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EXPLOITATION OF VARIABILITY THROUGH MUTAGENESIS IN CHRYSANTHEMUM (*CHRYSANTHEMUM MORIFOLIUM* RAMAT.) VAR. MAGHI

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ABSTRACT
Rooted cuttings of chrysanthemum variety 'Maghi' were treated with three concentrations each of EMS and DES (0.02, 0.03 and 0.04 %) and five doses of gamma rays (0.5, 1.0, 1.5, 2.0 and 2.5 kR) to exploit variability and evaluated for heritable effects on various parameters *viz*. survival rate, morphological and flowering. Reduction in survival rate and various morphological parameters was recorded with chemical mutagens and higher doses of gamma rays. All the mutagenic treatments delayed flowering up to 6 to 7 days whereas flowering duration was significantly reduced. Flower head diameter (3.44 and 3.57 cm), number of ray (130.27 and 132.60) and disc florets (27.93 and 26.33) were significantly increased with 0.5 and 1.0 kR gamma rays, respectively. Flower weight, number of flowers and flower yield per plant were highest at the lowest dose of γ-rays. Total abnormalities in vegetative and floral characters were higher in γ-rays as compared to EMS and DES. Variety 'Maghi' exhibited two foliage mutants with 0.03 per cent EMS and 1.0 kR gamma rays, and these mutants lost their flowering ability.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) one of the important cut flower and pot plant of world commonly known as 'Autumn Queen', is member of the Asteraceae family. It is native to Northern hemisphere, chiefly Europe and Asia (Anderson, 1987). It is one of the important cut flower in the international market and ranks 3rd in the global cut flower trade after rose and carnation (Datta and Gupta, 2012). Today, chrysanthemum has earned tremendous popularity due to its wide range of brilliant colour, shapes, size, long lasting flower life and diversity in height and growth. Conventional breeding in chrysanthemum has its own limitations including restricted gene pool, longer ray florets that prevent timely pollination, self-incompatibility, parental ploidy differences, etc.

For a modern and industrialized floriculture, there is always a demand and necessity for new varieties. Mutation breeding is an established method for crop improvement, and has played a key role in the development of many new colour/shape mutants in ornamental plants (Broertjes and Van Harten 1988). Physical mutagens such as X-rays, gamma-rays and neutron and chemical mutagens such as ethyl-methane-sulphonate and sodium azides have been applied to expand genetic resources in plants. A number of gamma-ray-induced floret mutants and other morphological mutants of chrysanthemum have been developed and commercialized (Datta *et al*. 2005).

The heterozygous nature of florist's chrysanthemum offers high mutation frequency. Mutation techniques by using ionizing radiations and chemical mutagens altered one or few dominant characters like flower colour, shape and size of an outstanding cultivar without changing the other characters. A number of gamma rays induced mutants and other morphological mutants of chrysanthemum have been commercialized (Broertjes 1966). In this experiment, an attempt was made to exploit variability for flower colour and form in yellow coloured chrysanthemum variety 'Maghi' through chemical (EMS and DES) and physical (γ-rays) mutagens and to study the mutagenic effect on morphological and flowering parameters.

MATERIALS AND METHODS

The present investigation was conducted at Floriculture Research Farm, Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari in the year 2013-2014.

Terminal cuttings of 6 to 7 cm long were treated with 0.1 per cent Carbendazim solution for 15 minutes and then planted in the sheltered beds in pure sand after quick dipping the basal ends in 500 ppm IBA for better rooting. Cuttings were ready in 30 days for treatment application. The uniform size of rooted cuttings of variety 'Maghi' were treated with three concentrations each of EMS and DES (0.02, 0.03 and 0.04 %) by immersing in the chemical solutions for 4 hours. After the treatment, these cuttings were immersed in STS solution (0.3 %) for 15 minutes to remove the EMS and DES solution sticking to leaves and other parts. Thereafter, these cuttings were washed in running tap water for 20 minutes. Whereas rooted cuttings were irradiated with five doses of gamma rays (0.5, 1.0, 1.5, 2.0 and 2.5 kR) in Gamma Cell-200 (Cobalt – 60 source emitting 3600 rads per minutes) at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai during October, 2013. The cuttings were planted in field with untreated rooted cuttings using control versus rest Randomized Block Design. All the standard cultural practices were followed, except pinching and disbudding operations. Heritable effects of various mutagens and their doses on vegetative growth, flowering and quality related aspects; vegetative and floral abnormalities were investigated and statistically analysed. Spectrum of mutation was also observed with critically visualization of each plant with any change in plant morphology, flower colour, flowering duration and chimera formation. The data were analysed with technical help received from computer centre of Soil and Water Management Research Unit, N.A.U., Navsari. The data was analysed as advocated by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Mutagenic effect on morphological parameters

It is apparent from the data presented in Table 1, that all the morphological characteristics relating to plant survival and growth were significantly affected by various mutagenic treatments. It is explicit from the data that decreasing trend in vegetative growth was observed with increasing levels of mutagenic treatments with respect to survival per cent of plant. Maximum survival (97.50 %) was recorded in control while maximum survival (94.17 %) was recorded with 1.0 kR followed by 0.5 kR (85.00 %) γ-rays in treated population. Significant reduction in survival after exposure to gamma rays was also observed by Kiran Kumari *et al.* (2013) reported similar results while treating rooted cuttings of chrysanthemum variety 'Otome Pink' were treated with 0, 10, 15 and 20 Gy of gamma rays. Minimum survival (28.33 %) was noted with 0.04 per cent EMS. Further, it also indicated that chemical mutagens resulted in higher reduction in per cent plant survival as compared to the gamma rays. Reduction in survival after exposure to gamma rays was explained due to inactivation and/or decrease in auxin content that affect cell division, it resulting in poor establishment and survival (Gordon 1957 and Mahure *et al.* 2010) or lethal effect of gamma rays caused due to chromosomal aberration (Datta and Banerji 1993). Dilta *et al.* (2003) reported that higher concentrations of EMS reduced the plant survival per cent in chrysanthemum. Misra and Bajpai (1983a) also observed up to 50 per cent reduction in survival percentage of plants in all the gladiolus cultivars

Table 1: Effect of different mutagens on morphological characters of chrysanthemum variety 'Maghi'.

Treatments	Survival percentage	Plant height (cm)	Plant spread (E-W) (cm)	Plant spread (N-S) (cm)	No. of branches per plant	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Leaf area (cm^2)	Vegetative abnormalities(%)
EMS @ 0.02 %	51.67 (45.96)	39.57 ± 2.21	21.07 ± 1.36	21.35 ± 1.40	23.30 ± 1.07	2.82 ± 0.43	2.28 ± 0.03	0.63 ± 0.04	4.87 ± 0.24	9.17 (17.59)
EMS @ 0.03 %	33.33 (35.11)	40.57 ± 0.38	20.47 ± 0.61	20.15 ± 0.58	19.60 ± 1.70	2.43 ± 0.14	2.12 ± 0.11	0.57 ± 0.04	4.86 ± 0.29	11.67 (19.80)
EMS @ 0.04 %	28.33 (32.13)	39.33 ± 1.45	18.53 ± 0.43	18.24 ± 0.93	17.40 ± 1.10	2.32 ± 0.11	1.89 ± 0.05	0.54 ± 0.03	4.51 ± 0.24	21.67 (27.64)
DES @ 0.02 %	42.50 (40.65)	39.80 ± 0.20	22.84 ± 0.60	22.05 ± 0.75	23.33 ± 1.02	2.45 ± 0.31	2.50 ± 0.26	0.62 ± 0.04	5.28 ± 0.66	10.00 (18.34)
DES @ 0.03 %	34.17 (35.75)	37.70 ± 1.68	18.96 ± 0.84	18.87 ± 0.61	21.73 ± 1.47	2.10 ± 0.09	2.45 ± 0.20	0.56 ± 0.08	5.12 ± 0.27	16.67 (24.00)
DES @ 0.04 %	30.00 (33.13)	38.23 ± 3.25	14.55 ± 1.69	14.38 ± 1.39	17.47 ± 1.16	0.97 ± 0.04	1.86 ± 0.17	0.47 ± 0.03	4.40 ± 0.16	22.50 (28.22)
Gamma rays - 0.5 kR	85.00 (68.01)	45.30 ± 2.80	26.81 ± 0.39	26.31 ± 0.48	26.90 ± 0.81	3.10 ± 0.37	3.08 ± 0.30	0.57 ± 0.02	6.52 ± 0.47	36.67 (37.24)
Gamma rays - 1.0 kR	94.17 (76.09)	49.30 ± 0.61	27.05 ± 1.35	26.57 ± 1.36	30.70 ± 0.94	2.52 ± 0.28	2.40 ± 0.17	0.53 ± 0.02	5.49 ± 0.26	38.33 (38.22)
Gamma rays - 1.5 kR	90.00 (72.58)	41.01 ± 1.74	21.83 ± 0.78	21.87 ± 0.80	26.10 ± 0.98	2.25 ± 0.19	2.20 ± 0.21	0.47 ± 0.03	4.85 ± 0.10	33.33 (35.26)
Gamma rays - 2.0 kR	57.50 (49.42)	31.75 ± 1.54	19.57 ± 0.38	19.33 ± 0.55	22.23 ± 0.64	1.48 ± 0.04	1.67 ± 0.08	0.41 ± 0.01	4.45 ± 0.22	30.00 (33.17)
Gamma rays - 2.5 kR	43.33 (41.16)	27.59 ± 3.19	16.36 ± 1.37	16.22 ± 2.00	19.00 ± 0.91	1.58 ± 0.19	1.93 ± 0.23	0.38 ± 0.04	3.85 ± 0.28	21.67 (27.64)
Control	97.50 (82.66)	43.38 ± 1.27	21.99 ± 0.17	21.63 ± 0.36	25.50 ± 0.68	2.91 ± 0.05	2.82 ± 0.01	0.59 ± 0.01	5.76 ± 0.10	0.83 (3.03)

Table 2: Effect of different mutagens on floral characters of chrysanthemum variety 'Maghi'.

Treatments	Days to flowering	Flowering duration (days)	Diameter of flower head (E-W) (cm)	Diameter of flower head (N-S) (cm)	Number of disc florets per head	Number of ray florets per head	Number of flowers per plant	Weight of flower (g)	Peduncle length (cm)	Floral abnormalities (%)
EMS @ 0.02 %	77.27± 1.18	26.57± 0.88	3.17± 0.07	3.15± 0.04	23.10± 0.68	123.63± 3.21	57.73± 1.98	3.03± 0.06	12.12± 0.34	4.17 (11.65)
EMS @ 0.03 %	76.87± 0.76	27.30± 0.58	2.86± 0.12	2.80± 0.10	20.80± 0.83	123.27± 1.98	53.67± 1.30	2.93± 0.10	11.48± 0.53	9.17 (17.35)
EMS @ 0.04 %	76.20± 2.52	24.93± 0.43	2.44± 0.07	2.49± 0.06	15.10± 1.19	113.10± 2.28	53.43± 3.03	2.84± 0.12	9.40± 0.63	15.00 (22.74)
DES @ 0.02 %	74.43± 0.52	28.37± 0.29	2.83± 0.08	2.85± 0.12	23.30± 1.06	125.47± 2.44	59.63± 2.05	3.17± 0.02	12.45± 0.55	9.17 (17.35)
DES @ 0.03 %	77.13± 0.76	27.53± 0.44	2.71± 0.19	2.73± 0.11	16.60± 1.08	112.37± 2.97	54.77± 1.83	3.01± 0.06	10.81± 1.39	12.50 (20.49)
DES @ 0.04 %	79.27± 0.55	26.23± 0.47	2.54± 0.04	2.55± 0.03	12.27± 0.58	102.33± 1.64	51.03± 1.87	2.73± 0.08	9.77± 0.60	16.67 (23.59)
Gamma rays - 0.5 kR	74.20± 0.61	27.93± 1.40	3.44± 0.11	3.43± 0.12	27.93± 0.82	130.27± 2.03	71.50± 1.33	3.22± 0.04	13.56± 0.64	27.50 (31.61)
Gamma rays - 1.0 kR	74.07± 0.42	28.90± 0.26	3.57± 0.11	3.50± 0.09	26.33± 0.64	132.60± 1.89	80.13± 1.56	3.23± 0.07	15.01± 0.89	20.00 (26.54)
Gamma rays - 1.5 kR	76.13± 1.66	27.97± 0.68	3.03± 0.12	3.01± 0.14	23.23± 0.61	121.47± 1.40	73.80± 0.95	3.17± 0.06	13.37± 0.56	23.33 (28.75)
Gamma rays - 2.0 kR	74.23± 0.90	27.77± 0.32	3.00± 0.08	3.03± 0.11	16.37± 1.48	108.60± 2.59	71.63± 1.24	3.09± 0.09	10.39± 0.44	25.00 (29.98)
Gamma rays - 2.5 kR	75.33± 0.78	25.93± 0.27	2.52± 0.12	2.50± 0.11	11.17± 0.79	95.50± 1.01	70.63± 1.72	2.91± 0.07	7.86± 0.42	20.00 (26.49)
Control	71.83± 0.35	30.13± 0.14	3.21± 0.08	3.19± 0.10	23.47± 0.61	126.10± 1.29	72.97± 0.62	3.19± 0.03	11.86± 0.33	0.00 (0.00)

over control when treated with different chemical mutagens *viz.,* EMS, DES and MNH. The drastic reduction in plant survival may be due to the formation of certain toxic substances by some biochemical substances which cause death of the cells ultimately resulting in the death of plants (Sax, 1955; D'Amato and Ostenhof 1956; Gordon, 1956).

Lower dose of gamma rays significantly influenced plant height, plant spread (E-W and N-S) and number of branches per plant whereas reduction was observed with higher doses. Reduction in vegetative characters by gamma rays treated plants depends on the nature and extend of chromosomal damage or due to physiological, morphological and cytological disturbance caused by irradiation (Banerji and Datta, 2002). While EMS and DES treated plants showed drastic reduction as compared to gamma radiation. Mishra and Bajpai (1983b) recorded similar results with chemical mutagens in gladiolus and explained that chemical mutagens proved to be injurious as promotes physiological disturbance, retarded cell division by arresting the mitotic division and had ill-effect on auxin thereby it resulting reduction in morphological characters.

Different mutagenic agents decreased the leaf length and leaf width over the control, except 0.5 gamma rays. Priya Misra *et al.* (2009) observed longer and broader leaves as compared to control with 0.5 and 1.0 Gy whereas Kiran Kumari *et al.* (2013) reported reduction in leaf size in terms of length and width of plants treated with higher doses of gamma rays in variety 'Otome Pink'. Petiole length was found shorter with increasing dose of mutagenic agents. All mutagenic agents decreased the leaf area over the control, except 0.5 gamma rays. Reduction in leaf area was observed with increasing dose of mutagens. Earlier Mahure *et al.* (2010) recorded that lower doses like 10 and 20 Gy increased leaf area but 30 Gy decreased leaf area over control.

Vegetative abnormalities included changes in plant morphology and branching habit, leaf shape, size margin, apex fission and fusion (Plate 1). Vegetative abnormalities were increased significantly over the control due to effect of different mutagenic treatments in chrysanthemum variety 'Maghi'. The frequency of abnormalities was increased with increase in doses of mutagens. It may possibly due to the inactivation and/or disturbances in the auxin synthesis (Gordon, 1957) and extent of chromosomal aberrations (Sparrow and Evan, 1961). Similar evidences have been documented by Priya Misra *et al.* (2009) in chrysanthemum.

Mutagenic effect on flowering and yield parameters

Physical and chemical mutagens significantly increased the number of days to flowering over the control in 'Maghi' (Table 2). Duration of flowering was found significantly higher in untreated plants as compared to chemical and physical mutagens. Generally, increasing doses of mutagens (chemical and physical) decreased flowering duration, but fluctuation was seen with 0.03 per cent EMS and γ-rays (0.5 and 1.0 kR). Ahirwar *et al.* (2014) reported that flowering was significantly delayed at 30 kR of gamma rays with 0.3 % of EMS mutagens in both the generations as compared to control in *Microsperma lentil* var. HUL-57. Days to flowering and its duration may be affected as a result of irradiation or mutagenic treatments because many biosynthetic pathways are believed to be altered, which are directly as well as indirectly associated with

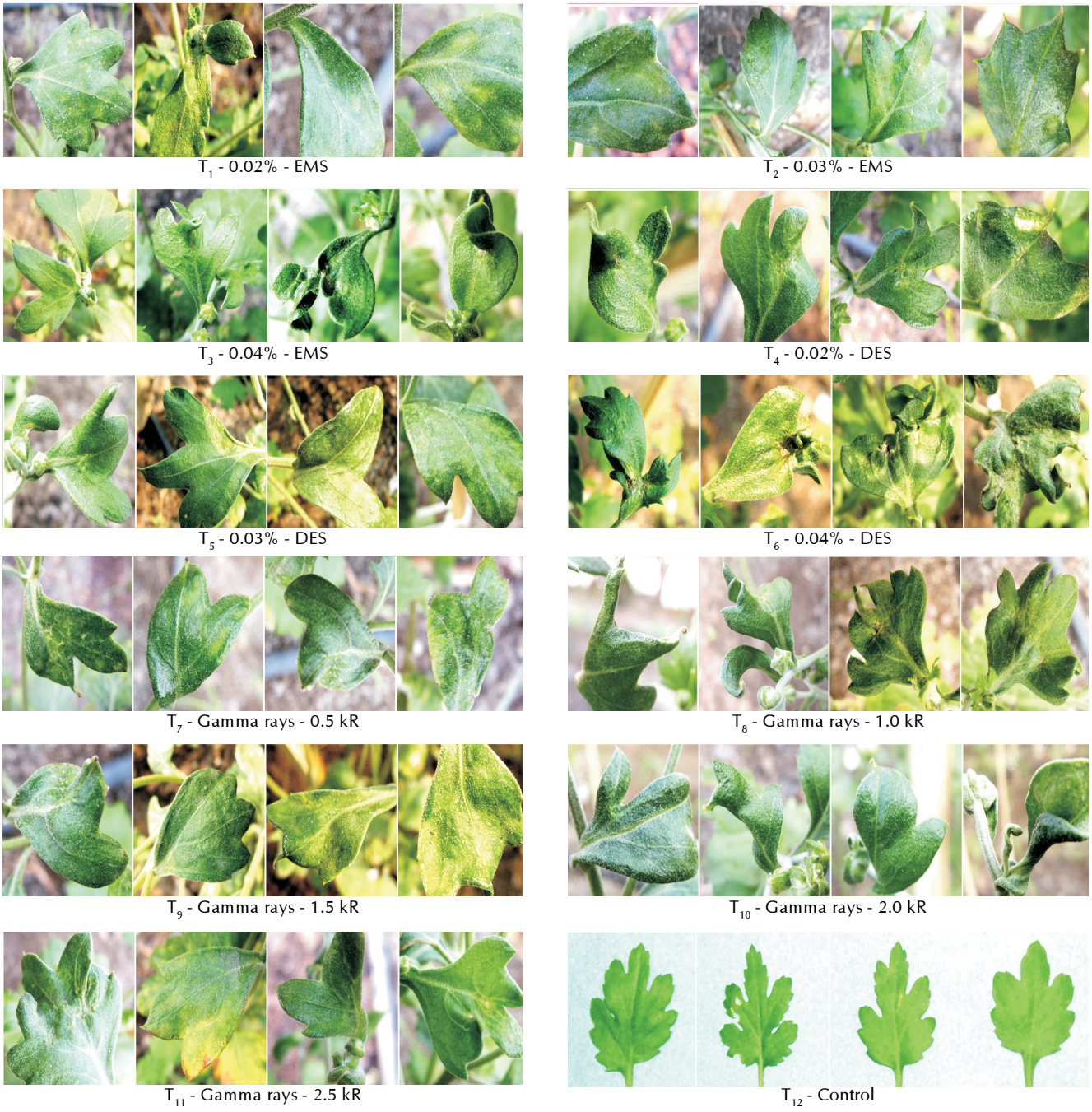

Figure 1: Vegetative abnormalities observed with different mutagens in chrysanthemum variety 'Maghi'.

the flowering physiology (Mahure *et al.*, 2010). Significant reduction in flower head diameter, number of disc and ray florets was found with increasing the levels of physical and chemical mutagens but increase in these parameters was noted with 0.5 and 1.0 kR gamma rays over control. Earlier Mahure *et al.* (2010) recorded similar observations in cultivar 'Red Gold'. Whereas, flower size was compared between gamma irradiated and chemically treated plants, smaller size flowers were found with EMS and DES treatments. Gaul (1970) also documented that physiological effects of mutagens are very different in nature and it has probably both chromosomal and extra chromosomal origin.

A significant reduction in the number of flower head was observed over the control after mutagenic treatments, except 1.0 and 1.5 kR γ-rays. This result is in conformity with Shukla and Datta (1993) and Banerji and Datta (2005). Maximum peduncle length was noted with 1.0 kR dose of γ-rays over the control which was followed by 0.5, 1.5 kR gamma rays and 0.02 per cent DES and EMS treated population. Similar results were observed by Zargar *et al.* (1998) in chrysanthemum cv. 'Satish Modi'.

Original plant | Foliage plants (0.03% EMS) | Foliage plants (1.0 kR gamma rays)

Figure 2: Radiation and chemically induced mutants in chrysanthemum var. 'Maghi'.

'Maghi' variety only exhibited two foliage mutants with 0.03 per cent EMS and 1.0 kR gamma rays (Plate 2). During entire season, these mutants did not produce any flower. Kaplan (1963) documented that small structural modification of DNA molecule as caused by the substitution of 5-bromoureacil for thymin have been shown to result in higher cell radio sensitivity and loss of reproductive ability. Yellow flower colour 'Maghi' variety did not produce any chimeras because yellow colour usually consist of pure carotenoid or xanthophyll compounds in the L_1 apical layer, but not in the L_2 (Langton, 1980) and they are homozygous recessive for both the water soluble (anthocyanin) and plastid pigment (carotenoid) genes which means that they will never sport to a colour other than yellow (Teynor *et al.*, 1989).

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