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COMPARATIVE STUDY FOR EFFECTS OF CHEMICAL MUTAGENESIS ON SEED GERMINATION, SURVIVABILITY AND POLLEN STERILITY IN M_1 AND M_2 GENERATIONS OF *DIANTHUS*

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, ethyl methane sulphonate (EMS), sodium azide (SA) and colchicine (COL) with three different concentrations (0.1%, 0.4% and 0.7%) were used to analyse their effect on seed germination behaviour, survivability and pollen sterility in both first (M_1) and second (M_2) mutant generations. 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. In M_1 and M_2, higher lethality over control (44.3 and 32.89, respectively) was shown by 0.7% of SA and EMS, respectively. Pollen sterility was also increased with increasing mutagenic doses. The maximum pollen sterility was 71.8% and 61.1% for 0.7% COL in M_1 and M_2, respectively. So, the effect of chemical mutagenesis on biological parameters with SA (0.7%) treatment in M_1 and EMS (0.7%) treatment in M_2 were much more beneficial as compared to colchicine. For each studied parameter, chemical mutagenesis was higher in M_1 than M_2. Hence, for the first time in *Dianthus*, we reported that these mutagens can be used for improving the germination behaviour and the metrical traits in *Dianthus* cultivar.

Key words: Chemical mutagen, *Dianthus caryophyllus*, mutant generation, pollen sterility, seed germination.

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INTRODUCTION

Mutation breeding has been widely used for the improvement of vital 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 (Micke, 1988). The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering major characteristics 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 (Laurie *et al*, 1968; Staby *et al.*, 1978; Roychowdhury and Tah, 2011).This genus is important by having pharmacological properties, aromatic things and polymorphism in morphology, genetics and hybridization (Facciola, 1990; Hughes, 1993; McGeorge and Hammett, 2002; Su Yeons, 2002; Lee *et al.*, 2005; Roychowdhury and Tah, 2011). 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 much difficult. The only alternative left with breeders is mutation breeding, which can be used as a potential source of creating variability (Novak and Brunner, 1992). Mutation can produce the development of *Dianthus* cultivars with more desirable floral characteristics and higher productivity (Roychowdhury, 2011; Roychowdhury and Tah, 2011a). For this purpose, inducible mutation is a suitable source of producing variation through mutation breeding procedure (Domingo *et al.*, 2007) which can produce several improved mutant varieties with high demanding economic values (Din *et al.*, 2004). 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 (Adamu and Aliyu, 2007). Mutation induction offers significant increase in crop production (Kharkwal and Shu, 2009) 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 (Adamu and Aliyu, 2007). Mutagenic agents have been used to induce useful phenotypic variations in plants for more than seventy decades (Vasline and Sabesan, 2011). During the past 70 years, more than 2543 mutant cultivars from 175 plant species including ornamentals, cereals, oilseeds, pulses, vegetables, fruits and fi-

bres have been officially released in 50 countries all over the world (Małuszyński *et al.*, 2000; Chopra, 2005). 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 (Singh and Singh, 2001). However the various workers emphasizes that artificial induction of mutation by ethyl methane sulphonate (EMS), sodium azide (SA) and colchicine (COL) provides tool to overcome the limitations of variability in plants especially Carnation that induces specific improvement without disturbing their better attributes (Mensah and Obadoni, 2007; Islam, 2010; Roychowdhury and Tah, 2011a). It might be considered that, these chemical induced growth abnormalities were mainly due to cell death and suppression of mitosis at different 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 workers (Tah, 2006; Khan and Goyal, 2009; Kozgar *et al.*, 2011; Mostafa, 2011; Roychowdhury *et al.*, 2011a; Roychowdhury *et al.*, 2011b; Roychowdhury *et al.*, 2012). The dose of an applied mutagen is an prime consideration in any mutagenesis programme. Generally, it was observed that higher the concentrations of the mutagen greater the biological damage to germination, seedling injury, pollen sterility and survival at maturity, which may be considered as an indication of mutagenic effect (Gaul, 1964). To enhance the seed germination, lethality, pollen sterility and other associated traits, more knowledge about the effect of time, pH value, temperature, seed soaking and various concentrations are required (Khan *et al.*, 2009).

The present study was conducted to understand the effect of three mutagens namely ethyl methane sulphonate, sodium azide and colchicine on above mentioned parameters. It is helpful in determining the effect and mechanism of action of the mutagen for further mutation breeding programme. Thus, induced genetic variability can be effectively exploited for evolving mutant strains possessing desirable attributes.

MATERIALS AND METHODS

Dry (15% moisture) and healthy seeds of experimental plant material i.e., *Dianthus caryophyllus* L. or Carnation, were obtained from Globe Nursery, Kolkata. It is suitable to grow in Burdwan agroclimatic conditions under timely

condition. Three different concentrations (0.1, 0.4 and 0.7 % as w/v) of three chemicals viz. Ethyl methane sulphonate (EMS), Sodium azide (SA) and Colchicine (COL) were freshly prepared using 1.0 M phosphate buffer (pH 7.0) for conducting the mutagenic treatments (Roychowdhury and Tah, 2011). For each chemical treatment, 300 healthy seeds were taken and 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 pre-soaked with double distilled water for 10 h 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. Equal seeds 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 3 h to remove the chemical present in them and then blotted to dry. For laboratory experimentation, treated seeds were then sown in absorbent cotton-wet Petri dish 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. The pollen sterility as well as fertility was observed at flowering stage on randomly selected ten plants per treatment and tested 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.

The field experimentation for the M_1 generation was conducted during the November- February of 2008-2009 at the Crop Research Farm, The University of Burdwan, Burdwan, India (23°53'N, latitude and 83°25'E, longitude and 86 m s.l.). Germinated seedlings of every treatment and untreated (control) were sown in the field in a complete randomized-block design (CRBD) with three replicates to raise the first mutant (M_1) generation. Plot size for a mungbean treatment in each replication was 3.5 m^2. Each plot had 3 m long three rows with row to row and plant to plant distance of 50 cm and 30 cm, respectively. Such a field design is most frequently used in plant breeding programmes. In M_1 generation the observations on germination, flowering, seedling survival and other characters were noted. For raising the M_2 generation in the next season

(2009-2010), ten M_1 progenies were selected which showed significant deviations in mean values in the positive direction from the mean values of the control, particularly for the yield and its associate components. Seeds from each selected M_1 progeny were bulked and thoroughly mixing them. A random sample of this bulk was sown to obtain M_2 progeny. Three replications of each M_2 mungbean treatment were maintained in the experimental field. Normal recommended cultural practices and plant protection measures were followed timely to raise good crop stand.

RESULTS AND DISCUSSION

Effects of different concentrations / doses of EMS, sodium azide (SA) and colchicine (COL) on biological parameters such as seed germination, seedling survivability, pollen sterility and fertility in both first (M_1) and second (M_2) mutant generation of *Dianthus* cultivar was studied. These two mutant generations of Carnation demonstrated differential responses towards the mutagenic treatments regarding the above parameters. The data on seed germination parameters and pollen sterility in M_1 and M_2 generation for aforesaid mutagens in *Dianthus caryophyllus* are given in Table 1 and Table 2, respectively.

Table 1

Effect of Ethyl Methane Sulphonate (EMS), Sodium Azide (SA) and Colchicine (COL) on seed germination, survivability and pollen sterility in M_1 generation of *Dianthus*

Mutagen	Concentration [%]	Total seed soaked	Germination [%]	Survival seedling	Survival at Maturity [%]	Lethality over control [%]	Pollen sterility [%]	Pollen fertility [%]
Control		300	79.0	237	91.98 (218)	0.00		
EMS	0.1	300	64.33	193	76.17 (147)	18.57	39.1	60.9
	0.4	300	56.33	169	69.23 (117)	28.70	51.2	48.8
	0.7	300	49.0	147	48.30 (71)	37.97	63.7	36.3
SA	0.1	300	62.33	187	74.33 (139)	21.10	32.9	67.1
	0.4	300	50.33	151	61.59 (93)	36.29	46.6	53.4
	0.7	300	44.0	132	43.94 (58)	44.30	59.3	40.7
COL	0.1	300	79.33	238	71.0 (169)	-0.41	38.2	61.8
	0.4	300	82.0	246	53.25 (131)	-3.80	56.4	43.6
	0.7	300	81.67	245	39.18 (96)	-3.38	71.8	28.2

It was evidenced from Table 1 and Table 2 that with increase in the mutagenic concentrations, the germination percentage had gone down except in COL, where it gears up; however, the effects of the chemicals differed consid-

erably from each other (Nandanwar and Khamankar, 1996; Singh *et al.*, 1997). In M_1 and M_2 Carnation population, as compared to the control (79% and 76%, respectively), the germination percentage was lower in EMS and SA treatments. In M_1 generation, it was noted that 64.33, 56.33 and 49.0% on 0.1, 0.4 and 0.7% of EMS; 62.33, 50.33 and 44.0% on 0.1, 0.4 and 0.7% of SA, respectively. On the other hand, M_2 population showed the germination percentage as 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.

Table 2

Effect of Ethyl Methane Sulphonate [EMS], Sodium Azide [SA] and Colchicine [COL] on seed germination, survivability and pollen sterility in M_2 generation of *Dianthus*

Mutagen	Concentration [%]	Total seed soaked	Germination [%]	Survival seedling	Survival at Maturity [%]	Lethality over control [%]	Pollen sterility [%]	Pollen fertility [%]
Control		300	76	228	91.67 (209)	0.00		
EMS	0.1	300	67.67	203	79.31 (161)	10.96	30.4	69.6
	0.4	300	64.67	194	73.2 (142)	14.91	43.7	56.3
	0.7	300	51	153	51.63 (79)	32.89	58.3	41.7
SA	0.1	300	69.67	209	79.43 (166)	8.33	21.1	78.9
	0.4	300	62.3	187	64.7 (121)	17.98	38.7	61.3
	0.7	300	52.67	158	46.2 (73)	30.7	53.6	46.4
COL	0.1	300	77.3	232	74.14 (172)	-1.75	28.7	71.3
	0.4	300	80.3	241	59.34 (143)	-5.7	40.4	59.6
	0.7	300	84.3	253	45.06 (114)	-10.96	61.1	38.9

In 0.1, 0.4 and 0.7% of Col., it was 79.33, 82.0 and 81.67% respectively in M_1 and 77.3, 80.3 and 84.3% respectively in M_2 generation, i.e., all values were higher than the control set. Similar results were also reported for EMS in soybean (Padavai and Dhanavel, 2004) and in mungbean (Singh and Kole, 2005). It was noted that 0.4% COL gave the highest germination%, then 0.7% and lastly 0.1% COL gave its lowest value in M_1, but in M_2 generation, germination % followed the magnitude of COL dose.

Similarly, the survivability during germination period of the treated seeds reduced with increased dose of mutagens in both generations, except in colchicine, where it was fully opposite. In M_1, the lowest laboratory germination of 44.0% with lowest survival seedling (132 out of 300) was recorded in 0.7% SA, whereas in M_2, lowest germination (51.0%) with lowest survival seedling (153

out of 300) was recorded in 0.7% EMS. For seedling survivability during germination period, COL showed the higher range (238-246), then EMS (147-193) and SA showed lower range (132-187) in M_1, whereas COL showed the higher range (232-253), then SA (158-209) and EMS showed lower range (153-203) in M_2.

In M_1, survival at flowering stage or at maturity due to different mutagenic doses was ranged 48.30-76.17% in EMS, 43.94-74.33% in SA and 39.18-71.3% in colchicine, whereas control at 91.98, that means compared to control, treatments were still less. This parameter was ranged 51.63-79.31% in EMS, 46.20-79.43% in SA and 45.06-74.14% in colchicine, whereas control at 91.67 in M_2 generation. 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 both M_1 and M_2 generations of *Dianthus* due to mutagenic treatments has also been reported in *Vigna mungo* (Mahna *et al.*, 1989) and in Rice (Afsar *et al.*, 1980). 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, the range of lethality over control (LOC%) were 18.57 – 37.97 for EMS, 21.10 – 44.30 for SA and -3.38 to -0.41 for COL (Table 1), whereas in M_2, this range were 10.96 – 32.89 for EMS, 8.33 – 30.7 for SA and -10.96 to -1.75 for COL (Table 2). It was revealed from the observation on M_1 and M_2 *Dianthus* populations 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 (44.30) was recorded in 0.7% SA, where 0.7% EMS showed second higher LOC value (37.97) in M_1. In M_2, LOC value (32.39) was high in 0.7% EMS and where 0.7% SA showed second higher LOC value (30.70). The behaviour in terms of lethality of EMS, SA and COL 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 (Nilan *et al.*, 1976; Singh *et al.*, 1997), delay in the onset of mitosis (Yadav, 1987), chromosomal aberrations induced enzyme activity such as catalase and lipase and hormonal activity resulted in reduced germination (Ananthaswamy *et al.*, 1971) 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 39.1 - 63.7 for EMS, 32.9 - 59.3 for SA and 38.2 – 71.8 for COL in M_1 (Table 1) and this parameter was ranged from 30.4 - 58.3 for EMS, 21.1 – 53.6 for SA and 28.7 to 61.1 for COL in M_2 (Table 2). In M_1 and M_2, the maximum pollen sterility (71.8% and 61.1%, respectively) was observed under the same treatment of 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 in both mutant generations. The relative sensitivity of *Dianthus* cultivars to various mutagenic treatments was assessed by studying the biological damage induced in M_1 and M_2 in terms of seed germination, survivability and pollen sterility. In the present study, reduction in seed germination and pollen fertility was concentration dose dependent and linear. Promoting effects of low doses of EMS, SA and Colchicine on biological parameters have been previously reported (Dubey, 1988). In most cases, meiotic abnormalities are responsible for pollen sterility (Mathusamy and Jayabalan, 2002; Khan and Wani, 2005). In addition to chromosomal aberrations, some genetic and physiological changes might have caused pollen sterility.

CONCLUSIONS

It is advocated that the chemical mutagenic action on seed germination, survivability and pollen sterility behaviour with SA (0.7%) treatment in M_1 and EMS (0.7%) treatment in M_2 are much more beneficial as compared to colchicine. In both generations, increase in colchicine concentration firstly gears up the germination at seedling stage, but they were not survived till maturity. For each studied parameter, chemical mutagenesis was higher in M_1 than M_2. Hence, these chemical mutagens and their respective doses can be useful to improve the genetic background of *Dianthus* cultivar, especially for germination and survivability and their component traits.

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