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Full Length Research Paper

Mutagenic effectiveness and efficiency in varieties of sunflower (*Helianthus annuus* L.) by separate and combined treatment with gamma-rays and sodium azide

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The effect of single treatment with gamma-rays, sodium azide and combination treatments of gamma-rays and sodium azide on seed germination, seedling survival, pollen fertility and seed set in sunflower (*Helianthus annuus* L.) M_2 generation was studied in the varieties of USH-430 and SHSF-333. There was gradual decrease of seedling survival and pollen fertility with an increase in the dose of mutagen in both varieties. The percentage of lethality and achene sterility gradually increased with an increased dose. In USH-430 variety, the seed set percentage was significantly increased at 6kR, 4 mM and 6 kR + 6 mM, respectively. Mutagenic effectiveness decreased with an increased dose or concentration of gamma-rays and sodium azide in both varieties. In combined treatments, mutagenic effectiveness gradually increased with an increased dose. Mutagenic efficiency increased with an increased dose in case of gamma-irradiated seeds in both varieties. Mutagenic efficiency decreased gradually with an increased dose in both varieties. The linear correlation co-efficient was positive in case of gamma-rays and sodium azide separated, whereas in combined treatment, negative correlation was observed.

Key words: Sodium azide, gamma-rays, seed set, pollen sterility, mutation frequency, mutagenic effectiveness, mutagenic efficiency.

INTRODUCTION

The increasing value of sunflower (*Helianthus annuus* L.) oil use in India is gaining great importance because of multifarious uses in enhancing the good health of the people. The production of vegetable oil of sunflower is the third largest in the world following soyabean and palm oil. Sunflower oil contains linoleic and linolenic acid which lowers blood cholesterol and manage the blood glucose levels. The mutagenic effect of a mutagen, which is an index for the appropriate choice, can be evaluated in terms of “mutagenic effectiveness and efficiency” (Konzak et al., 1965). Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen. Mutagenic efficiency refers to the proportion of mutation in relation to other associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility, induced by the mutagen in question. Generating variability by mutagenic agents is of paramount importance in sunflower crop improvement when seed unfilling is a major problem. A crop plant can be improved in productivity; resistance to biotic and abiotic stress etc. when the genetic variability for the specific trait is available to the respective population or species. The induced variation and quantitative characters in the application of mutagenesis in plant breeding has been carefully investigated by several authors. Mutation breeding is the most useful and vital technology of sunflower (Jaya and Selva, 2003). Induction of mutations by gamma-rays and sodium azide are widely used in plant breeding programmes. The higher doses of gamma-rays were deleterious and lethal to sunflower crop and therefore, the lower doses were considered as significant (Giriraj et al., 1990).

Azide, a well-known respiratory, catalase and peroxidase inhibitor is shown to be a potent chemical mutagen

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in both higher and lower organisms (Nilan et al., 1973). The best use of mutation breeding is the possi-bility of improving one or two characters without changing the rest of the genotype with physical and chemical mutagens. Hence, in these experiments, gamma-rays and sodium azide were effectively used to generate genetic as well as morphological mutants.

MATERIALS AND METHODS

Seeds of two varieties of common sunflower (*H. annuus* L.), USH-430 and SHSF-333, were obtained from Satya Sai Agribiotech, Kurnool, A. P. and used in the experiments. Seeds with 11% moisture content were exposed to 2, 4, 6, 8 and 10 kR of gamma-rays (Cobalt ^{60}) at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Tamilnadu. The seeds were also presoaked in distilled water for 10 h and then subjected to treatment with sodium azide at different concentrations of 2, 4, 6, 8 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 days of sowing the seedlings were transplanted from seedbeds to field plots in randomized block design (RBD) and labeled. In M_2 generation, seedling survival, growth, morphological changes, pollen fertility and seed setting were carefully observed. Mutation frequency was deduced with characters specified by Gustafsson (1940), Stubbe and Wettestein (1941). Gaul et al. (1971) and Blixt and Gottschalk (1975) methods was followed here for categorizing the mutagenised population into micro and macro-mutations adopted in these experiments. Palynological studies (through acetolysis method) descriptions and pollen terminology were made according to the methods suggested by Erdtman (1969).

Achene sterility

The removed seeds from capitulum, the filled and unfilled seeds were tested with two fingers by touch. The filled seeds were considered as fertile and unfilled as sterile.

Mutagenic effectiveness and efficiency of gamma-rays and sodium azide was calculated by using the following formulae as given by Konzak et al. (1965):

$$\text{Mutagenic Effectiveness (Physical mutagens)} = \frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{Dose in kilo Roentgen (kR)}}$$

$$\text{Mutagenic Effectiveness (Chemical mutagens)} = \frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{Concentration of mutagen (mM)} \times \text{time of treatment}}$$

$$\text{Mutagenic Effectiveness (Combination Treatment)} = \frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{Dose in kilo Roentgen (kR)} \times \text{concentration of mutagen (mM) x time of treatment}}$$

$$\text{Mutagenic Efficiency} = \frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{\% Pollen sterility (Ps) or \% lethality or achene sterility}}$$

RESULTS AND DISCUSSION

The effect of the different doses or concentrations of gamma-rays, sodium azide and combined treatments on seedling survival, pollen fertility, seed set, mutation frequency, mutagenic effectiveness and efficiency of USH-430 and SHSF-333 varieties are presented in Tables 1 and 2.

A reduction percentage of seedling survival with an increasing gamma-ray dose was observed in the sunflower varieties of USH-430 and SHSF-333 as depicted in Tables 1 and 2. This was also observed by Ratnam et al. (1992), Ahmed (1979), Jambulkhar and Joshua (1999) in sunflower, Swaminathan and Gupta (1967) in *Brassica compestris*, Khan (1979) in mungbean and Cheema and Atta (2003) in rice. The seedling survival also decreased with an increasing concentration of sodium azide that was also observed in the sunflower varieties of USH-430 and SHSF-333 as it was corroborated with the results of Wang et al. (1996) in sunflower and Mensah and Obadoni (2007) in groundnut. Determination of mutagenic-sensitivity of germinating seeds constitutes an important aspect in mutation breeding, since healthy crop growth and yield depends on seedling establishment.

Pollen sterility increased as the dose concentration of gamma-rays increased in both varieties as reported by Sinha and Godward (1972) in *Lens culinaris*, Singh and Roy (1971) in *Trigonella*, Awnindra (2007) in *Nigella sativa* L., Sushil and Dubey (1998) in *Lathyrus sativus* L. Pollen sterility was also increased as the concentration of sodium azide increases in both varieties as mentioned in Tables 1 and 2. It was previously reported by Phogat et al. (2004) in *L. culinaris* and Giriesh and Priyanka (2007) in black cumin.

The reduction of seed setting was observed with the increase of dose concentration of gamma-rays in USH-430 and SHSF-333. It was also reported by Yamakawa and Sparrow (1966) in tomato and *Capsicum* and Sushil and Dubey (1998) in khesari. Similar results were also obtained with sodium azide by Mensah and Obadoni (2007) in groundnut. The mutation frequency gradually increased as dose concentration of gamma-rays and sodium azide in both varieties of sunflower. The results were corroborated by Jaya and Selva (2003) in sun-flower, Awnindra (2007) in mungbean by gamma-rays and with sodium azide in cowpea by Dhanavel et al. (2008).

Mutagenic effectiveness means the frequency of mutations induced by unit dose concentration of a mutagen, while efficiency means undesirable biological effects like lethality and sterility caused by the mutagen (Konzak et al., 1965). The utility of a particular mutagen depends not only on its effectiveness and inducing mutation but also on its efficiency. The mutagenic effect-tiveness decreased with increased dose concentration of gamma-rays and sodium azide in both varieties. This was reported by Gupta and Yashvir (1975) in foxtail millet, Deepalakshmi and Ananda (2003) in *Vigna mungo* L.,

Table 1. The effectiveness and efficiency of gamma-irradiation, sodium azide and combined treatment on sunflower variety cv.USH-430.

Treatment with gamma rays, sodium azide and combined	% of Seedling survival on 40th day	% of Pollen fertility	% of seed set	% of Lethality (L)	% of Pollen sterility (S)	Mutation frequency (M)	Mutagenic effectiveness. M\dose or M\C × t or M\ kR × C × t	Mutagenic efficiency		
								Lethality M/L	Pollen sterility M/S	Achene sterility M/A
2 kR	73.00	90.00	76.50	27.00	10.00	25.18	12.59	0.93	2.51	1.07
4 kR	66.00	88.00	74.30	34.00	12.00	32.77	8.19	0.96	2.73	1.27
6 kR	50.00	86.00	82.50	42.00	14.00	53.66	8.94	1.27	3.83	3.06
8 kR	42.00	75.00	78.70	55.00	25.00	54.20	6.77	0.98	2.16	2.54
10 kR	25.00	60.00	61.67	70.00	40.00	37.00	3.70	0.52	0.92	0.96
2 mM	60.00	81.20	72.60	32.00	18.80	28.35	141.75	0.88	1.50	1.03
4 mM	56.00	78.00	78.80	38.00	22.00	54.63	136.57	1.43	2.48	2.57
6 mM	39.00	73.20	73.30	54.00	26.80	30.07	50.11	0.55	1.12	1.12
8 mM	20.00	67.00	66.00	75.00	33.00	22.00	27.50	0.29	0.66	0.64
2 kR + 2 mM	68.00	92.00	75.68	30.00	8.00	21.20	212.00	0.70	2.65	0.87
4 kR + 4 mM	65.00	86.43	78.91	33.00	13.57	26.45	264.50	0.80	1.94	1.25
6 kR + 6 mM	58.00	80.72	80.20	38.00	19.28	29.00	290.00	0.76	1.50	1.46
8 kR + 8 mM	49.00	75.00	74.00	45.00	25.00	31.60	316.00	0.70	1.26	1.21
10 kR + 10 mM	30.00	68.00	62.50	62.00	32.00	32.73	327.30	0.52	1.02	0.88

M/Dose = gamma-irradiation, M/C × t = sodium azide, M/kR × C × t = combined treatment (C = concentration of sodium azide, t = time, kR= Kilo Roentgen of gamma-rays)

Table 2. The effectiveness and efficiency of gamma-irradiation, sodium azide and combined treatment on sunflower variety cv.SHSF-333.

Treatment with gamma rays, sodium azide and combined	% of Seedling survival on 40th day	% of Pollen fertility	% of seed set	% of Lethality (L)	% of pollen sterility (S)	Mutation frequency (M)	Mutagenic effectiveness. M\dose or M\C × t or M\ kR × C × t	Mutagenic efficiency		
								Lethality M/L	Pollen sterility M/S	Achene sterility M/A
2 kR	67.00	90.00	70.30	28.00	10.00	19.50	9.75	0.69	1.95	0.65
4 kR	61.00	88.20	73.65	32.00	11.80	23.30	5.82	0.72	1.97	0.88
6 kR	60.00	80.70	84.72	35.00	19.30	28.67	4.77	0.81	1.48	1.87
8 kR	49.00	76.50	80.00	44.00	23.50	32.00	4.00	0.72	1.36	1.60
10 kR	37.00	69.00	72.70	55.00	31.00	41.35	4.13	0.75	1.33	1.53
2 mM	71.00	92.00	73.62	22.00	8.00	22.50	112.50	1.02	2.81	0.85
4 mM	65.00	87.35	79.80	30.00	12.65	28.35	70.87	0.94	2.24	1.40
6 mM	58.00	85.60	82.57	35.00	14.40	29.67	49.45	0.84	2.06	1.70
8 mM	51.00	78.00	68.35	43.00	22.00	25.66	32.07	0.59	1.16	0.81
10 mM	35.00	72.72	59.80	57.00	27.28	24.00	24.00	0.42	0.87	0.50

Table 2. Contd.

2 kR + 2 mM	72.00	89.30	74.42	24.00	10.70	23.42	234.20	0.97	2.18	0.91
4 kR + 4 mM	61.00	86.50	80.23	31.00	13.50	28.75	287.50	0.92	2.12	1.45
6 kR + 6 mM	58.00	82.66	83.50	37.00	17.34	30.60	306.00	0.82	1.76	1.85
8 kR + 8 mM	46.00	76.00	72.67	48.00	24.00	26.00	260.00	0.54	1.08	0.95
10 kR + 10 mM	32.00	70.82	60.60	60.00	29.18	24.36	243.60	0.40	0.83	0.61

C = M/Dose gamma-irradiation, M/c × t = sodium azide, M/kR ×C × t = combined treatment (concentration of sodium azide, t = time, kR= Kilo

Table 3. Correlation between mutagenic effectiveness and efficiency in USH-430 and SHSF-333 sunflower varieties.

Variety	Treatment with gamma-rays, sodium azide and combined	Linear correlation		
		ME and M/L	ME and M/S	ME and M/A
USH-430	Gamma-rays	0.58	0.63	0.04
	Sodium azide	0.87	0.83	0.63
	Combined	- 0.48	- 0.99	0.22
SHSF-333	Gamma-rays	- 0.58	0.80	- 0.83
	Sodium azide	0.90	0.94	0.19
	Combined	0.29	0.27	0.94

ME = Mutagenic effectiveness; M/L = lethality; M/S = pollen sterility; M/A = achene sterility.

Jaya and Selva (2003) in sunflower and Sreeramulu (1970) in S*orghum*. Similar results were also observed in the case of sodium azide by Phogat et al. (2004) in *Macrosperma lentil* and Dhanavel et al. (2008) in cowpea. Mutagenic effectiveness increased as the dose concentration increased in case of combined treatment of gamma-rays and sodium azide in both varieties as it was reported in rice by Seetharamireddi and Ramamohan (1988).

Mutagenic efficiency increased with an increased dose of gamma-rays in USH-430 and SHSF-333. Similar results were also produced by Sharma and Sharma (1979) in lentil, Jaya and Selva (2003) in sunflower and Awnindra (2007) in mung-bean. Mutagenic efficiency decreased with an increased concentration of sodium azide in both varieties as it was corroborated with the results of Mensah and Obadoni (2007) in groundnut and Dhanavel et al. (2008) in cowpea.

Linear correlation co-efficient was statistically analyzed between mutagenic effectiveness and efficiency (lethality, pollen sterility and achene sterility). In USH-430 variety, the linear correlation co-efficient was positive in case of gamma-rays and sodium azide separately, whereas in combined treatment, negative correlation was observed. Significant correlation was obtained in case of sodium azide at ME and M/L (0.87), ME and M/S (0.83), respectively, as shown in Table 3. In SHSF-333 variety, positive correlation was observed with sodium azide and combined treatment while negative correlation was indicated with the gamma-irradiation treatment. The significant correlation was observed in sodium azide treatment at ME and M/L (0.90), ME and M/S (0.94) and in combined treatment at ME and M/A (0.94), respectively, as shown in Table 3.

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