgson and Lee-74 with 100 to 500 Gy gamma rays and 5 to 30 Gy fast neutrons and reported that growth inhibition increased with increasing doses and germination was inhibited only at the higher doses. Lee-74 was the most sensitive variety to gamma radiation and Bragg the most sensitive to fast neutron doses above 20 Gy.

Zakri and Jalani (2013) treated two cultivars (Palmetto and Acadian) of soybean with EMS and gamma rays and reported that the cultivar Palmetto showed higher survival percentages following either treatment.

Li et al. (2015) observed marked effects on seedling height and survival percentage in M1 following the treatment of dry seeds of soybean with various doses of electron beams.

Balakrishnan (2013) observed gradual reduction in germination and survival per cent due to increasing concentration of mutagen in soybean varieties Co 1 and Co 2. Kundi et al. (1997) reported differential sensitivity within crop and even within the genotype to different mutagenic treatments. It is obvious from the reports of Kozlova and Enken (1981), Geetha and Vaidyanathan (2000), Harb (1990) and Karthika and Subbalakshmi, 2006) that differences exist in the mutability of a variety.

Archana et al. (2017) observed the reduction in plant height and seed weight per plant at higher doses of mutagen and their combination treatments and they also observed the reduction in pods per plant due to reduced vigour.

Girija and Dhanavel (2015) reported that the frequency of mutation was more in combination treatments of gamma rays and EMS than in individual treatments. It was found that with increasing doses of EMS or Gamma rays the values obtained for all the biological criteria in M1 generation were decreased.

III. Materials And Methods

Seed treatments

This research employed eight distinct sunflower inbred lines from the Institute of Field and Vegetable Crops' genetic collection in Novi Sad (Table 1). Approximately 500 seedlings were exposed to gamma rays (70-160 Gy), fast neutrons (Nf: 3-5 Gy), and ethyl-methane-sulfonate mutagens (EMS: 0.1 and 0.25 percent , for 3.5 h). Treatments were carried out at Seibersdorf, Austria, at the Joint IAEA/FAO Laboratories. The doses/concentrations were determined based on Gvozdenovi et alLD30 .'s values (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 (M1) and untreated (control) seedlings were sown at the Institute of Field and Vegetable Crops' experimental field in Novi Sad. M2 seeds were gathered after the plants were self-pollinated. Control plants were treated in the same way. Seeds were sown in the following generation based on observed changes in individual plants. This M2 generation was cultivated in the field, and the M3 seeds were harvested following self-pollination. Individual plants were chosen in the M2 and M3 generations based on changes in plant height, blooming period, branching, and oil content. In subsequent generations, the permanence of new features was confirmed (M4, M5 and M6).

Agronomic evaluation

Selected mutants (M6) and original lines were planted in a comparison trial to see how productive and stable they were, as well as their morphological and biological properties. The trail was set up in a three-replication randomised block form. On 10 plants from each entry, the height and diameter of the heads were

measured at maturity. Days to flowering were determined as the number of days from the emergence of the plant and the onset of complete blooming (UPOV - stage F3.2). Seed yield was assessed for each plant independently after harvesting. NMR was used to determine the oil content of each plant's seed. Statistica 8 was used to analyse the findings statistically. The differences between mutants and original lines were assessed using a t-test with significance levels of 0.05 and 0.01.

IV. Results And Discussion

Induced mutagenesis changed the properties of sunflower inbred lines. The selection of suitable mutant plants began in the M2 generation, with the assumption that the altered traits were inherited genetically. In the field, many mutations were studied, and promising mutants were chosen based on early blooming, short and tall height, branch appearance, and oil content. Four were early flowering, nine had short stature and high, two had higher oil content, and one was branching. Mutants were planted in M3 generation, and seventeen were directly produced from mutant forms; four were early flowering, nine had short stature and high, two had higher oil content, and one was branching (Table 2). All eight sunflower inbred lines were used to create mutants. The majority of mutations were created by female line L1 and restorer line R2 (3 each). In the case of gamma irradiation, almost all mutants (13) for various plant characteristics were detected. Fast neutrons (3 mutations) and ems were less effective agents (only one mutant). During selfing and selection in subsequent generations (M4, M5, M6), a few mutants were rejected since the phenotype was not totally fixed or genetically inherited, as most traits are quantitative and influenced by the environment. In the M6 generation, seven mutants were fixed: Early-1, Shorty-5, Shorty-6, Shorty-7, Shorty-8, Shorty-9, Shorty-10, and Shorty.

Table 2: Types and values of morphological and physiological mutations in M3 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-γ60 (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 M6 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

Branchy-1, Shorty-9, Tally-2, Oily-3, Oily-5, and Shorty-9 These mutants were bred from a variety of original lines. Mutants, unlike their parents, have improved one or a few qualities that were put to the test in comparison trials (Table 3). For mutated qualities, significant changes were found between mutants and original lines, although in most instances, differences were found for other features.

Early flowering mutant

On line L3, line Early-1 was produced utilising fast neutrons at a dosage of 3 Gy. For roughly 5 days, statistical analysis indicated that mutant Early-1 blossoms sooner than the parent line L3. This mutation had no effect on other qualities, particularly plant height, which is known to have a significant association (kori, 1989), indicating that the mutation separated these two traits. Many writers have written about early mutants used the pedigree technique to extract potential mutant lines, which they then used in a heterosis breeding programme to create hybrids with varied age groups.

Short stature mutants

Gamma rays, dosages of 120 Gy and 100 Gy, were used to create two low stature mutant lines. The stem of the mutant line Shorty-5 was around 15 cm shorter than that of the parent line L2, which is a tall line. Despite having a smaller head, mutant Shorty-5 exhibited a considerably better seed output per plant than the normal line. Shorty-9, a short mutant, was created from the R1 high-oleic restorer line. Aside from their lower size, the mutants displayed a broad variety of additional characteristics. In comparison to the original line, Shorty-9 had a substantially shorter time to flowering and a smaller head. Plant height is one of the most studied morphological features, with and Kalaydzhyan et al. reporting its decrease by induced mutations (2007). Reduced plant height may result in increased sunflower yield owing to greater stand-ability seen in the instance of the 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. Agronomic ally, mutant had an advantage concerning seed yield and hybrid production.

Mutants with higher oil content

Chemical and statistical investigations revealed that mutant lines Oily-3 and Oily-7 have higher and more stable oil content than their parent lines L1 and R2. Fast neutrons (Nf) were used to create mutant Oily-3 at a dosage of 3 Gy. Other mutant Oily 7 exhibited lower height and a smaller head than their parent line R2, indicating that this mutant line was stable in other features. In compared to the originals, which contained 44.62 percent (L1) and 35.97 percent (Oily-7), the seed oil content was 49.69 percent (Oily-3) and 46.13 percent (Oily-7) respectively (R2). The findings demonstrate that mutation induction is not definitive, although no significant changes in sunflower seed oil content have been documented (Vrânceanu, 1991). The statistics, however, reveal significant alterations were caused, and more testing is planned. Oil yield was much greater in mutant line Oil-7, which had higher oil content.

Branching mutant

Seed of the single-head female line L4 was treated with 120 Gy gamma rays to produce a branching mutant. Earlyness and smaller heads were seen as a result of this mutation. Mutations in genes involved in apical dominance might cause branching mutants, which can be utilised in hybrid development. The obtained

findings resulted in extremely helpful genetic diversity in key economically important features in several sunflower inbred lines. When sunflower lines were mutated, they revealed a lot of phenotypic and genotypic diversity, which matches prior results.

V. Conclusion

Induced mutagenesis resulted in sunflower inbred lines with genetically inherited diversity, making them amenable for breeding operations. Testing novel mutant lines in hybrid combinations, as well as ways of mutant trait inheritance, should be the focus of future research. Because created mutant lines vary in one or more features, they may be employed instead of their original lines in hybrid development.

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