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Research Note

Mutation breeding in *Dianthus caryophyllus* for economic traits

Rajib Roychowdhury and Jagatpati Tah
Genetics and Plant breeding Laboratory, Botany Department (UGC-CAS), The University of Burdwan, Burdwan – 713104, West Bengal, India.
E-mail id.: rajibroychowdhurybu@gmail.com

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Abstract:
Dianthus caryophyllus is a commercial season based floricultural crop which grows well in temperate climate all over the world and popular as cut-flowers for its variegated petal's colour. So, it has terrific market demands in international trading. In this modern era, an agronomic demand of high yielding cultivar of this crop was noticed. Development of cultivars with more desirable floral characteristics and higher productivity are also very important in this crop. Keeping this view in our mind, we undertook the venture of mutation breeding programme by selecting chemical mutagens, viz. Colchicine (Col), Ethyl Methane Sulphonate (EMS), and Maleic Hydrazide (MH) at different concentrations, which were applied on the seeds and leaves. The first mutation generation (M_1) seeds of the crop were harvested properly and kept in dessicator for its proper restoring. Thereafter, the second mutation generation (M_2) seeds were grown in the next season for availing M_2 populations. Various biometrical characters viz. number of tillering per plant, total number of flowers, length (cm) of flowers, length (cm) of petals and weight (g) of 1000 seeds were studied and the obtained raw data were analyzed following the ANOVA model.

Key words:
cut-flower, mutation breeding, chemical mutagen, M_2 population, biometrical characters.

Dianthus is a floricultural crop species which grows well in temperate climate in all over world. This crop is very profitable and has a good market demand worldwide due to its vase quality and variegated colour of petal. In western countries, such as USA, it ranks next only to Rose in popularity (Laurie *et al*., 1968; Staby *et al*., 1978). This genus is important by having pharmacological properties, aromatic things and polymorphism in morphology, genetics and hybridization (McGeorge and Hammett, 2002; Facciola, 1990; Hughes, 1993; Lee *et al*., 2005; Su Yeons, 2002). Many new mutant cultivars have been directly or indirectly derived through mutation induction by using chemical or physical mutagens. From applicational point of view, it is quite possible to induce gene-mutation artificially with the help of some potent chemical mutagens like Colchicine (Col), Ethyl Methane Sulphonate (EMS), Maleic hydrazide (MH), etc. to create any new variety. The mutation breeding performance in *Dianthus* species can significantly accelerate many breeding endeavours, which have proven difficult with classical *Dianthus* breeding techniques. This aim is achieved by a tedious and sustainable mutation breeding programme, which permit an effective screening of large plant population leading to generate demanding mutation lines. The present study was conducted with the objective of developing a high quality, and better yielding new *Dianthus* varieties to increase its diversity and sustain market demand.

The field trial of *Dianthus caryophyllus* cultivar for the mutation breeding programme was conducted in the Horticulture farm of Botany Department, Burdwan University (latitude 23.53^0 N, 22.56^0 S and longitude 83.25^0 E, 86^0 W), West Bengal, India. This experiment was started from November (2009) and extended to April (2010).

The study on M_2 generation (second mutant generation) of *Dianthus* cultivar was obtained from previously collected M_1 seeds of mutagenic treatment. In that case, the pure line seeds were treated by some chemical mutagens such as, Colchicine (Col), Ethyl methane sulphonate (EMS) and maleic hydrazide (MH) as both seed and foliar treatment. The seeds were presoaked in distilled water for a few hours to initiate metabolic activities, and then placed in freshly prepared solutions of Col. and EMS at two different concentrations for each, say at 0.5% and 1.0% for 4 and 6 hours. Foliar treatment

was operated on the leaves, after some period of control seed germination, by using different concentrations (0.1%, 0.4% and 0.7% for each) of Col., EMS and MH. To avoid dissociation of chemicals, the treatment was generally given at low-temperature and the acidity of solution was controlled by the use of buffer solutions. The treated seeds were then washed in running tap water to remove the chemical present in them and placed in wet petridishes for recording the germination percentage. The germinated seeds were finally transferred to experimental plots. After the foliar spray of chemicals, tap water was sprayed on those treated leaves. The obtained seeds of M_1 plants were dried in dessicators and then were sown in the well prepared field separately, depending upon the concentration of chemicals with proper marking. This sowing process followed randomized block design (RBD) layout design having three replications for each treatment, where spacing between the plants in rows and columns is 2 X 2 ft. Uniform agronomical measures were provided for the M_2 crop in the field experimentation. The parameters such as no. of tillering per plant, total number of flowers per plant, length (cm) of flowers, length (cm) of petals and Weight (g) of 1000 seeds. The obtained analytical data from various biometrical characters were processed and represented by ANOVA (Analysis of Variance), which employed to obtain, assemble, classify and to interpret voluminous quantitative data that might have been influenced by many external environmental factors. New codes were given to each mutagenic treatment such as:

The recorded data on different economically important agronomic parameters were subjected to analysis of variance (ANOVA) to confirm the differences among *Dianthus* cultivar which were treated by three chemical mutagens and their different concentrations. Mean squares from analysis of variance of five indicated traits of *Dianthus* are presented in Table No. 1 & 2. The tables depicted highly significant differences among mutagenic treatment of *Dianthus* cultivar for all the characters studied. The value of variance ratio was as calculated significant at 1% level in case of number of tillering per plant, total number of flowers per plant and length of petals (foliar treatment) & length of flowers, length of petals (seed treatment) and at 5% level in total number of flowers per plant and weight of 1000 seeds (in foliar treatment) & number of tillering per plant and total number of flowers per plant (in seed treatment). The critical difference values (C.D values) were also calculated in all these five metrical traits. It is evident from this combined ANOVA table that no significant F value has been found in all other metrical traits in case of length of flowers for foliar treatment and weight of 1000 seeds for seed treatment, neither in replication nor in variety source of variance. It is nothing but due to the plantation of cultivars in proper seasonal time and also environmental effects. But the significant C.D. value indicates that *Dianthus* cultivar is no doubt suitable in this location where prevailing agro-climatic factors provided plantation of crop is in proper time. The fluctuations of various metrical characters are varied treatment to treatment. Now, it was indicated that the cultivar was more or less suitable in the location. There were lots of minute variations during growth and developmental phases in the field condition.

Foliar treatment gives the better result than the seed treatment. It is due to the easy penetrance power of used three chemical mutagens in the form of solution. In case of foliar treatment, increase in Colchicine concentration gear up the number of flowers (especially at 0.4% Col.) and reducing the seed weight when compared with control plant. This effect of Colchicine is somewhat reversed in EMS treatment. For their similarity, the numbers of flower, length of petal and seed weight are to be considered. MH treatment is negatively affected for these traits. On the other hand, seed treatment only shows the action of EMS in different concentration and treatment duration. Better result is observed in ST_2 (1% EMS treatment for 4 hrs.) and ST_5 (0.5% EMS treatment for 6 hrs.). Treatment by EMS 0.5% for 4 hrs gives very poor satisfaction which is reflected by ST_4.

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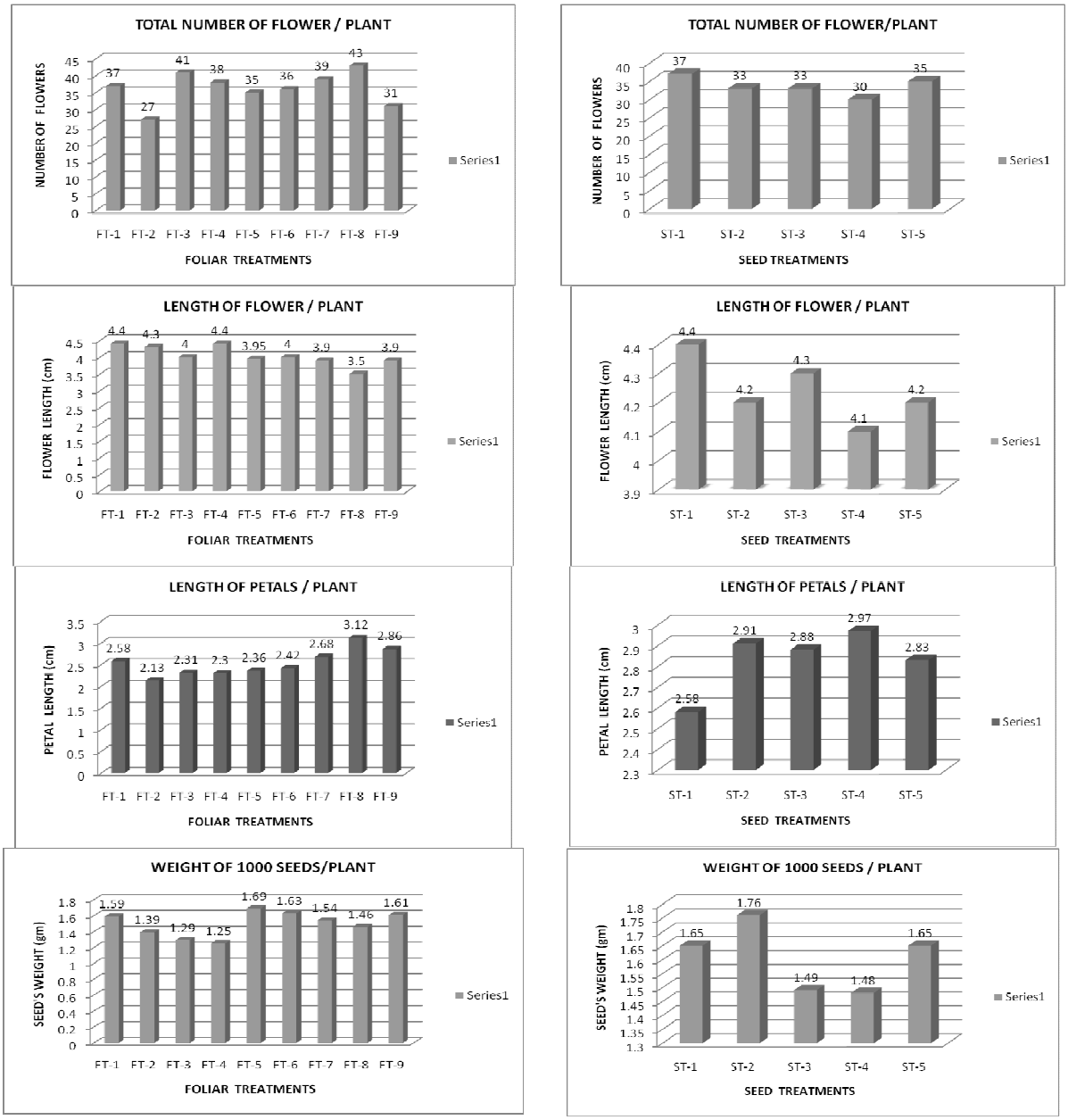
Figure 1. Graphical representation of various treatments

FOLIAR TREATMENT **SEED TREATMENT**



Codes for Foliar treatment: Control= FT_1, 0.1% Colchicine=FT_2, 0.4% Colchicine= FT_3, 0.7% Colchicine= FT_4, 0.1% EMS=FT_5, 0.4% EMS=FT_6, 0.7% EMS=FT_7, 0.1% MH=FT_8, 0.7% MH=FT_9.
Codes for Seed treatment: Control= ST_1, 1% EMS for 4 hours= ST_2, 1% EMS for 6 hours= ST_3, 0.5% EMS for 4 hours= ST_4, 0.5% EMS for 6 hours= ST_5.

Table 1. ANOVA for various characters of foliar treatment in *Dianthus caryophyllus*.

CHARACTER	Source of variance	Degree of freedom (df)	Sum Square (SS)	Mean Sum Square (MSS)	F Value	Critical Difference (CD)
No. of tillers per plant	Replication	2	6.89	3.445	13.405 **	1.21
	Observation	8	40.67	5.084	19.782**	
	Error	16	4.11	0.257		
Total number of flowers per plant	Replication	2	6.74	3.37	2.618*	1.964
	Observation	8	84.97	10.62	8.25 **	
	Error	16	20.59	1.287		
Length (cm) of flowers	Replication	2	0.048	0.024	0.242	---
	Observation	8	0.228	0.0285	0.288	
	Error	16	1.584	0.099		
Length (cm) of petals	Replication	2	0.04	0.02	0.671	0.412
	Observation	8	0.85	0.106	3.557**	
	Error	16	0.476	0.0298		
Weight (g) of 1000 seeds	Replication	2	0.02	0.001	0.068	0.21
	Observation	8	0.21	0.026	1.769*	
	Error	16	0.235	0.0147		

*, **Significant at 5 and 1% level respectively

Table 2. ANOVA for various characters of seed treatment in *Dianthus caryophyllus*.

CHARACTER	Source of variance	Degree of freedom (df)	Sum Square (SS)	Mean Sum Square (MSS)	F Value	Critical Difference (CD)
No. of tillers per plant	Replication	2	2.53	1.265	4.72*	0.975
	Observation	4	4.26	1.065	3.974*	
	Error	8	2.14	0.268		
Total number of flowers per plant	Replication	2	6.93	3.465	1.106	3.33
	Observation	4	51.73	12.933	4.127*	
	Error	8	25.07	3.134		
Length (cm) of flowers	Replication	2	0.3	0.15	11.538 **	0.687
	Observation	4	0.142	0.0355	2.73	
	Error	8	0.104	0.013		
Length (cm) of petals	Replication	2	0.018	0.009	1.765	0.196
	Observation	4	0.221	0.055	10.784**	
	Error	8	0.041	0.0051		
Weight (g) of 1000 seeds	Replication	2	0.004	0.002	0.4	---
	Observation	4	0.041	0.0103	2.06	
	Error	8	0.04	0.005		

*, **Significant at 5 and 1% level