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Macro and micro-morphological mutants in varieties of sunflower (*Helianthus annuus* L.) by using gamma-rays, sodium azide and combined treatment

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SUMMARY

Two varieties of viable seeds of sunflower (USH-430 and Nidhi-999) treated with gamma-rays, sodium azide and combined treatments. The following micro-mutants were scored *viz*: patchy albino, white margin, virescent, darker green, xantha purple, albino. The micro-mutants were increased with increased dose concentrations with all three treatments in both the varieties. The micro-mutations rate was high with sodium azide followed by combined and gamma-rays treatment. The macro-mutants include, branched, basal stem bifurcation, rosette and compact leaf arrangement, double headed, dwarf early-I, dwarf early-II and mosaic leaf arrangement. The macro-mutations were scored high at gamma-rays followed by combined and with sodium azide treatments.

Key words : Mutation breeding, Macro and micro-mutations, Gamma-rays and sodium azide

Sunflower has become an important oil seed in the Indian vegetable oil pool following its introduction from Russia in 1969, and in such a imported varieties, some valuable traits have been lost due to poor farming techniques by poor farmers. Characters not known in the natural variability of species (or population) concerned may be created by induced mutation in up to date variety. Macro-mutations technique has been used for continuous improvement of newly introduced varieties and in this way to extend their life span. In mutation breeding programmes, macro-mutants may be used directly. Macro-mutants may be very useful in cross-breeding since they may carry desired characters which otherwise are to be found only in non adapted varieties such as primitive types or even in wild forms. The desired goal of plant mutagenesis has been to control and direct the induced mutation process to generate mutation at specific loci and alter the mutation spectrum. For mutation breeding this goal is more specific, since the desire here is to produce "at will mutations" that having a beneficial value for crop improvement.

A wide range of morphological and physiological mutants have been reported in barley (Nilan *et al.*, 1973 and Kleinhofs *et al.*, 1978) with sodium azide which is very potent mutagen. Gamma-rays induced chlorophyll and macro-mutations in rice (Miah *et al.*, 1970), in barley (Swaminathan *et al.*, 1962). Some combinations of mutagens have been tested in rice by gamma-rays and combined with sodium azide (Reddi and Rao, 1988). Further Gaikward and Kothekar (2004) with combined treatment scored the macro-mutants and chlorophyll mutants in lentil. Hence, the present investigation was carried out to assess the effect of gamma-rays and sodium azide independently and with combined treatments on two varieties of sunflower in generating the macro and micro-mutations in employing them in this crop improvement.

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MATERIALS AND METHODS

Genetically pure and viable seeds of two varieties of common sunflower (*Helianthus annuus* L.) *viz*., USH-430 and Nidhi-999 were procured from Acharya N.G. Ranga Agricultural Research Centre (ANGRARC), Anakapalli, A.P. and were used in the experiments. Seeds with 11 per cent moisture content were exposed to 2kR, 4kR, 6kR, 8kR and 10kR of gamma-rays ($Cobalt^{60}$) at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Tamil Nadu. The seeds were also presoaked in distilled water for 10 hours and then subjected them to the treatment with sodium azide at different concentrations of 2mM, 4mM, 6mM, 8mM and 10 mM. Gamma-irradiated seeds were also again (combined) treated with sodium azide at (2kR + 2mM, 4kR + 4mM, 6kR + 6mM, 8kR + 8mM, 10kR + 10mM), 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 termed as control plants. After 15 days of sowing the seedlings were transplanted to separate field plots and labeled. All mutant progenies were sown and grown in separate lines. The experiments were conducted adopting Randomized Block Design (RBD).

The concept of micro-and macro-mutations and its importance for mutation breeding has been utilized in these experiments (Gaul 1965, Gaul *et al.*, 1971). Micro-morphological mutants comprising leaf characters including chlorophyll mutants (patchy albino, white margin, virescent, darker green, xantha purple, albino) and broad leaves were identified followed by criteria established by Gustafsson (1940) and Stubbe and Wettestein (1941). The scored data of micro-morphological mutants were computed for the plants produced from gamma-rays, sodium azide alone and combined treatments.

RESULTS AND DISCUSSION

The control and treated M_2 population were scored out for the micro- and macro-mutations and are being described as follows:

Micro-mutations: (Table 1)

Patchy albino:

A sector of the leaf can be seen as albino and the condition persisted throughout the crop growth of sunflower varieties. (Fig. 1 A).

Table 1 : Micro-morphological mutants of sunflower varieties in response to gamma-irradiation, sodium azide and combined treatment in M_2 generation

Name of the variety	Treatment with Gamma rays, Sodium azide and Combined	Patchy albino	White margin	Virescent	Darker green	Xantha purple	Albino	Broad leaf	Total no. of mutant plants	Sub-total
USH-	Control	-	-	-	-	-	-	-	-	
430	2kR	-	-	-	-	1	2	-	3	
	4kR	1	-	-	1	3	1	-	6	
	6kR	1	1	2	1	1	1	1	8	33
	8kR	2	-	-	1	2	-	1	6	
	10kR	-	2	2	-	3	3	-	10	
	2mM	2	-	-	-	2	1	1	6	
	4mM	2	1	-	-	3	2	1	9	
	6mM	-	-	2	2	5	2	1	12	56
	8mM	1	1	2	1	6	2	-	13	
	10mM	-	2	2	1	5	6	-	16	
	2kR+2mM	-	1	-	-	2	1	1	5	
	4kR+4mM	-	-	-	1	5	-	1	7	
	6kR+6mM	1	-	3	-	4	1	1	10	47
	8kR+8mM	1	1	1	-	7	2	-	12	
	10kR+10mM	-	1	3	-	6	3	-	13	
Nidhi-	Control	-	-	-	-	-	-	-	-	
999	2kR	-	-	-	-	1	2	1	4	
	4kR	1	-	-	1	2	1	-	5	34
	6kR	-	1	2	1	2	1	1	9	
	8kR	1	-	2	1	4	-	1	11	
	10kR	-	1	1	-	2	1	-	5	
	2mM	-	-	1	-	2	1	1	5	
	4mM	1	1	-	1	4	1	1	9	
	6mM	1	-	2	2	3	1	1	10	51
	8mM	-	2	2	1	5	3	-	13	
	10mM	-	2	4	1	5	2	-	14	
	2kR+2mM	-	-	-	-	2	1	1	4	
	4kR+4mM	-	-	-	1	4	-	1	6	
	6kR+6mM	1	-	2	-	5	1	1	10	45
	8kR+8mM	1	1	1	-	6	1	1	11	
	10kR+10mM	-	3	2	-	7	2	-	14	

(A) Patchy albino (USH430-4mM) **(B) Xantha purple (Nidhi999-4kR+4mM)**
(c) Different leaf shaped mutants **(D) Lanceolate leaf (Nidhi999-6kR)**

Fig. 1 : Micro-mutations in sunflower varieties of USH-430 and Nidhi-999

Virescent:

The leaves after emergence were white and patches of yellowish green. Initially these plants were slowly growing and less vigorous but gradually they regained normal growth and at the time of maturity they were as moderately vigorous as the normal plants in both the varieties of sunflower.

Xantha purple:

The leaf tips, petioles, capitulum's involucre bracts, calyx and stem parts were shining with purple pigments giving the appearance of xantha purple character in both the varieties of sunflower (Fig.1 B).

Albino:

The seedlings of albino had very small completely white first pair of leaves which died within 7-10 days.

Other micro-mutations such as darker green and white margins were observed in both the varieties with all three treatments. Some plants exhibited broad darker green leaves in both the varieties and were not affected by pests and fungal diseases throughout the growth period. Leaves exhibited lanceolate and obovate shapes unlike to the control leaves which exhibited cordate shape. (Fig1 C and D). In this present investigation, the obtained micro-mutations in response to gamma-rays were corroborated with the previous reports of Arvind *et al.*, (2007) in blackgram, Mishra *et al.*, (2007) in okra, Cheema and Atta (2003) in rice and Lyakh *et al.*, (2005) in sunflower. Rybinski (2003) in grass pea with sodium azide. Whereas in combined treatment same was reported by Kaul and Bhan (1977) in rice. The frequency of micro-mutations was much higher with sodium azide than with gamma-rays by Ando and Motalvan (2001) in rice as it was corroborated with the present results. (Table1). Swaminathan (1964) and Goud (1967) demonstrated the chlorophyll mutations in pea seedlings; chlorophyll development seems to be controlled by many genes on several chromosomes which could be adjacent to centromere and proximal segment of chromosome. The chlorophyll deficient mutants were back crossed with control plants, majority of chlorophyll deficient types segregated in a simple Mendelian 3:1 ratio.

Macro-mutations: (Table 2)

Branched:

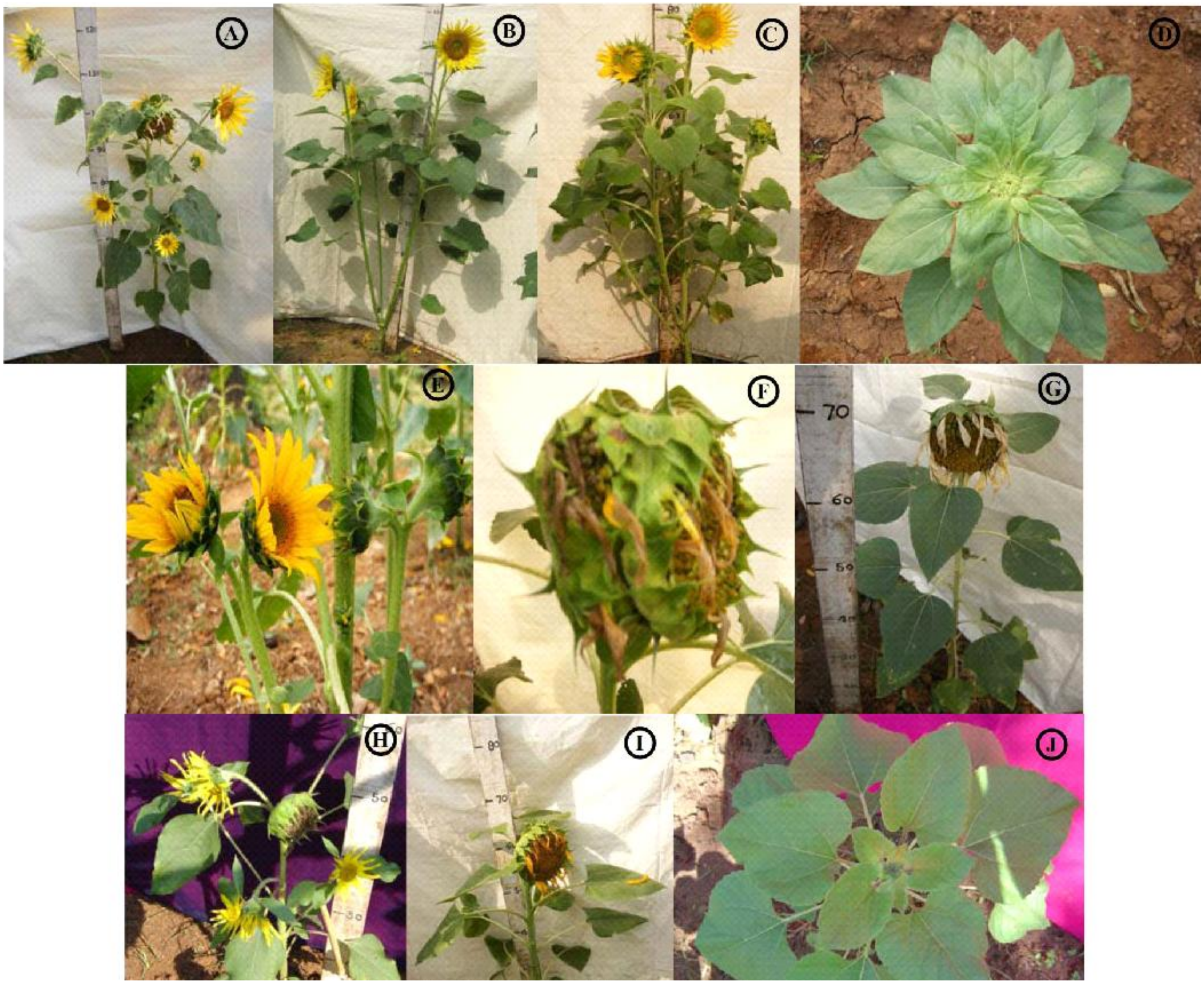
Generally sunflower plant is monocauly producing main capitulum, after production of main capilutum axillary buds will be initiated evidently produces axillary flowers whereas in this present investigation some of the plants exhibited branches before capitulum production in both the varieties, which gave the appearance of branched. (Fig. 2 A). The increase in the degree of branching was considered to increase the yield (Gottschalk and Wolff, 1983). Biswas and Datta (1988) in *Trigonella* with gamma-rays. Singh *et al.* (2006) in *Vigna unguiculata* L. with sodium azide treatment.

Basal stem bifurcation:

These mutants were observed with gamm-rays than with sodium azide and combined. Each bifurcated stem produced capitulum of normal, both of equal size and with 88% seed setting. (Fig. 2 B and C). Thirty nine plants exhibited the basal stem bifurcation out of them 21plants with gamma-rays, 7 plants with sodium azide and 11 plants with combined treatment as it was also corroborated with the results of Gunckel and Sparrow (1961) in sunflower. They further attributed the bifurcation of stem was due to actual forking of shoot growth and death of cells or group of cells in short apical meristem caused by radiation induced chromosomal aberrations.

Rosette and compact leaf arrangement:

The intermodal length was greatly reduced and gave the appearance of a compact and condensed stem with rosette leaf arrangement. Nine plants showed larger leaves with condensed petioles than the control and also the plants exhibited more vigorous growth and the leaves of these plants were devoid of fungal diseases. (Fig. 2 D). A very few rosette and compact leafy mutants were



(A) Branched (USH430-6kR) (B) Stem bifurcation (USH430-6kR) (C) Stem bifurcation (Nidhi999-6kR)
(D) Rosette and compact leaf (USH430-4kR+4mM) (E) Double headed (Nidhi999-4kR+4mM)
(F) Double headed (USH430-6kR) (G) Dwarf early I-50 cm (Nidhi999-8kR) (H) Dwarf early II-70 cm (USHA-4mM)
(I) Dwarf early II-60 cm (Nidhi999-6kR) (J) Mosaic leaf (USH430-4kR)

Fig. 2 : Micro-mutations in sunflower varieties of USH-430 and Nidhi-999

observed in all three treatments. The shortened petioles of leaves gave the compact appearance which was controlled by polygenic as against the vertical petiole controlled by monogenic and recessive character (Bockovai, 1980) in sunflower.

Double headed:

Sixteen plants exhibited double heads in two varieties of sunflower with three treatments at various dose concentrations.(Fig2 E and F). The double headed nature was heritable in sunflower by Ahmed and Goud (1978) in barley. The doubling tendency was due to the physiological disorder (Dubinin, 1964) as it was corroborated with the present results (Table 2).

Dwarf early I and early II:

Dwarf early maturing mutants I plants measured 35 cm to 45cm. They had eleven to fourteen leaves only. The internodes were condensed to give the dwarf appearance. These plants exhibited blooming at about 30

Table 2 : Macro-morphological mutants of sunflower varieties in response to gamma-irradiation, sodium azide and combined treatment in M_2 generation

Name of the variety	Treatment with Gamma rays Sodium azide and combined	Branched	Bifurcation	Rosette and compact leaf arrangement	Double headed	Dwarf early-I	Dwarf early-II	Mosaic leaf arrangement	Total no. of mutant plants	Sub-total
USH-	Control	-	-	-	-	-	-	-	-	
430	2kR	-	2	--	-	2	3	-	7	
	4kR	-	2	1	-	3	6	-	12	95
	6kR	5	5	2	2	6	8	1	29	
	8kR	2	1	1	-	5	11	2	22	
	10kR	3	1	-	1	10	10	-	25	
	2mM	1	1	-	-	2	2	-	6	
	4mM	4	2	1	2	5	7	-	21	
	6mM	2	-	-	-	5	6	-	15	65
	8mM	-	-	-	-	9	9	-	22	
	10mM	-	-	-	-	2	5	-	7	
	2kR+2mM	2	1	-	-	4	4	-	11	
	4kR+4mM	5	2	1	2	4	6	-	20	
	6kR+6mM	1	1	-	2	5	6	-	15	70
	8kR+8mM	-	-	-	1	8	6	-	15	
	10kR+10mM	-	-	-	-	4	5	-	9	
Nidhi-	Control	-	-	-	-	-	-	-	-	
999	2kR	-	1	-	-	3	2	-	6	
	4kR	2	2	-	2	4	5	-	15	85
	6kR	5	4	1	-	5	8	2	25	
	8kR	3	1	-	1	6	6	1	18	
	10kR	2	2	-	-	8	9	-	21	
	2mM	-	1	-	-	3	2	-	6	
	4mM	3	2	1	1	4	3	-	14	
	6mM	2	1	-	-	5	8	-	16	57
	8mM	-	-	-	-	6	8	-	14	
	10mM	-	-	-	-	2	5	-	7	
	2kR+2mM	2	2	-	-	3	4	-	11	
	4kR+4mM	5	2	1	1	4	4	-	17	
	6kR+6mM	1	3	-	1	6	8	-	19	65
	8kR+8mM	-	-	-	-	5	6	-	11	
	10kR+10mM	-	-	-	-	3	4	-	7	

days followed by seed setting (85%) and the whole seed set process was completed within 60 days. Dwarf early II plants measured 47cms to 67cms with seven to twenty five leaves. These plants reached blooming within 35 days. Seed setting was completed within 65 days. (Fig. 2 G, H and I). The dwarf mutants were recorded as follows: 120 with gamma-rays, 98 with sodium azide and 99 with combined treatment in both the varieties with various dose concentrations. The dwarf early I and II were reported by Sanjeev (2007) in rice. An extreme and severe dwarf mutant have been isolated and studied in sunflower by Jambhulkar (2002) and Jagadeesan *et al.*(2008). Gunckel (1957) reviewed the morphological effects of ionizing radiation and opined that the possible influence of phytohormones and other physiological disturbances caused the stunted growth of the plants. Halvey and Shoub (1965) opined that the irradiation retarded the growth in Wedge weed *Iris* plants may be caused by inhibition in auxin synthesis. According to Weber and Gottschalk (1973) dwarfness was due to the shortening of internodes may be resulted in decrease of cell number, cell length or both. From the dwarf mutant plants, the mean height ranges between 30±1.05 to 70±0.85.

Mosaic leaf arrangement:

These were keenly observed in 6kR and 8kR of gamma-rays in both the varieties. (Fig2 J). The leaves are larger and leave some gaps among them which the younger smaller leaves occupy. All the leaves come to the same level and present uninterrupted green when looked at from above. As the dose concentration increases the macro-mutations were increased in both the varieties of sunflower. 170 macro-mutations were noticed with gamma-rays, 122 with sodium azide and 135 with combined treatment in both the varieties of sunflower at different dose concentrations.

These phenotypic changes are presumably brought about by genotypic and mutational events among different loci of chromosomes providing the fundamental variability required for plant improvement through mutation breeding. In these mutagenised populations of two varieties dwarf early I (30 days) and dwarf early II (35 days) were registered exhibited 85 and 88 per cent seed set which was desirable character and it was intended to make crosses with compact leaf arrangement disease resistant plants anticipating in developing vigorous early varieties.

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