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Chemical mutagenic action on seed germination and related agro-metrical traits in M_1 *Dianthus* generation

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Abstract

Chemical mutagenesis is an efficient tool used in mutation breeding programme for improving various vital characteristics in floricultural crop, like *Dianthus*. In this study, colchicine (Col), ethyl methane sulphonate (EMS) and sodium azide (SA) with three different concentrations like 0.1%, 0.4% and 0.7% were used to analyze their effect on seed germination behaviour, lethality, pollen sterility and other related agro-metrical traits. Mutagen treated pure-line seeds were sown in experimental field following randomized block design (RBD) layout to raise first mutant (M1) generation. It was noted that increase in the dose of EMS and SA, germination percentage and survivability were decreased; whereas colchicine doses were proportional to increase germination percentage at seedling stage, but they were not survived till maturity. Higher lethality over control (32.89) was shown by 0.7% EMS. Pollen sterility also increased with increasing mutagenic doses. The maximum pollen sterility (61.1%) was observed under 0.7% colchicine. So, the effect of chemical mutagenesis on seedling and pollen sterility with EMS (especially 0.7%) treatment is much more beneficial as compared to colchicine and SA. 0.4% colchicine is effective for other agronomical characters. Hence these mutagens can be used for improving the germination behaviour and the metrical traits in *Dianthus* cultivar.

Keywords: Agro-metrical traits, chemical mutagen, *Dianthus caryophyllus*, M_1 generation, pollen sterility, seed germination.

INTRODUCTION

Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base [1]. The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering one or two major agronomic metrical traits which limit their productivity or enhance their quality. *Dianthus caryophyllus* L., commonly known as Carnation, belongs to the angiospermic family Caryophyllaceae, is an important floricultural crop all over the world and ranks just next to Rose in popularity [2,3]. This genus is important by having pharmacological properties, aromatic things and polymorphism in morphology, genetics and hybridization [4-8]. In this modern era, an agronomic demand of high yielding cultivar of this crop was noticed. One way of creating variability in such a self-pollinated crop is attempting crosses between two genotypes complementing the characters of each other but, due to autogamous nature of this crop, hybridization at appropriate time is a difficult process. The only alternative left with breeders to create variability is mutation breeding. This method can be used as a potential source of creating variability [9]. Mutation can produce the development of *Dianthus* cultivars with more desirable floral characteristics and higher productivity [10,11]. It is a tool and being used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops [12]. Mutation induction offers significant increase in crop production [13] and the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evaluation. Treatment with mutagens alters genes or breaks chromosomes. Gene mutations occur naturally as errors in DNA replication. Most of these errors are repaired but some may pass to the next cell division to become established in the plant offspring as spontaneous mutations. Gene mutations without phenotypic expressions are usually not recognized. Consequently, genetic variation appears rather limited and breeders have to resort to mutation induction [12]. Mutagenic agents have been used to induce useful phenotypic variations in plants for more than seventy decades [14]. During the past 70 years, more than 2543 mutant cultivars from 175 plant species including ornamentals, cereals, oilseeds, pulses, vegetables, fruits and fibers have been officially released in 50 countries all over the world [15,16]. Chemical mutagenesis (the non-GMO approach) is a simple approach to create mutation in plants for their improvement of germination behaviour and other related potential agronomic traits. In any mutation breeding programme, selection of an effective and efficient mutagen is very essential to produce high frequency of desirable mutation. Many chemical mutagens have been employed for obtaining useful mutants in various crop species [17]. However the various workers emphasizes that artificial induction of mutation by colchicine (Col), ethyle methane sulphonate (EMS) and sodium azide (SA) provides tool to overcome the limitations of variability in plants especially Carnation that induces specific improvement without disturbing their better attributes [11,18,19]. It might be considered that, these chemical induced growth abnormalities were mainly due to cell death and suppression of mitosis at different

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exposures. 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. The role of mutation breeding in increasing the genetic variability for desired traits in various crop plants have been proved beyond doubt by a number of scientists [20-23]. The dose of a mutagen applied is an important consideration in any mutagenesis programme. Generally, it was observed that higher the concentrations of the mutagen greater the biological damage. To enhance the seed germination, lethality, pollen sterility and metrical traits, more knowledge about the effect of time, pH value, temperature, seed soaking and various concentrations are required [24]. Carnation offers many opportunities exploitation of mutations, recombination and of increasing genetic variability in quantitatively inherited agronomic characters. Induced mutations are also useful when it is desired to improve easily identifiable characters. The present studies have provided evidence on the induction of genetic variability connected with germination behaviour and metrical traits in *Dianthus* crop. Thus, induced genetic variability can be effectively exploited for evolving mutant strains possessing desirable attributes.

MATERIALS AND METHODS

The genotype used for mutagenic treatment was Carnation (*Dianthus caryophyllus* L.), a promising and leading *Dianthus* variety of which dry (10% moisture) and healthy seeds were obtained from Globe Nursery, Kolkata. It is suitable to grow in Burdwan agro-climatic conditions under timely and late sown condition. Three different concentrations (0.1, 0.4 and 0.7 % as w/v) of three chemicals viz. colchicine (Col), ethyl methane sulphonate (EMS) and sodium azide (SA) were freshly prepared using phosphate buffer (pH 7.0) for conducting the mutagenic treatments [10,11]. For each chemical treatment, 300 healthy seeds were taken and were at first surface sterilized by 0.01% (w/v) mercuric chloride ($HgCl_2$) for 5 minutes and thoroughly washed thrice with single distilled water for 10 minutes in each and then presoaked with double distilled water for 10 hours to initiate metabolic activities. After pre-soaking the seeds were blotted, dry and then placed in freshly prepared solutions of aforesaid three mutagens with their three different concentrations. The seeds were kept in the mutagenic solution for 6 h at room temperature 28±2°C with intermittent shaking for providing uniform treatment to the dipped seeds. An equal number of same genotypes were soaked in distilled water which served as control. To avoid dissociation of chemicals, the acidity of the solutions was controlled by using buffer solution. After the treatment time is over, the seeds were thoroughly washed in running tap water for three hours to remove the chemical present in them and then blotted dry. For laboratory experimentation, treated seeds were then sown in absorbent cotton-wet petridish for recording the germination behaviour like germination percentage, survival after germination and maturation, lethality over control (LOC). The germination percentage per treatment with three replicates was counted and recorded on 21st day after seed sowing. Percent inhibition or stimulation over control (lethality over control, LOC) were calculated as [Control-Treated/Control] X 100. Pollen fertility as well as sterility was tested for each treatment by using 2.0% (w/v) freshly prepared Aceto-carmine solution and examined under the low power (X15) of compound light microscope (Olympus). Dark stained and normal sized pollen grains were considered as fertile and those of irregular shaped and sized with light or no stain were considered as sterile. The number of plants survived till maturity, i.e., at the time of flowering phase, were scored from each treatment and recorded as per cent survival and compared with the control. The germinated seeds were finally transferred to experimental plots.

In field experimentation, seeds of ten treatments of Col, EMS and SA as well as untreated (control) sown during winter (2009-2010) for raising M_1 generation was done in three different plots having 4 m length and 25 cm apart by adopting 25 x 20 cm spacing following Randomized Block Design (RBD) layout with three replications for each genotypes/treatments at Crop Research Farm, Botany Department (UGC-CAS), The University of Burdwan, Burdwan. This experimental site is situated at 23.53^0 N, 22.56^0 S latitude and 83.25^0 E, 86^0 W longitude and 86 meter above the mean sea level (msl). In M_1 generation the observations on germination, flowering, seedling survival and other characters were noted. The all normal recommended cultural practices and plant protection measures were followed timely to raise good crop stand. Uniform agronomical measures were provided for this M_1 crop in the field experimentation. The data were recorded on five randomly selected plants from each replication for some agro-economic traits studied viz. days to Seed germination, shoot height (cm) at 21 days after planting, number of leaves/plant, stem diameter (cm), leaf area (cm^2), fresh weight (g) of vegetative growth, dry weight (g) of vegetative growth, days to flowering, number of flower/plant, flower longevity (days),seeds/inflorescence, 1000 seed weight (g).

RESULT AND DISCUSSION

The data on seed germination parameters and pollen fertility in first mutant (M_1) generation for colchicine (Col), EMS and sodium azide (SA) treatments in *Dianthus caryophyllus* are given in table 1. It was evidenced from table 1 that with increase in the mutagenic concentration or dose, the percentage germination had gone down except in Col, where it gears up; however, the effects of the chemicals differed considerably from each other [25, 26] . In Carnation, as compared to the control (76%), the germination percentage was lower in EMS and SA treatments. It was noted that 67.67, 64.67 and 51% on 0.1, 0.4 and 0.7% of EMS; 69.67, 62.3 and 52.67% on 0.1, 0.4 and 0.7% of SA, respectively. In 0.1, 0.4 and 0.7% of Col., it was 77.3, 80.3 and 84.3%, respectively, i.e., all values were higher than the control set. Similar results were also reported for EMS in soybean [27] and in mungbean [28]. Similarly, the survival rate during germination period of the treated seeds reduced with increased dose of mutagens in M_1 generation, except in colchicine, where it was fully opposite. The lowest laboratory germination of 51% with lowest survival seedling (153 out of 300) was recorded in 0.7% EMS. Survival at flowering stage or at maturity due to different mutagenic doses was ranged 38.9-71.3% in colchicine, 41.7-69.6% in EMS and 46.4-78.9% in sodium azide, whereas control at 91.67, that means compared to control, treatments were still less. We see that, colchicine treatment firstly gears up the germinability, but they were not survived no longer till the maturity. A reduction in germination and plant survival in M_1 generation of *Dianthus* due to mutagenic treatments has also been reported in *Vigna mungo* [29] and in Rice [30]. They observed that, in general, an increase in SA concentration resulted in decrease in germination; the plant survival was also decreased with the mutagenic dose increase, which is in accordance with the present findings.

Mutagens are known to induce lethality at the seedling stage in M_1 generation (Table 1). It was revealed from the observation that colchicine have the negative value of lethality over control (LOC) when compared to the control set (0.00) indicating that low lethality rate (i.e. higher survival rate) at the seedling stage. LOC value of both EMS and SA were positive higher value than that of control indicating their higher rate of lethality. Higher LOC (32.89) was recorded in 0.7% EMS, where 0.7% SA showed second higher LOC value (30.7). The behavior in terms of lethality of Col, EMS and SA at highest concentrations was noted.

The above results could be attributed to the effect of mutagens on the meristematic tissues of the seeds. These may be due to physiological and acute chromosomal damage [26, 31], delay in the onset of mitosis [32], chromosomal aberrations induced enzyme activity such as catalase and lipase and hormonal activity resulted in reduced germination [33] and survivability. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by Col, EMS and SA leading to decrease in germination. Reduced growth due to higher doses was also explained differently by different workers. It may be attributed to one or more of the following reasons (i) the increase in growth promoters, (ii) the sudden increase in metabolic status of seeds at certain levels of dose, (iii) the increase in destruction of growth inhibitors, (iv) drop in the auxin level or inhibition of auxin synthesis and (v) decline of assimilation mechanism. Taking these as the preliminary consideration.

In the present investigation, the pollen sterility among all the mutagenic treatments shows gradual increase with respect to the increase in concentrations, whereas pollen fertility gradually decreases. Pollen sterility ranged from 28.7 to 61.1 for Col, 30.4 - 58.3 for EMS and 21.1 – 53.6 for SA (Table 1). The maximum pollen sterility (61.1%) was observed under the treatment 0.7% Col. The dose treatment of Col and EMS was found to be more effective to produce maximum pollen sterility as compared to SA. The relative sensitivity of *Dianthus* cultivars to various mutagenic treatments was assessed by studying the biological damage' induced in M_1, in terms of seed germination, pollen sterility and fertility. In the present study, reduction in seed germination and pollen fertility was concentration dose dependent and linear. Promoting effects of low doses of Col, EMS and SA on biological parameters have been previously reported [34]. In most cases, meiotic abnormalities are responsible for pollen sterility [35, 36]. In addition to chromosomal aberrations, some genetic and physiological changes might have caused pollen sterility.

Different responses of various agronomical characters, which are very much important in any crop improvement programme, by applied doses of three mutagens are represented in table 2. It reveals differences among character values that fluctuate treatment to treatment. It was observed that under all three treatments, most of the characters were decreased with increasing concentration. For all the characters studied, 0.4% colchicine treatment gave the better value than that of control. Effect of 0.1% EMS was more or less non-effective, indicating nearest values to the control set. Increase concentration of SA negatively affect to the characters.

Table 1. Effect of colchicine, EMS and sodium azide on seed germination and pollen fertility in M_1 generation of *Dianthus*.

Mutagen	Concentration (%)	Total seed soaked	Survival seedling	Germination percentage (%)	Lethality over control (%)	Survival at flowering (%)	Pollen fertility (%)	Pollen sterility (%)
Control	---------	300	228	76	0.00	91.67 (209)	--	--
COL	0.1	300	232	77.3	-1.75	84.48 (196)	71.3	28.7
COL	0.4	300	241	80.3	-5.7	75.52 (182)	59.6	40.4
COL	0.7	300	253	84.3	-10.96	68.77 (174)	38.9	61.1
EMS	0.1	300	203	67.67	10.96	79.31 (161)	69.6	30.4
EMS	0.4	300	194	64.67	14.91	73.2 (142)	56.3	43.7
EMS	0.7	300	153	51	32.89	51.63 (79)	41.7	58.3
SA	0.1	300	209	69.67	8.33	79.43 (166)	78.9	21.1
SA	0.4	300	187	62.3	17.98	64.7 (121)	61.3	38.7
SA	0.7	300	158	52.67	30.7	46.2 (73)	46.4	53.6

Table 2. Effect of Colchicine (COL), Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) on seed germination, flower characters and some other vital biometrical characters of *Dianthus caryophyllus* in M_1 generation.

CH.	C.	DS	SH	NL	SD	LA	FW	DW	DF	NF	FL	SI	SW
CON	-	5± 0.33	24.33±1.9	56.9±1.9	0.22±0.2	3.35±0.4	36.83±1.6	3.75±0.5	27.33±1.6	37.00±1.4	45.04±1.6	26.8±1.3	1.59±0.1
COL	0.1	5± 0.14	19.63±2.6	72.4±1.8	0.29±0.1	4.34±0.6	34.60±1.5	3.04±0.3	25.31±1.9	31.00±1.7	44.20±2.3	23.1±1.6	1.60±0.4
	0.4	3± 0.07	26.47±2.2	82.8±2.1	0.51±0.2	4.66±0.5	45.38±2.3	4.19±0.7	26.67±2.2	41.33±1.3	53.18±2.7	27.4±1.8	1.67±0.3
	0.7	4± 0.03	23.20±2.25	84.3±2.1	0.34±0.2	3.35±0.3	39.61±2.1	3.13±0.7	24.33±1.4	38.23±1.7	47.52±1.6	29.6±1.3	1.35±0.3
EMS	0.1	5± 0.72	20.53±2.0	42.7±2.5	0.31±0.1	4.50±0.4	36.39±1.9	3.13±0.8	25.00±1.8	34.67±1.6	42.34±1.9	26.1±1.4	1.45±0.4
	0.4	6± 0.48	22.53±2.3	36.2±2.2	0.26±0.3	3.92±0.6	33.23±1.7	2.87±0.4	25.82±2.1	36.12±1.3	38.40±1.4	24.4±1.9	1.63±0.2
	0.7	8± 0.09	21.20±2.5	52.5±1.8	0.30±0.2	3.65±0.8	30.04±1.9	2.64±0.3	25.83±1.5	39.03±2.1	33.28±2.1	19.2±1.5	1.61±0.4
SA	0.1	4± 0.97	21.60±1.8	64.6±2.7	0.21±0.1	3.76±0.5	31.65±2.4	3.01±0.4	25.00±1.4	42.67±2.5	42.09±2.4	24.3±1.8	1.52±0.2
	0.4	6± 0.12	18.27±2.1	51.4±2.4	0.19±0.2	3.35±0.4	29.42±2.1	2.70±0.7	26.03±2.2	31.90±1.8	39.51±1.4	21.7±1.5	1.49±0.3
	0.7	6± 0.93	15.30±2.4	41.7±1.9	0.18±0.3	3.10±0.3	26.25±1.5	2.96±0.4	27.21±1.9	33.52±1.5	36.39±1.7	17.8±1.8	1.54±0.3

Legends:
CH. = Chemical, C. = Concentration (%), CON = Control, COL = Colchicine, EMS = Ethyl methane sulphonate, SA = Sodium azide, DS = Days to Seed germination, SH = Shoot height (cm) at 21 days after planting, NL = Number of leaves/plant, SD =Stem diameter (cm), LA = Leaf area (cm^2), FW = Fresh weight (g) of vegetative growth, DW = Dry weight (g) of vegetative growth, DF = Days to flowering or Maturity time, NF = Number of flower/plant, FL = Flower longevity (days), SI = Seeds/inflorescence, SW = 1000 seed weight (g), ± = Standard error.

CONCLUSION

It is advocated that the chemical mutagenic action on seed germination behaviour and pollen sterility with EMS (0.7%) treatment is much more beneficial as compared to colchicine and sodium azide. Increase in colchicine concentration firstly gears up the germination at seedling stage, but they were not survived till maturity. For other biometrical traits, 0.4% colchicine dose was found to be most effective than the other mutagenic doses. Hence, these chemical mutagens and their respective doses can be useful to improve the genetic background of *Dianthus* cultivar, especially in seed yield and its component major traits.

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