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Chromosomal Induced Aberrations in Sunflower (*Helianthus annuus.* L) with Gamma-irradiation, Sodium Azide and Combined Treatments

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Summary The seeds of 2 varieties of common Sunflower (*Helianthus annuus* L.) viz:USH-430 and KL-675 were treated to estimate the mutagenicity of both gamma-rays, sodium azide alone and in combination with a view to generate morphological macro-mutations and screen and ascertain the chromosomal aberrations followed by reduction of pollen fertility with increased doses. An attempt was made to know the genetic basis of the chromosomal aberrations. Such aberrations are a source of changes in the pattern of gene regulation at the time of differentiation leading to the formation of cultivars.

Key words Gamma-rays, Sodium azide, Meiospore mother cells, Chromosomal aberrations, Sunflower.

The increasing value of vegetable oil use in India is gaining great importance because of multifarious uses for good health of people. Sunflower oil contains linoleic acid and linolenic acid which lowers blood cholesterol and manages blood glucose levels. The cytological investigation is an important factor in breeding programs. This involves the development of new original forms with the help of empherical mutagenesis. The usual and amicable course of meiosis secures gamete viability, if changed the same has a great effect on yield. Meiosis is a regular process that leads to evolutionary stability and plays a great role in having the chromosome number in order to maintain the same ploidy levels of progeny and also secure the viability of gametes. Meiosis is a major aspect in the life cycle of an individual. The normal and regular completion of the cytological action is controlled by a large number of genes which proceed from pre-meiotic to post-meiotic stages. The chromosomal rearrangements are a source of changes in the pattern of gene regulation at times differentiates to form cultivars (Wilson 1975, King and Wilson 1975, Prazer, Fowler and Wilson 1976). The objective of this work was to investigate the meiotic behaviour in pollen mother cells of sodium azide and gamma-rays treated sunflower plants.

The different concentration of gamma-rays and sodium azide caused meiotic abnormalities but affected the pollen fertility. The usefulness of gamma-rays and sodium azide mutagens on sunflower was investigated by ascertaining and assessing the type of chromosomal aberrations correlating with pollen viability and seed set.

Materials and methods

Seeds of 2 varieties of common sunflower (*Helianthus annuus* L.) viz. USH-430 and KL-675 were obtained from Satya Sai Agribiotech, Kurnool, A.P. India and used in the experiments. Seeds with 11 percent moisture content were exposed to 2kR, 4kR, 6kR, 8kR and 10kR of gamma-rays (Cobalt-60) at Centre for Nuclear Techniques (CNT), Andhra University, Visakhapatnam. The

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seeds were also presoaked in distilled water for 10 h and then subjected to treatment with sodium azide at different concentrations of 2 mM, 4 mM, 6 mM, 8 mM and 10 mM. Gamma-irradiated seeds were also again (combined) treated with sodium azide at (2 kR+2 mM, 4 kR+4 mM, 6 kR+6 mM, 8 kR+8 mM, 10 kR+10 mM) respectively.

The seeds were sown in Botany Experimental Farm separately in seed beds and watered as per schedule. The seeds without exposure to the gamma-rays were sown in separate seed beds and were termed as control plants. After 15 d of sowing the seedlings were transplanted to separate plots as well as in field plots and labeled. In M_1 and M_2 generation, germination, survival, growth, morphological changes, chromosomal behaviour, pollen fertility and seed setting were carefully observed. The experiments were conducted adopting randomized block design (RBD). The procedures for the cytological studies were carried out according to the method described by Sharma and Sharma (1980). The pollen mother cells with evenly spread chromosomes on slides were sealed with wax for preventing the entry of air and from drying. The selected slides were used for analysis, microphotography and image analysis.

Results

From control plants of both varieties, meiotic studies were carried out. The chromosome pairing behaviour was observed at pachytene and Diakinesis (Figs. 1. a–c) in which chromosomes paired normally. The chromosomal count indicated 17 bivalents giving a total number of $2n=34$. Among 17 bivalents, ring bivalents, rod bivalents and V-shaped bivalents were recorded.

In the mutagenised population some selected plants were subjected for cytological analysis. From them, some of the meiospores cells exhibited chromosomal aberrations which included

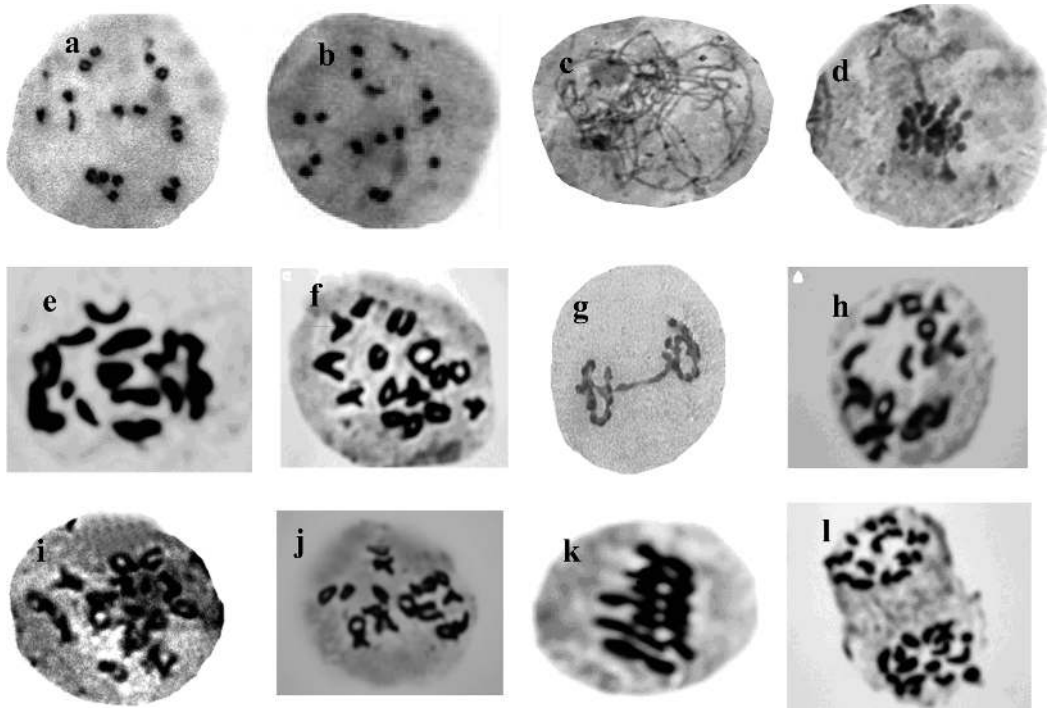

Fig. 1. a. diakinesis (USH430-control), b. diakinesis (KL675-control), c. pachytene (USH430-control), d. metaphase-I (USH430-4mM), e. pro-metaphase-I (USH430-6kR), f. diakinesis (KL675-6kR), g. anaphase-I (USH430-6kR), h. diakinesis (USH430-10kR), i. diakinesis (USH430-10kR), j. diakinesis (KL675-2 mM+2 kR), k. metaphase-I (KL675-6 mM+6 kR), l. anaphase-I (KL675-6 kR).

Table 1. Mean Chromosome chiasmata at diakinesis and Pollen stainability Sunflower varities of USH-430 and KL-675

Plant variety/ Treatment	Chromosome number	Cells analysed	Bivalents		Total bivalents	Chiasma frequency	%Pollen stainability
			Ring	Rod			
USH-430							
Control	34	70	9.86±0.05	7.14±0.05	17	24.28±0.05	95.00
Gamma-irradiation							
2 kR	34	72	11.15±0.04	5.85±0.04	17	23.60±0.4	90.00
4 kR	34	73	8.12±0.4	8.88±0.52	17	23.28±0.32	88.00
6 kR	34	75	8.03±0.49	6.97±0.02	15	20.00±0.07	86.30
8 kR	34	80	9.85±0.50	7.15±0.03	17	21.24±0.51	73.00
10 kR	34	82	9.74±0.60	7.26±0.06	17	20.72±0.72	69.50
Sodium azide							
2 mM	34	73	10.10±0.61	6.90±0.51	17	23.28±0.32	81.20
4 mM	34	74	10.96±0.53	4.04±0.05	15	20.27±0.43	78.00
6 mM	34	75	7.97±0.49	9.03±0.04	17	22.66±0.07	73.20
8 mM	34	80	12.90±0.06	4.10±0.06	17	21.24±0.43	67.00
Combined							
2 kR+2 mM	34	74	8.09±0.23	8.91±0.37	17	22.97±0.26	92.00
4 kR+4 mM	34	74	9.32±0.04	7.68±0.53	17	22.96±0.64	87.00
6 kR+6 mM	34	76	11.03±0.34	5.97±0.22	17	22.36±0.45	84.40
8 kR+8 mM	34	78	10.80±0.42	6.20±0.53	17	21.78±0.53	79.00
10 kR+10 mM	34	80	11.90±0.04	5.10±0.07	17	21.24±0.52	66.00
KL-675							
Control	34	70	11.68±0.25	5.32±0.32	17	24.28±0.27	85.00
Gamma-irradiation							
2 kR	34	74	11.32±0.02	5.68±0.06	17	22.96±0.56	82.00
4 kR	34	75	12.45±0.34	4.55±0.43	17	22.66±0.66	73.00
6 kR	34	77	10.38±0.29	4.62±0.38	15	19.48±0.77	68.00
Combined							
2 kR+2 mM	34	73	8.63±0.35	8.37±0.35	17	23.28±0.05	78.00
4 kR+4 mM	34	75	10.80±0.42	6.20±0.53	17	22.66±0.53	73.20

interchanges, associations of 4. Through terminalization of the chiasmata the bivalents proceeded and oriented at metaphase I in 4 mM of sodium azide as shown in Fig. 1. d. In USH-430 6 kR treatment the analysed cells of meiospores of 1 cell exhibited 6 ring bivalents, 5 rod bivalents, 4 V-shaped bivalents and 1 quadrivalent as shown in Fig. 1. e. In KL-6kR in 1 plant the smeared meiospores exhibited 7 ring bivalents, 4 rod bivalents; 4 V-shaped bivalents and 1 quadrivalent as shown in (Fig. 1. f). Under 6 kR treated plants of some USH-430 variety the smeared microspore mother cells exhibited some laggard chromosomes which formed a connecting bridge between 2 chromosome anaphase poles as shown in Fig. 1. g. In some 10 kR treated population of USH-430 variety, the meiospore mother cells exhibited 3 ring bivalents, 7 rod bivalents and 7 V-shaped bivalents amounting $2n=34$ as exhibited in Fig. 1. h. Under the same treatment, some meiospores exhibited 6 ring bivalents, 6 V-shaped bivalents, and 5 rod bivalents as depicted in Fig. 1. i. In KL-675 variety under the combined (2 kR+2 mM) treatment some meiospores mother cells exhibited 7 ring bivalents, 7 rod bivalents and 3 open bivalents as shown in Fig. 1. j. In KL-675 variety some plants which were treated at 6 kR+6 mM, the meiospores mother cells under the metaphase I, has 7 ring bivalents, 7 rod bivalents and 3 open bivalents were assembled at the equatorial plate properly as observed in Fig. 1. k. In the same variety at same dose, the meiospores mother cells and the chromosomes are evenly distributed to the opposite poles 17+17 (anaphase I) as depicted in Fig. 1. l.

Under the meiotic behaviour studies of plants, the different types of chromosomes pairing in which chiasmata indicate the proximity and closeness of the chromosomes. For establishing the chiasmata the ring and rod bivalents were scored and the mean values and standard error were statistically calculated from which chiasma frequency was calculated to indicate the chromosomal homology and pairing behaviour. Here the chiasma frequency is correlated with that of the pollen stainability. As the chiasmata decreases the pollen fertility decreases along with the increased dose. The pollen stainability is decreased proportionately as shown in the Table 1. Therefore under the individual treatment along with the controls the mean of rod and ring bivalents were established statistically in control variety of USH-430 as 9.86±0.05 ring, 7.14±0.05 rod bivalents that were scored amounting 17 bivalents which has a chiasma frequency 24.28±0.05 with pollen fertility of 95%. The chiasma frequency gradually decreases from 2 kR to 10 kR in USH-430 variety *i.e.*, in 2 kR 23.60±0.4 with 90% pollen fertility and in 10kR 20.72±0.72 with 69.5% pollen fertility respectively as shown in the Table 1. The mean chiasma frequency and pollen fertility decreases as the dose increases in case of sodium azide treatment in USH-430 variety. In 2 mM, the chiasma frequency and pollen fertility were 23.28±0.32 and 81.2% respectively, and 21.24±0.43 and 67% in 8 mM as mentioned in the Table 1. In combined treatment of USH-430 variety, the chiasma frequency and pollen fertility slightly decreases as the dose increases from 22.97±0.26 (2 kR+2 mM) to 21.24±0.52 (10 kR+10 mM) and 92% to 66% respectively.

Whereas in KL-675 variety in the mutagenised and control population the meiotic behaviour studies and pollen fertility studies were made and these include 24.28±0.27 chiasma frequency and 85% pollen fertility respectively. In KL-675 variety, 2 kR, 4 kR and 6 kR treated plants of which certain cells were studied for meiotic behaviour and pollen fertility. The mean chiasma frequency and pollen fertility gradually decreased as the dose increased. *i.e.*, 2 kR (22.96±0.56 and 82%) to 6 kR (19.48±0.77 and 68%) as presented in the Table 1. In combined treatment of the KL-675 variety at low dose of 2 kR+2 mM there was a slight variation in chiasma frequency as recorded (23.28±28±0.05) which is a slight deviation to the 4 kR+4 mM (22.66±0.53) along with that of pollen fertility 78% to 73.2% respectively.

Discussion

The basic chromosome number of genus *Helianthus annuus* L. is 17 (Geisler 1931, Darlington and Janaki Ammal 1945) as it is also confirmed in the present investigation. Both physical and chemical mutagenic agents produce the classes of chromosomal mutations as reported by Gecheff (1996), Ratnam and Madhava Rao (1992) also in this crop. Generally the gamma-rays treated seeds used to induce the chromosomal variation as it was observed by (Brock 1980, Friebe *et al.* 1991, Sanamyan *et al.* 2000) in cotton. Sodium azide which is a very potent mutagen induced the chromosome breakage in barley has been reported to be very low as reported by many authors (Sideris *et al.* 1969, Nilan *et al.* 1973, Nilan and Pearson 1975, Kleinhofs *et al.* 1974, Walther 1975, Prina and Favret 1983), Vig in soybean (1973) as it is also corroborated in *Helianthus annuus* L. in this present investigation.

In analysed meiospores of control plants normal bivalent formation was observed as also reported by Srivastava and Kavita Kapoor (2008) in *Trigonella foenum-graecum*, Pagliarini (1990) in *Aptenia cordifolia*, Defani Scoarize *et al.* (1995a, b) in maize, Consolaro *et al.* (1996) in *Centella asiatica* L. As the radiation doses increases, the percentage of abnormal cell increased by Zeerak (1992) in tomato, Ahmad (1993) and Khare (1994) in *Cicer arietinum* L., as it is also reported in sunflower in the present investigation. In mutagenised population of USH-430 at 6 kR and KL-675 at 6 kR treatment the analysed meiospores in 1 plant exhibited quadrivalents as it was reported by Bose and Saha (1970), Ratnam and Madhava Rao (1992). The single bridge without fragment could result from failure of division of end genes of chromosomes brought about by nucleic acid upset

(Bose and Saha, 1970) which is also concorded in this present investigation. Nevertheless Walters (1950) reported that bridges and fragments could also result from breakage and reunion of chromosomes; but McClintock (1941, 1984) reported that such bridges may be formed due to consequence of crossing over associated with inversions or chromosomal rings. Such bridge formation is also due to delayed separation of the chiasmata, and also due to later replication of heterochromatin or chromosome stickiness (Sinha and Godward, 1972).

In the present sunflower studies with the increase of dose concentration the pollen sterility increased as shown in (Table 1) as has been corroborated by Patil and Bora (1961) in groundnut, Dhanraj (1971) in tomato, Sing and Roy (1971) in *Trigonella foenum-graecum* L., Sinha and Godward (1972) in *Lens culinaris*, Ratnam and Madhava Rao (1992) in sunflower. The decrease in seed setting in USH-430 and KL-675 was associated with the occurrence of chromosomal aberrations as it was also recorded by Ratnam and Madhava Rao (1992) in sunflower. In sunflower crop the higher doses of gamma-rays were deleterious and hence lower doses were considered useful (Giriraj *et al.* 1990, Ratnam and Madhava Rao, 1992).

In the present investigation on sunflower with gamma-rays and sodium azide treatment, chromosomal aberrations helped in understanding the levels of pollen sterility, seed set via chiasma frequency. The above cytogenetic studies provided some instances with a morphogenetic interest.

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