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Effect of chemical mutagens on growth and flowering of chrysanthemum varieties in VM_1 generation

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Abstract
A field experiment was carried out at Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth Parbhani. (Maharashtra) during *kharif* season of the year 2017-2018. The experiment was laid out in Factorial Randomised Block Design with thirty treatment combinations. The treatments comprised of two factors i.e. factor A and factor B. Factor A consist of 3 varieties (V_1-Raja Pandharpuri,V_2- Brown and V_3-Shinaton) and factor B consist of 10 levels of chemical mutagen (colchicine T_1 -0.01%, T_2 -0.02%, T_3 -0.03%, T_4 - Ethylmethanesulphonate (EMS) 0.01%, T_5 -0.05%, T_6 -0.1%, T_7 -0.5%, T_8 -1.0%, T_9 -1.5%,T_{10} – Control) in Chrysanthemum. The different treatments to rooted cuttings of chrysanthemum varieties with colchcine and EMS had significantly influenced the vegetative and flowering characters and also create the variability. Significant reduction occurs in both colchicine and EMS treated seedlings of chrysanthemum varieties in terms of plant height, branches plant^{-1}, plant spread, Diameter of main stem and Number of leaves plant^{-1} Whereas leaf area and Petiole length was increased due to the colchicines and decreased due to EMS treated population of chrysanthemum varieties over control. The higher dose of colchicine and EMS delayed the flower bud initiation, flower opening and 50% flowering.

Keywords: Chrysanthemum, varieties, chemical mutagen, growth, flowering

Introduction
Chrysanthemum is one of the most beautiful and perhaps the oldest flowering plant, commercially grown in different parts of the world. It is commonly known as the “Queen of the East” and “Autumn Queen” and is the symbol of royalty in Japan. Chrysanthemums, are one of the prettiest varieties of perennials that start blooming early in the fall. This is also known as favorite flower for the month of November

Now a days the test of people for flower in terms of colour, different shape, size, novelty of flower structure have change and improved, so in accordance to full the wish and demand of people the newly improved variety is required continuously. Mutation breeding plays an important role in plant breeding and helps in the creation of genetic variation to create novelty. However, various workers emphasizes that artificial induction of mutation by colchicine (Col), ethyl methane sulphonate (EMS) and sodium azide (SA) provides tool to overcome the limitations of variability in plants that induces specific improvement without disturbing their better attributes. Colchicine is a chromosome doubling agent that possesses antimicrotubular action. EMS is a common alkylating agent, whereas sodium azide is responsible for creating point mutation in DNA level. However, these chemicals have also proved their worth as mutagens to induce genetic variability. Thus, they become important tool to enhance agronomic traits of crop plants (Mostafa, 2011) [13]. The mutagenic effectiveness was maximum in EMS. The highest mutagenic efficiency was recorded in Col. The effectiveness of the three chemicals on *Dianthus* is ranked as EMS>Col>SA (Roychowdhury and Tah 2011) [18]. Colchicine (0.0625 per cent) has been successfully used for development of flower colour mutation in Chrysanthemum Cv. Sharad Bahar. The original colour of Sharad Bahar was purple whereas mutant colour was Terracotta Red. The mutant has been released in the name of ‘ColchiBahar’ (Datta, 1987) [3]. The use of colchicine as a means of chromosome doubling has opened a large reservoir of possibilities in plant breeding work. The fact that numerical changes in chromosome number fundamentally entail a mutation which may be expressed in a

number of characters of the plant indicates the significance of the above statement (Derman, 1990) [4]. Therefore keeping in view these facts, the present investigation was undertaken with following objectives.

Material and Method

Chrysanthemum seedlings raised by using shoot tip cuttings of 6 to 8 cm have been collected. Shoot tip cuttings of Chrysanthemum *cv.* Raja Pandharpuri were collected from the Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (Dist. Parbhani), Maharashtra State and other two verities *cv.* Brown and Shinaton were collected from College of Agriculture, Nagpur. Dr. PDKV, Akola Cuttings were first treated with 0.2% Bavistin for 5 min. and then planted in pot filled with coco peat and sand. All inter cultural operations like weeding, watering, application of Humic acid and plant protection measures were carried out as when required.

An experimental land was ploughed one to two times followed by harrowing were given to bring the soil to the fine tilth. The soil then after was loosen and ridge and furrow were prepared at 45 cm apart, recommended dose of farm yard manure and chemical fertilizers for chrysanthemum is 15 tones ha^{-1} FYM and 300:200:200 NPK kg ha^{-1}. The one third dose of nitrogen and full dose of phosphorous and potassium will be applied at the time of transplanting. The remaining dose of nitrogen (N) will be applied one month after transplanting.

Uniform and healthy rooted cuttings were selected for transplanting. The rooted cuttings were treated with different concentrations of colchicine and Ethyl methane sulphonate (EMS) by immersed in colchicine and Ethyl methane sulphonate (EMS) solution for 4 and 2 hours respectively. In control the rooted cuttings were immersed in distilled water for 2 hours. After the treatments, these cuttings were immersed in STS (sodium thio-sulphate) solution (0.3%) for 15 minutes to remove stresses of solution on plant parts. Then, these cuttings were washed in running tap water for few minutes. The field should be irrigated one day prior to transplanting. The treated cuttings were transplanted on main field. These cuttings were planted at 45 X 30 cm distance on experimental field in Factorial Randomized Block Design (FRBD) with three replications. All the standard cultural practices were followed, except the pinching and disbudding operations

Results and Discussion

Growth parameters

Plant height

Significant reduction occurs in both colchicine and EMS treated seedlings of chrysanthemum varieties in terms of plant height, branches $plant^{-1}$, plant spread, Diameter of main stem and Number of leaves $plant^{-1}$ Whereas leaf area and Petiole length was increased due to the colchicines and decreased due to EMS treated population of chrysanthemum varieties over control. Among the chemical mutagen maximum plant height (57.14 cm) was recorded at 0.01% colchicine which was at par with colchicine 0.02% (53.32 cm), EMS 0.01% (55.18 cm), 05% (52.68 cm) and minimum (43.99 cm) in EMS 1.5% (T_9) over control (60.40 cm). However, among the varieties maximum plant height (54.13 cm) was recorded in variety Shinaton (V_3) and minimum (47.27 cm) in Brown (V_2) in VM_1 generation. The decrease in plant height after exposure of the rooted cuttings to the colchicine and EMS has been due to disturbances of auxin synthesis and chromosomal aberrations. It was agreed with the results of Gupta and Gill (1985) [5] in *Chrysanthemum coronarium* when treated with colchicine and Vaidya *et al.* (2016) [9] and Kapadiya *et al.* (2014) [8] also have similar opinion when treated with EMS in chrysanthemum. There was significant decreased in number of branches $plant^{-1}$ over control T_{10} (19.63) in chemical mutagen maximum branches $plant^{-1}$ **(**17.61) was recorded at 0.01% EMS (T_4) which was at par with T_1 (17.11), T_5 (16.16) and minimum (11.46) branches $plant^{-1}$ were observed in 0.03% colchicine T_3. Among the varieties maximum branches $plant^{-1}$ was recorded in variety Shinaton V_3 (18.31) and minimum in Raja Pandharpuri V_1 (11.31). Increase in concentration of colchicine and EMS prove to be injurious by promoting physiological disturbances and retarded cell division by arresting the mitotic division and ill effects thereby reduction of branches per plant. These results are in conformity with the findings of Kazi (2013) [10] and Rafiq *et al.* (2017) [16] who observed that there is decreased in number of branches $plant^{-1}$ with increase concentration of EMS and colchicine.

Plant spread was decreased due to the colchicines and EMS treated population of chrysanthemum varieties over control T_{10} (19.63). Among the chemical mutagen maximum plant spread (42.41cm) was recorded at 0.01% colchicine (T_1) which was at par with T_4 (42.36 cm), T_2 (40.26 cm), T_6 (38.93 cm), T_7 (38.20 cm), and minimum (36.02 cm) in EMS 1.5% (T_9). However, among varieties maximum plant spread was recorded in variety Shinaton (V_3) 44.84 cm and minimum (36.37 cm) in Raja Pandharpuri (V_1). mutant spread was reduced with increasing concentration of EMS and colchicine. The reduction in the plant spread with increase in concentration of colchicine and EMS prove to be injurious by promoting physiological disturbances and retarded cell division by arresting the mitotic division and ill effects. There is reduction in number of branches due to colchicine and EMS ultimately reduced the plant spread in chrysanthemum varieties. The results obtained in present investigation are in conformity with the findings of Verma *et al.* (2018) [12, 20] observed that plant spread have been reduced with the colchicine treatment in *Chrysanthemum carinatum.* Kaul (1975) [9] also have same opinion. Similarly in EMS plant spread reduced at treated plants in *Dendranthema grandiflora* Tzvelev, reported by Padmadevi and Janaharlal (2011) [14].

Among the chemical mutagen maximum diameter of main stem (0.69 cm) was recorded at 0.01% EMS (T_4) which was at par with T_5 (0.65 cm) and minimum (0.48 cm) in EMS 1.5% (T_9). Whereas, among the varieties maximum diameter of main stem (0.66 cm) was recorded in variety Shinaton V_3 and minimum (0.53 cm) in Raja Pandharpuri V_1 as against control (0.79 cm). The results obtained are in conformity with the findings of Verma *et al.* (2018) [12, 20], Kaul (1975) [9] when treated with colchicine. And in EMS treated plant population Hridhya and Remesh (2016) [7], Prabhukumar *et al.* (2015) [15] found similar result.

Number of leaves $plant^{-1}$ was decreased due to the colchicines and EMS treated population of chrysanthemum varieties over control T_{10} (282.76). Among the chemical mutagen maximum number of leaves $plant^{-1}$ (269.90) was recorded at 0.01% EMS (T_4) which was at par with T_1 (263.07), T_5 (260.81), T_2 (254.96), T_6 (252.75) and minimum (218.91) in EMS 1.5% (T_9). However, among varieties maximum number of leaves (275.33) $plant^{-1}$ was recorded in variety Brown (V_2) and minimum (231.18) in Raja Pandharpuri V_1. Leaf area of leaves has been increased (Table 1) due to the increased dosed of colchicine treatments but the number of leaves $plant^{-1}$ has

been reduced. This was might be due to phenotypic characters and cytological characters sometimes work vice versa. The results obtained in this study are in conformity with the findings of Rathod (2018) [17], Rafiq *et al.* (2017) [16], Amiri *et al.* (2010) [1] in colchicine. While in EMS Kapadiya *et al.* (2014) [8], Bhajantari and Patil (2013) also have same observations.

Among the chemical mutagen maximum leaf area (34.51 cm^2) and petiole length (3.22 cm) was recorded at 0.03% colchicine (T_3) and minimum (18.09 cm^2) (3.22 cm) in EMS 1.5% (T_9) respectively. Whereas, among varieties maximum leaf area (32.42 cm^2) and petiole length (2.80 cm) was recorded in variety Raja Pandharpuri V_1 and minimum (24.03 cm^2) (2.26 cm) in Brown (V_2) over control T_{10} (32.39 cm^2) (2.78 cm) respectively. Derived mutant plantlets have fewer large stomata and larger leaves and more petiole length as compare with main control diploid plants. Increased in leaf area with higher dose of colchicine this might was probably due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume. This increase in size may translate to an increase in plant and its organs. Kushwah *et al.* (2018) [12] He *et al.* (2016) [6] reported that plants developed larger, thicker leaves when treated with colchicine *Chrysanthemum carinatum* and *Dendranthema indicum* var. *aromaticum* respectively. The reduction in leaf area and petiole length with higher dose of EMS might be due to inactivation or decreased in auxin content or disturbances in auxin synthesis. The results obtained in the present study are in conformity with the findings of Vaidya *et al.* (2016) [19], Kapadiya *et al.* (2014) [8] stated that, reduction in leaf area in chrysanthemum when treated with EMS.

The interaction effect of chrysanthemum varieties (V) and chemical mutagens (T) on growth parameters was found to be non significant.

Table 1: Effect of chemical mutagens on growth and flowering parameter of chrysanthemum varieties in VM_1 generation

Treatments	Plant height (cm)	Number Branches $plant^{-1}$	Plant spread (cm)	Diameter of main stem (cm)	Leaf area (cm^2)	Petiole length (cm)	Number of leaves $plant^{-1}$	Days to first flower bud initiation	Days to fully opened flower from bud emergence	Days to 50% flowering
Factor A – Varieties (V)										
V_1 - Raja Pandharpuri	52.92	11.31	36.37	0.53	32.42	2.80	231.18	69.49	31.00	106.70
V_2 - Brown	47.27	14.86	38.62	0.61	24.03	2.26	275.35	86.60	28.91	121.97
V_3 - Shinaton	54.13	18.31	44.84	0.66	26.06	2.64	248.17	92.28	29.80	128.35
SE (m) ±	1.00	0.41	0.81	0.01	0.53	0.05	5.12	1.66	0.62	2.45
CD at 5%	2.83	0.82	2.31	0.03	1.51	0.14	14.52	4.71	N/S	6.95
Factor B – Chemicals (T)										
T_1 - Colchcine 0.01%	57.14	17.11	42.41	0.62	33.16	2.91	263.07	79.29	28.14	114.23
T_2 - Colchcine 0.02%	53.32	13.56	40.26	0.57	34.14	3.12	254.96	81.34	30.12	118.20
T_3 - Colchcine 0.03%	49.26	11.46	37.99	0.51	34.51	3.22	235.15	85.53	31.52	123.28
T_4 - EMS 0.01%	55.18	17.61	42.36	0.69	29.83	2.56	269.9	78.9	27.04	112.10
T_5 - EMS 0.05%	52.68	16.16	40.97	0.65	27.46	2.42	260.81	80.23	29.14	115.49
T_6 - EMS 0.1%	49.78	15.18	38.93	0.6	24.04	2.3	252.75	81.97	30.39	118.67
T_7 - EMS 0.5%	47.22	13.46	38.2	0.56	22.10	2.26	242.42	85.58	31.48	122.92
T_8 - EMS 1.0%	45.41	12.43	36.59	0.51	19.31	2.12	234.92	86.53	32.26	125.23
T_9 - EMS 1.5%	43.99	11.64	36.02	0.48	18.09	1.97	218.91	91.33	33.08	131.25
T_{10} - Control	60.40	19.63	45.70	0.79	32.39	2.78	282.76	77.15	25.86	108.72
SE (m) ±	1.82	0.74	1.49	0.02	0.97	0.09	9.34	3.03	1.13	4.47
CD at 5%	5.17	1.49	4.22	0.06	2.77	0.26	26.52	8.60	3.20	12.69
Interaction effect (VXT)										
SE (m) ±	3.16	1.29	2.58	0.04	1.69	0.16	16.18	5.24	1.96	7.74
CD at 5%	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S

Flowering parameters

The higher dose of colchicine and EMS delayed the flower bud initiation, flower opening and 50% flowering. Among the chemical mutagen minimum days to first flower bud initiation (78.90 days) and days to 50% flowering (112.10 days) was recorded at 0.01% EMS (T_4) and maximum days to first flower bud initiation (91.33) and days to 50% flowering (131.25 days) was in EMS 1.5% (T_9). Among the varieties minimum (69.49 days) days to first flower bud initiation and days to 50% flowering (106.70 days) was recorded in variety Raja Pandharpuri V_1 and maximum (92.28 days) (128.35 days) in Shinaton (V_3) over control T_{10} (77.15 days) (108.72 days) respectively. Days to fully opened flower from bud emergence was delayed due to the colchicines and EMS treated population of chrysanthemum varieties over control T_{10} (25.86 days). Among the chemical mutagen minimum days to fully opened flower from bud emergence (27.04 days) was recorded at 0.01% EMS (T_4) which was at par with T_1 (28.14 days), T_5 (29.14 days), T_2 (30.12 days), and maximum days to fully opened flower from bud emergence (33.08) was in EMS 1.5% (T_9). Shoot induction of chrysanthemum showed slow and low growth when treated with chemical mutagen. Kushwah *et al.* (2018) [12] reduction in the rate of various physiological processes of the plant after the chemical treatment. All the treatments of colchicine and EMS prove to be injurious by promoting physiological disturbances and retarded cell division by arresting the mitotic division and ill effects which had delayed the flower bud initiation, flower opening and 50% flowering in chrysanthemum plants. Kolar *et al.* (2015) proposed that, the treated populations showed delayed flowering in *Chrysanthemum carinatum.* Rafiq *et al.* (2017) [16] also found similar results. In chrysanthemum Kapadiya *et al.* (2014) [8] and Padmadevi and Janaharlal (2011) [14] reported maximum delayed in days to flowering when treated with EMS and minimum in control.

The interaction effect of chrysanthemum varieties (V) and chemical mutagens (T) on flowering parameters was found to be non significant.

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