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INDUCTION OF PLANT INJURY, CHIMERA, CHLOROPHYLL AND MORPHOLOGICAL MUTATIONS IN SUNFLOWER USING GAMMA RAYS

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SUMMARY

Seeds of the sunflower variety 'Surya' were irradiated with 4 doses of gamma rays. Effects of gamma rays on the induction of plant injury and morphological mutations were studied. Increasing doses of gamma rays increased the plant injury in the M_1 generation. Two sectorial male sterile chimera were found in the M_1 generation. In the M_2 generation, 27 morphological mutants were isolated. Among them, 3 each were for chlorophyll and stem, 9 for leaf, 8 for capitulum and 4 for seed coat colour. Some of the mutants possessed more than one mutated character. Mutations like yellow leaf vein, fasciation, wrinkled leaf, zigzag stem, zigzag ray florets, stigma emergence and brown patch mutants are novel characteristics. Among the 4 doses of gamma rays, 200 Gy was the most effective dose for induction of mutation in sunflower.

Key words: sunflower, gamma rays, mutation

INTRODUCTION

Among the cultivated crops of India, the sunflower is a recent introduction. This important oilseed crop has limited variability. Morphological variability at different stages of crop growth plays an important role to identify specific plants or recombinants in cross breeding programme. The objectives of the breeder is to link as many characters as possible with economically important traits resulting in an early identification and development of superior strains. The pre-requisite for this is variability of germplasm. If sufficient variability is not available, it could be generated through cross breeding and induced mutations. Mutation studies in sunflower carried out by Vrânceanu and Stoenescu (1982), Vrânceanu and Iuoras (1990) and Vrânceanu *et al.* (1993) reported the effect of gamma rays on plant injury and induction of variability for different characters. Efforts on generating variability for

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morphological characters in sunflower are limited (Luczkiewicz, 1975; Miller, 1992) and very few studies have been carried out in the Indian sub-continent (Giriraj *et al.*, 1990; Jambhulkar, 1996). Here we present a study on the effect of gamma rays on plants in the M_1 generation and its effectiveness and efficiency in inducing morphological mutations in the M_2 generation.

MATERIALS AND METHODS

The high-yielding variety 'Surya' was selected for mutation study. This is the first Indian variety released for cultivation in Maharashtra state which possesses black and white zebra stripes on the seed coat. Seeds of an inbred line of 'Surya' containing less than 5% moisture were irradiated with 50, 100, 150 and 200 Gy doses of gamma rays. The M_1 generation was grown along with control and observations on germination, chlorophyll sectors, leaf deformities and chimeras were taken during crop growth. All plants were selfed at the time of flowering, using muslin cloth bags. Their plant-to-row progenies were raised in the M_2 generation where chlorophyll and morphological mutations were isolated. True breeding nature of these mutants was studied in subsequent generations. The dosewise frequency of plant injury in M_1 and mutations in M_2 were calculated on the basis of their respective plant populations. The mutagenic effectiveness was calculated on the basis of the frequency of M_2 chlorophyll mutations and gamma ray dose (Msd/Gy) and mutagenic efficiency on the basis of M_2 chlorophyll mutations and reduction in the percent of germination (Msd/lethality).

RESULTS AND DISCUSSION

Plant injury

Increasing doses of radiation decreased germination, however, almost all germinated plants survived to maturity (Table 1). The plant injury, like chlorophyll sectors and leaf deformities, increased with increasing radiation dose in the M_1 generation. Maximum chlorophyll sectors and leaf deformities like flecking, bifurcation and wrinkling were found with the 200 Gy dose of gamma rays. It provided a good index of radiation effect. Reduction of germination up to 20-30% in 200 Gy dose of gamma rays was observed by Vrânceanu and Iuoras (1990) and Vrânceanu and Stoenescu (1982). The present study showed the maximum plant injury at 200 Gy dose.

Table 1: Plant injury in the M_1 generation and chlorophyll and morphological mutations, their frequency and mutagenic efficiency and effectiveness in the M_2 generation

	M_1 variation (Plant injury %)						M_2 mutations (%)				
Dose (Gy)	Germination	Survival	Chlorophyll sectors	Chimera (disc florets)	Leaf (deformities)	Number of M_2 plants	Chlorophyll	Mutagenic effectiveness	Mutagenic efficiency	Morphol. mutations	Chimera (seed coat)
0 (C)	97.3	96.5	-	-	-	-	-	-	-	-	
50	77.0	76.2	0.33	-	1.74	3758	0.42	0.0084	0.0182	0.47	-
100	83.4	82.3	1.71	-	1.03	4531	0.41	0.0041	0.0246	0.77	0.02
150	68.6	66.2	1.87	-	2.25	4112	0.09	0.0060	0.0028	0.82	-
200	62.6	59.9	3.18	0.78	5.90	3590	1.02	0.0051	0.0272	1.05	-

Chimera

Two plants (0.78%) in 200 Gy dose were found as sectorial male sterile in the M_1 generation (Table 1) where one plant had $1/4^{th}$ sector of the disc possessing sterile disc florets with smaller ray florets and $3/4^{th}$ sector with fertile disc florets and normal ray florets. Another plant had the capitulum where half of the disc florets were sterile and the other half were fertile and ray florets were absent at the site of the sterile sector (Figure 1). This chimera affected both ray and disc florets. Male sterility is a common phenomenon in the M_1 generation due to chromosomal abnormalities and this phenomenon has been extensively studied in a number of the crops. Male sterile plants in the M_1 generation were reported by Vrânceanu and Iuoras (1990) in sunflower. However, sectorial male sterility was not reported. In the M_2 generation, one plant in 100 Gy dose had no ray florets in $1/8^{th}$ part of the disc (Table 1). At the time of harvest, it was found that the same portion carried a chimera for seed coat colour. Seeds in the chimeric region possessed only two white stripes on the seed coat whereas seeds in the remaining portion had 5-8 white stripes like normal zebra stripes in the parent variety 'Surya'. Seeds from the chimeric and normal portions were harvested separately. There were 92 seeds in the chimeral sector and 962 seeds in the normal portion. When these seeds were sown separately in the M_3 generation, none of the plants were chimeric. However, Hermelin *et al.* (1987) reported sectorial seed coat colour mutations in F_1M_1 plants of sunflower and suggested that the sampling of seeds from chimeric region leads to mutant plant in the M_2 generation. Chimera formation is a common phenomenon following mutagenic treatments of any meristem whether in seeds, buds or tissue culture (D'Amato, 1965; IAEA, 1983).

Table 2: Morphological mutations and their brief description

Num.	Mutations	Characteristic
I Chlorophyll mutations		
1	Xantha	Completely yellow seedling survived for 8 days
2	Virescent	Younger leaves yellow which gradually turns to green
3	Yellow leaf veins	Leaf veins turned yellow looking like virus infection leading to leaf yellowing
II Leaf mutations		
1	Fasciation	Large number of small leaves, up to 175; compressed internode, flattened stem and fused flower
2	Wrinkled leaf	Dark green highly wrinkled lamina, short and thick petiole; dissected ray florets
3	Spinach leaf	First 4-6 leaves appear like spinach leaf and zigzag; elongated ray florets
4	Light green leaf	Light green leaves; small close serration, axillary branches at the base and very small ray florets
5	Involuted leaf	Boat-shaped lamina
6	Elongated leaf	Elongated leaf lamina; acuminate, flower buds closed by younger leaves forming cabbage like structure
7	Erect leaf	Less angle of petiole to stem, more leaves towards capitulum and older leaves turn yellow before maturity
8	Smooth leaves	Light green smooth leaves without serration and less venation
9.	Bushy plant	More number of leaves (45-48) as against 30-36
III Stem mutations		
1	Light yellow stem	Light yellow stem, at maturity complete plant looks like whitish yellow
2	Erect stem	Stem does not bent after flowering and angle of head/capitulum ranged from 0-15^{o}
3	Zigzag stem	Zigzag growth of stem at flower bud initiation
IV Capitulum mutations		
1	Very small ray florets	Ray florets 3-4 cm long
2	Less number of ray florets	Number of ray florets ranged from 3-10
3	More number of ray florets	Number of ray florets ranged from 70-75 having stigma in some florets as against 35-40 ray florets without stigma
4	Dissected ray florets	Single ray floret dissected into 2-3 tiny parts
5	Zigzag ray florets	Narrow, elongated and zigzag growth of ray florets
6	Narrow ray florets	Narrow and elongated ray florets
7	Stigma emergence	Stigma protrude out form base of the anther tube
8	Male sterility	Very small anther tube with sterile pollen
V Seed coat colour mutations		
1	White seed	White seed coat with 2 - 3 very thin black stripes
2	Brown seed	Brown seed coat
3	Black seed	Black seed coat
4	Brown patch	Brown patch in the middle of seed coat with black and white striped background

Morphological mutations

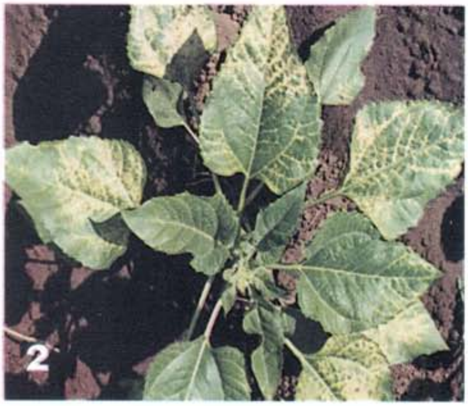
Twenty-seven morphological mutants were isolated from the large M_2 population. Among these, 3 were for chlorophyll, 9 for leaf, 3 for stem, 8 for capitulum morphology and 4 for seed coat colour. Their brief description is given in Table 2. True breeding nature of these mutants were confirmed in the M_3 and M_4 generations.

Chlorophyll mutations

In the M_2 generation, the frequency of chlorophyll mutations like chimera, xantha and virescent was increased in the 200 Gy dose of gamma rays (Table 1). Besides xantha and virescent, a distinct mutant was isolated. It has green leaves in initial stages of plant growth and after 25-30 days, leaf veins of older leaves starts yellowing giving the appearance of virus infection leading to leaf yellowing (Figure 2). Generally, mutation studies resulted in common chlorophyll mutations like albina, virescent, xantha, etc. However, a mutation having yellow leaf veins has not been reported so far in sunflower.

Leaf mutations

The sunflower variety 'Surya' possesses 30-36 leaves with serration, smooth lamina and long petiole. In the present study, 9 distinct mutants deviating from the normal leaf morphology were isolated. Among them fasciated, wrinkled, spinach, involute and light green leaf mutants possessed distinct morphology. The fasciated mutant had the maximum of 175 small leaves with disturbed phyllotaxy, flattened stem and fused capitulum (Figure 3). The largest number of small leaves was located in the vicinity of the capitulum. A genetic study revealed that it is governed by a single recessive gene (unpublished). Fasciated mutants were isolated in peas (Gottschalk, 1977) and chick pea (Knight, 1993). So far, there has been no report on fasciated mutant isolated through induced mutation in sunflower. In the wrinkled leaf mutant, the lamina was dark green and highly wrinkled. The petiole was shortened and thick. The ray florets were dissected, looking like the fusion of 3-4 small ray florets (Figure 4). Thus, a single mutant has three distinct morphological markers. The short petiole is a desirable character in sunflower breeding (Vrânceanu *et al.*, 1988). In the spinach leaf mutant, first 4-6 leaves were similar to that of spinach and therefore it was termed so. The ray florets were elongated and zigzag. This mutant has two morphological markers. The light green leaf mutant possessed light green leaves, smaller and denser serration, axillary branches at the base and very small ray florets 2.8-3 cm long. All these mutants had more than one morphological marker which has not been reported so far. Genetic studies of these mutants are in progress. Various mutants for leaf morphology were isolated using X-rays and their inheritance was studied (Luczkiewicz, 1975). Some of the mutants in the present study are similar to involute leaf, wrinkled leaf, thick and short petiole and the increased number of leaves isolated by Luczkiewicz (1975). Genetic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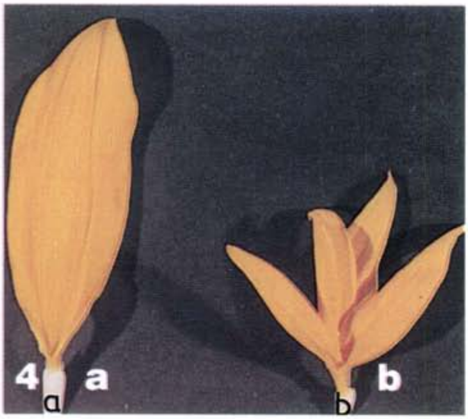
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Figures 1 to 6

Figure 1: A capitulum without ray florets and sterile disc florets in one half (left) and another half (right) with normal fertile disc florets and ray florets
Figure 2: A mutant showing green younger leaves and yellow veins on older leaves leading to leaf yellowing
Figure 3: The fasciated mutant with many small leaves, disturbed phyllotaxy and flattened stem
Figure 4a: A normal ray floret
Figure 4b: A single ray floret dissected into 4 small ray florets
Figure 5: A mutant showing zigzag growth of stem instead of straight
Figure 6: A mutant with straight stem and capitulum looking towards the sky

studies revealed that the wrinkled, erect and cup-shaped leaves are controlled by single recessive gene each whereas the thick and shortened petiole is controlled by complementary genes while the number of leaves is a quantitative character (Luczkiewicz, 1975).

Stem mutations

Three mutants were isolated for stem morphology. These were a light green stem, a straight stem and a zigzag stem. In the light green stem, besides a reduced chlorophyll content, young leaves were yellow which gradually turned to normal green-like virescent. At maturity, the whole plant looked whitish-yellow. The zigzag stem mutant was characterized by zigzag growth of the stem at flower bud initiation (Figure 5). Both these mutants have not been reported so far. Generally, the capitulum of sunflower inclines towards the sun after flowering. However, in the straight stem mutant, the stem and the capitulum did not droop down even after flowering (Figure 6). The head inclination ranged from 0 to 15°. A similar mutant was isolated by Luczkiewicz (1975). He found that the head did not incline up to flowering but at achene ripening, the capitulum drooped down. The leaves were erect and this character was controlled by a single recessive gene. The head inclination normally ranges from 0 to180° which is conditioned by 12 genes of which 4 act in an additive manner. Each gene contributed approximately 15° (Kováčik and Škaloud, 1990).

Capitulum mutations

Sunflower capitula consisted of 35-40 ray florets and many disc florets. Eight mutants deviating from the standard florets morphology were isolated in the M_2 generation. These were very small ray florets (Figure 7), smaller number of ray florets (Figure 8), increased number of stigmoid ray florets, dissected ray florets, zigzag ray florets, narrow ray florets (Figure 9), abnormal stigma emergence and male sterile disc florets mutant. Most of these mutants had been reported by Luczkiewicz (1975). Genetic studies of reduced number and length of ray florets revealed that these mutations were governed by complementary genes (Luczkiewicz, 1975). However, there have been no reports so far on zigzag ray florets, narrow ray florets,


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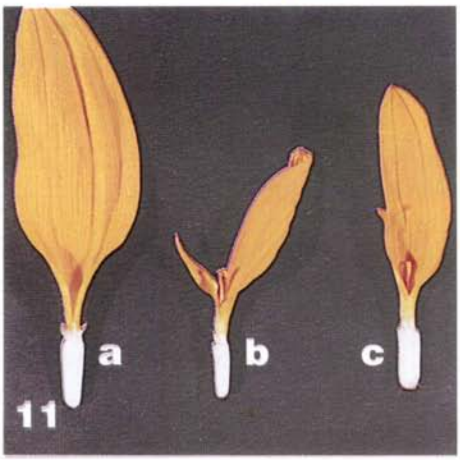
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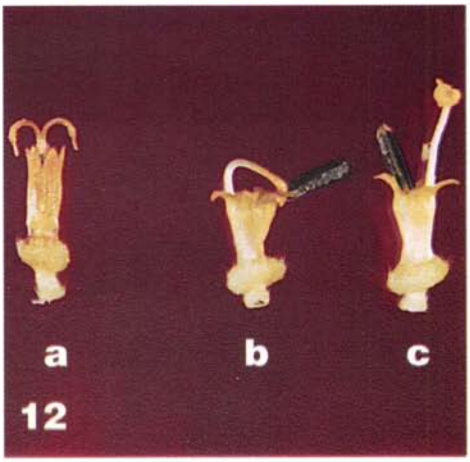
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Figures 7 to 12

Figure 7. A capitulum with small ray florets of 3-4 cm length
Figure 8. A mutant showing only 10 ray florets against 35-40 ray florets occurring normally
Figure 9. The narrow and small ray florets mutant
Figure 10. A capitulum showing narrow long and zigzag ray florets
Figure 11a. A normal ray floret without the stigma
Figure 11b., Figure 11c. Ray florets with stigmata
Figure 12a. A normal disc floret showing the stigma emerging from the top of the anther tube
Figure 12b. A disc floret with the stigma protruding from the base of the anther tube
Figure 12c. A fully emerged stigma with intact anther tube

increased number and stigmoid ray florets and mutant for stigma emergence. The zigzag ray floret mutant was characterised by elongated and zigzag growth of ray florets (Figure 10). Generally, ray florets in the sunflower are sexless, however, in the stigmoid ray floret mutant, the stigma emerged from the tubular structure of the ray floret (Figure 11). It appeared intermediate between the ray and disc florets. This character was governed by a single recessive gene (Luczkiewicz, 1975). In the disc florets, the stigma emerges vertically from the anther tube, however, in the abnormal stigma emergence mutant, the stigma protrudes out from the base of the anther tube (Figure 12). Genetic studies of some of these mutants were carried out by Miller (1992) and Kováčik and Škaloud (1990).

Seed coat colour mutations

The variety used in the present study possessed black and white stripes on the seed coat looking like zebra stripes. Four seed coat colour mutations viz. black, brown, white seed coat and one with brown patch in centre surrounded by black and white stripes on seed coat were isolated. The black, brown, and white seed coat colour mutations were true breeding in subsequent generations. However, selfed seeds of the brown patch mutant segregated into normal zebra stripes and brown patch seed coat in subsequent generations. It shows the possibility of the existence of a controlling element for this particular trait, but this requires confirmation.

Mutagenic effectiveness and efficiency

Mutagenic effectiveness was calculated on the basis of the number of chlorophyll mutations in the M_2 generation (Table 1). Maximum reduction in germination (50%), highest frequency of leaf deformities in the M_1 generation and maximum chlorophyll and morphological mutations in the M_2 generation with the 200 Gy dose clearly indicated that expected mutations could be isolated with this dose of gamma rays. Though the mutagenic frequency was high, the mutagenic effectiveness was lower with the 200 Gy dose. This could be attributed to a comparatively low plant stand in the M_2 generation and higher dose whereas, mutagenic efficiency was higher in 200 Gy dose of gamma rays. Mutation studies by Vrânceanu and Stoenescu (1982) clearly indicated that gamma ray dose for dry seed of sunflower is 50-

200 Gy. Our results also support this. In our study, it was observed that the mutation recovery was maximum in the 200 Gy dose of gamma rays, therefore it could be the most effective dose for mutation studies in sunflower.

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INDUCCIÓN DE LESIONES, QUIMERAS, MUTACIONES DE CLOROFILAS Y MUTACIONES MORFOLÓGICAS EN EL GIRASOL POR LA UTILIZACIÓN DE RAYOS GAMMA

RESUMEN

Las semillas de la variedad de girasol "Surya" era irradiado por 4 dosis de rayos gamma. Era estudiada la influencia de los rayos gamma sobre la inducción de lesiones y mutaciones morfólogicas en las plántas. Por el aumento de dosis de rayos gamma se aumentaba también el número de lesiones en la generación M_1. Dos quimeras masculinas esteriles de sector fueron encontradas en la generación M_1. En la generación M_2 fueron aislados 27 mutantes morfológicos. Entre ellos, tres eran mutantes de clorofila y tronco, 9 eran mutantes de hoja, 8 mutantes de cabeza y 4 mutantes de color de graño. Algunos mutantes tenian más de una característica mutante. Las características nuevamente descubiertas son la nervatura amarilla de hoja, tallo achatado, plegadura de hoja, tallo sinuoso, flores linguiformes sinuosas, peciolo y manchas brunas. De 4 dosis de rayos gamma investigadas, esa de 200 Gy fué la más eficaz con respecto a la suscitación de mutaciones en el girasol.

INDUCTION DE LESIONS, CHIMERES, MUTATION DE CHLOROPHYLLE ET MUTATIONS DE MORPHOLOGIE DANS LE TOURNESOL AU MOYEN DE RAYONS GAMMA

RÉSUMÉ

Des semences de tournesol de l'espèce "Surya" ont été irradiées par quatre doses de rayons gamma. L'effet des rayons gamma sur l'induction de lésions et de mutations morphologiques de la plante a été observé. L'augmentation des doses de rayons gamma a augmenté le nombre de lésions de la génération M_1. Deux chimères sectorielles mâles stériles ont été trouvées dans la génération M_1. Dans la génération M_2, 27 mutants morphologiques ont été isolés. Parmi eux, trois étaient mutants pour la chlorophylle et la tige, 9 pour les feuilles, 8 pour la tête et 4 pour la couleur de la graine. Certains mutants avaient plus d'une caractéristique de mutation. Les caractéristiques nouvellement découvertes sont la nervure jaune de la feuille, la fasciation, le flérissement de la feuille, la sinuosité de la tige, les fleurs linguiformes et sinueuses, l'émergence du stigmate et des taches brunes. Des 4 doses de rayons gamma étudiees, la dose de 200 Gy s'est révélée la plus efficace dans l'induction de mutations chez le tournesol.