EFFECTS OF GAMMA RADIATION ON SEED GERMINATION, PLANT SURVIVAL AND GROWTH CHARACTERISTICS IN Dianthus caryophyllus var. Chabaud

Article *in* Journal of Global Biosciences · November 2018

CITATION
1

READS
288

2 authors:

Prashant Deshmukh
Sant Gadge Baba Amravati University
4 PUBLICATIONS **1** CITATION
SEE PROFILE

Satish Malode
Sant Gadge Baba Amravati University, Amravati, India Govt. Vidarbha Institute of …
25 PUBLICATIONS **34** CITATIONS
SEE PROFILE

Journal of Global Biosciences
ISSN 2320-1355
Volume 7, Number 3, 2018, pp. 5403-5410
Website: www.mutagens.co.in

Research Paper

EFFECTS OF GAMMA RADIATION ON SEED GERMINATION, PLANT SURVIVAL AND GROWTH CHARACTERISTICS IN *Dianthus caryophyllus* var. Chabaud

Deshmukh, P.D. and S.N. Malode

Cytology and Genetics Laboratory
Department of Botany,
Govt. Vidarbha Institute of Science and Humanities,
Amravati-444604, (M.S), India.

Abstract

The present study was conducted to evaluate the performance of *Dianthus caryophyllus* L. var. Chabaud for agronomic traits through induced mutation by gamma radiation. Seeds were exposed to different doses of gamma radiations 0, 80Gyres, 160Gyres, 240Gyres, 320Gyres, 400Gyres, 500Gyres, 600Gyres and 700Gyres to examine their effect on germination and survival percentage, growth traits and morphological variations. Highest germination percentage recorded in control, whereas increase in dose of gamma radiations there is a considerable decrease in germination percentage and plant survival percentage. Higher gamma radiations doses there are considerable decrease in plant height. 320Gyr dose recorded dwarf plant type 1 (0.02%) with increased in number of branches and number of flowers per branches. Lower doses showed variations in plant height and other morphological parameters. High yield and good plant type mutant with more number of flowers (18) was recorded in 240Gyr and 400Gyr dose respectively in M_2 generations. Doses 240Gyr, 320Gyr and 400Gyr caused morphological variations and growth traits in *Dianthus caryophyllous.* The effect of these radiations is dose dependent, as these rays stimulate growth in plants at lower doses. Therefore, these radiations are important in modifying the plant genome for *Dianthus caryophyllus* crop improvement programme.

Key words: *Dianthus caryophyllus*, germination, plant survival, agronomic traits, gamma irradiation.

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) 2n = 30 belongs to the angiospermic family Caryophyllaceae, is an important floriculture crop all over the world and ranks just next to rose in popularity in western countries (Staby *et al*., 1978). It is well known cut flower with its variegated petals colour, high spicy fragrance and long shelf life of flowers. It is mostly found in temperate climate throughout the world with high

worldwide demand. In western countries, such as USA, it ranks next only to rose in popularity.This genus is important due to its pharmacological properties, aromatic things and polymorphism in morphology, genetics and hybridization (Facciola, 1990 and Hughes, 1993). Any sudden change either in the amount or in the arrangement of structure of DNA of a living being is called mutation. As the frequency of natural mutation is very low in nature, mutation can also be artificially induced for bringing the desired attributes in living organisms. Such mutation is called as induced mutation.

Induce mutation is highly effective in enhancing natural genetic resources and have been used to develops improved cultivars of cereals, fruits and other crops. Similarly it is simple relatively cheap to perform and equally usable on a small and large scale (Siddiqui and Khan, 1999). A huge range of chemical and physical mutagens have been investigated for their use in crop improvement. Induced mutation by using physical and chemical mutagens is a way to produce genetic variation, resulting in the creation of new verities with better characteristic (Wongpiyasatid, 2000).Physical mutagens especially the ionizing radiation i.e. Gamma rays, have been widely and routinely used to generate variability in various crop species including pulses (Tomlekova, 2010). A highest majority of mutant varieties i.e. 64% were developed by the use of gamma radiations (Ahlowalia *et al*., 2004). The effects of gamma radiation have been well studied and it is known to generate point mutation mostly. Due to this, they may lead to a complete or partial loss of gene function or less frequency to some other alterations in normal gene function. A high degree of mutational saturation can be achieved by gamma rays that does not cause a lot of collateral DNA damage (Bhosale and More, 2013 b).

MATERIALS AND METHODS:

Seeds of *Dianthus caryophyllus* L. var., Chabaud were collected from Universal Seed Company, Pune,(M.S.) India. These seeds were treated with Co^{60} radio isotope for gamma irradiation at Sophisticated Instrumentation and Analytical Faculty, RSTM Nagpur University, Nagpur .The doses like 80Gy, 160Gy, 240Gy, 320Gy, 400Gy, 500Gy, 600Gy and 700Gy were selected for experimental work. The experiments were performed in the Cytology and Genetics Laboratory, Department of Botany, Govt. Vidarbha Institute Of Science And Technology, Amravati (M.S.). Seeds were sown immediately after irradiation in petriplate as well as in germination slots at 21 ^{0} C with 90% moisture. The rhythmic light of 9hrs was provided by using 40watts tungsten bulb. In each petriplate, 50 seeds were sown in triplicates, observed for germination percentage and also fixed for mitotic studies. The slots (15cm X 4 cm) were prepared from blotting papers and seeds of equal numbers (10seeds/slot) were allowed and observed for germination and survival percentage. Similarly seeds were sown in field (in triplicates) and analyzed for growth traits and other morphological variations for M_1 and M_2 generations. Desirable mutants were screened from M_2 generation, characterized and selected for raising M_3 generations. The values of seed germination and survival percentage were determined by using the following formulae.

$$\text{Germination percentage} = \frac{\text{Total seeds germinated}}{\text{Total seeds sown}} \times 100$$

$$\text{Survival percentage} = \frac{\text{Total plants survived}}{\text{Total plant germinated}} \times 100$$

RESULT AND DISCUSSION

It was noted that mutagenic effectiveness and efficiency increased with the decreased in dose or concentrations. Similar finding were noted by V. Ravichandran and S. Jayakumar (2015) in sesame (*Sesamum indicum* L.). They also reported that gamma rays and EMS are produced a high frequency as well as a wide spectrum of mutation and rays was proved to be more effective and efficient in causing mutations as compared to EMS treatments. Hence effect of gamma radiations on seed germination percentage, seedling survival, M_2mutant screening, its characterization and frequency were tabulated and studied in following discussion.

1) Seeds germination percentage:

Gamma radiation doses 0 (control), 80Gyr, 160Gyr, 240Gyr, 320Gyr, 400Gyr, 500Gyr, 600Gyr and 700Gyr were selected and evaluated for germination and survival percentage study. It was noted that mutagenic doses and germination percentage was inversely proportional to each others. The values of germination percentage were decreased with corresponding increased in gamma doses. Dose 80Gyr showed 75% germination, 160Gyr showed 69% , 240Gyr showed 64% , 320Gyr showed 61%, 400Gyr showed 58% and 500Gyres showed 53%, 600Gyr showed 51% and 700Gyr showed 48% germination over control 82%. Dose 80Gyr shows maximum germination percentage (75%) followed by 160Gyr (69%), 240Gyr (64%), 320Gyr (61%), 400Gyr (58%) and 500Gyres (53%), 600Gyr (51%) and 700Gyr (48%) germination. Similar report were also noted by (corresponding decreased in germination percentage with increased doses) Bharti *et al*.,(2013) in *Withania,* in *Solanum lycopersicum* L. by Sikder et al.,(2013), (Bhosale and More,2014) in *Coriandrum sativum* L.,(Sikder et al.,2013) in Isabgol, The minimum germination percentage was recorded for 700Gyr (42%).

Table1.1: Effect of different concentrations of gamma irradiations on seed germination percentage and shoot length in *Dianthus caryophyllus* L. var. Chabaud.

Treatment	Germination (%)	Seedling Survival (%)
Gamma Irradiations		
Control	82	76
80Gyr	75	71
160Gyr	69	64
240Gyr	64	61
320Gyr	61	57
400Gyr	58	54
500Gyr	53	51
600Gyr	58	45
700Gyr	53	42

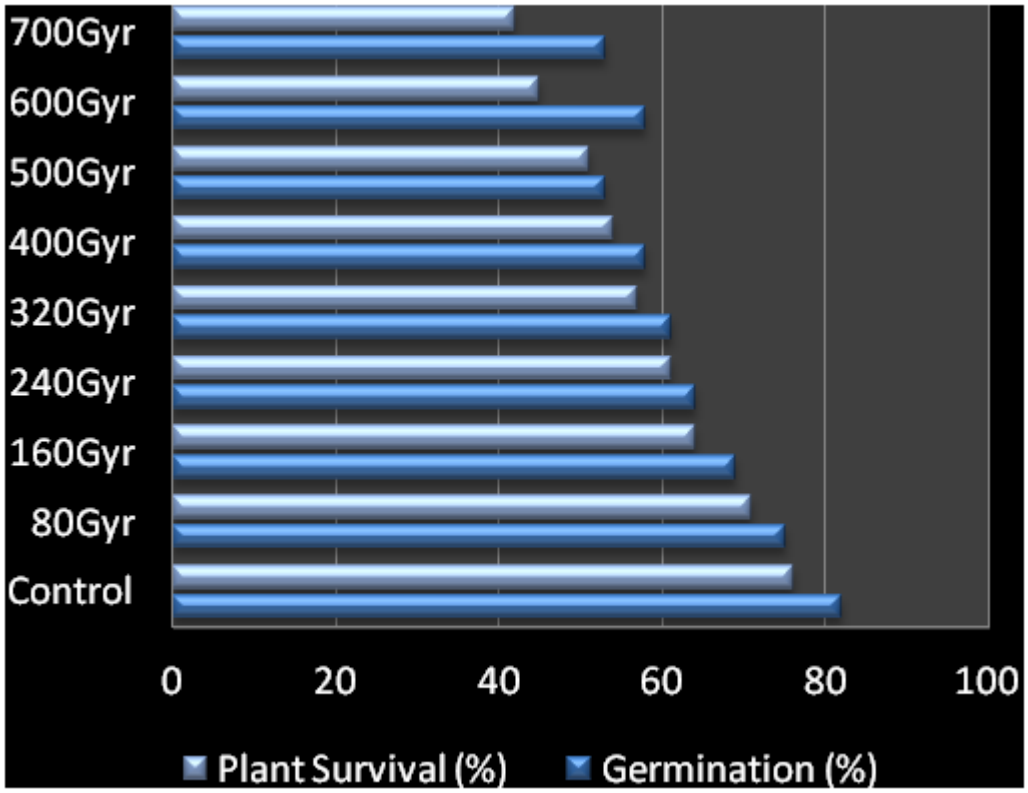


Fig.1.1: Showing effect of different concentrations of gamma irradiations on seed germination and plant survival in *Dianthus caryophyllus* L. var. Chabaud.

2) Seedling survival percentage:

Seedling survival percentage were also found to be decreased with increase in gamma doses. Control showed 76% survival percentage followed by 80Gyr (71%), 160Gyr (64%), 240Gyr (61%), 320Gyr (57%), 400Gyr (54%), 500Gyr (51%), 600Gyr (45%) and 700Gyr (42%). Lower doses showed corresponding decreased in value of germination percentage and seedling survival but doses 600Gyr and 700Gyr were recorded for prominent difference in the value of germination percentage and plant survival percentage.

3) Screening and characterization of mutants:

Mutants were recorded from M_2 genration on the basis of phenotypic characterization. Three mutants (Good Plant Type, High Yield Mutant and Dwarf Mutant) were scored after screening M_2 population for all the agronomic traits like plant height, total number of branches per plant, total number of leaves per plant, total number of flowers per plants, size of flowers, weight of flower, shelf life of flower, total number of seeds per plant, total number of pods in one season, seeds per pod, weight of single seed and weight of 100 seeds. S. J. Jambhulkar (2002) also reported an extreme dwarf mutant observed in the M_9 generation of the diploid sunflower variety 'Surya' is reported through gamma irradiation. High yielding mutants in *Ocimum sanctum* Linn. was reported by P.N. Nasare (2011) through physical mutagen (gamma rays), as well as the chemical mutagens (SA and EMS).Maximum mutants were recorded for doses 400Gyres followed by 320Gyres and 240Gyres.

Table 1.2: Screening of gamma irradiated M_2 population for mutants of *Dianthus caryophyllous* L. var. Chabaud during 2014 - 2016.

Sr. No.	No. of plant screened	Types of Mutant scored	Total No. of Mutants recorded	Frequency of Mutants (%)	Non-segregating mutants	Segre-gating mutants
1	3654	**High yield Mutant**	08 (240gyres)	0.70	3	5
2		**Dwarf mutant**	11 (320gyres)	0.02	3	8
3		**Good plant Type**	15 (400gyres)	0.30	2	13

Fig 1.2: Showing frequency and types of mutants recorded in M_2 generation of *Dianthus caryophyllus* L. var. Chabaud in respective gamma doses.
Fig 1.3: Graph showing phenotypic characterization of three mutants with respect to control.

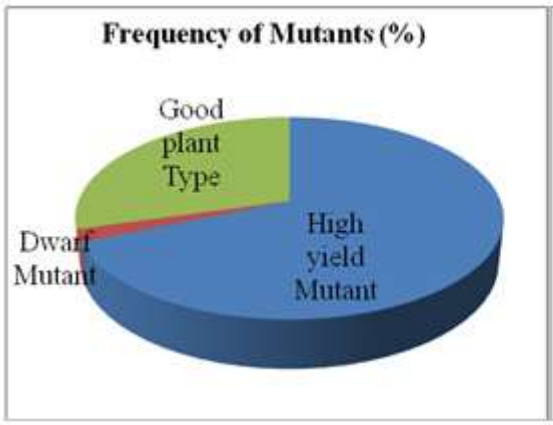


Fig: 1.2

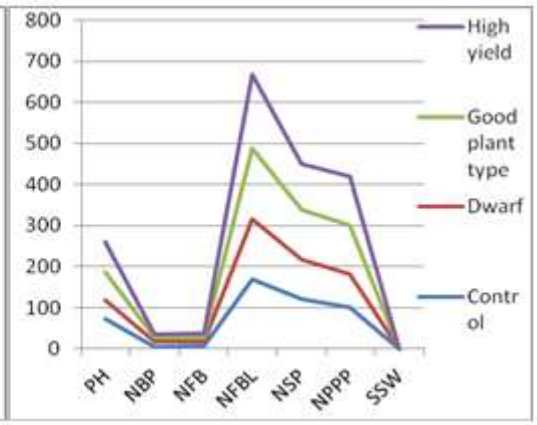


Fig:1.3

(PH - Plant Height, NBP-No. of branches per plant, NFB - No. of flowers per branch, NFBL - No. of flower per bloom, NSP - No. of seeds per plants, NPPP - No. of pod per plant, SSW- Single seed weight).

Maximum mutant were recorded for Good Plant Type (frequency 0.30) followed Dwarf Mutant (0.02) and High Yield Mutants (0.70). Mutants characterization showed that Dwarf mutant recorded increased in number of branches per plant (11) over control (06) followed by Good plant type (10) and High yield mutant (09). Total number of flowers per plant were also recorded maximum for Dwarf mutant (11) followed by Good plant Type (10) and High Yield (09) over control (07).

Reduction in plant height and incresed in primary branches per plant were noted in M_2 generation.The same result were also recorded in rapeseed (B*rassica napus* L.) by **Siddiqui *et al.,***2009. Maximum plant height were recorded for control (73.5) followed by High Yield (71.5), Good Plant Type (69.3) and Dwarf mutant (45.2). Maximum yield were showed by High Yield Mutant (seeds per pod value 112 and total number of pods121) followed by Good plant type mutant (seeds per pod value 120 and total number of pods 118), Dwarf (seeds per pod value 96 and total number of pods 81) over control (seeds per pod value 122 and total number of pods101). Maximum flower size

were recorded for mutant Good plant type (3.1) followed by Dwarf (3.1), High yield (2.9) over Control (3.1).

Table 1.3: Phenotypic characterization of M_3 non-segregating mutants of *Dianthus caryophyllous* L. Var. Chabaud during 2015 – 2016

Sr. No	Character	Control	High yield	Dwarf	Good plant type
1	Plant height (cm)	73.5	71.5	45.2	69.3
2	No. of branches per plant	6	9	11	10
3	Total no. of flowers per branches	07	09	11	10
3	Total no. of leaves per plant	276	295	187	286
4	Total no. of flower per bloom	169	179	148	171
5	Size of flower (cm)	3.2	2.9	3.1	3.2
6	Weight of single flower (gm)	1.3	1.3	1.1	1.4
7	Shelf life of flower (days)	9	9	8	9
8	No. of seeds per pod	122	112	96	120
9	Total no. of pods per plant	101	121	81	118
10	Single seed weight (gm)	0.0031	0.0029	0.0025	0.0026
11	100 seeds weight (gm)	0.3476	0.3521	0.0032	0.0034

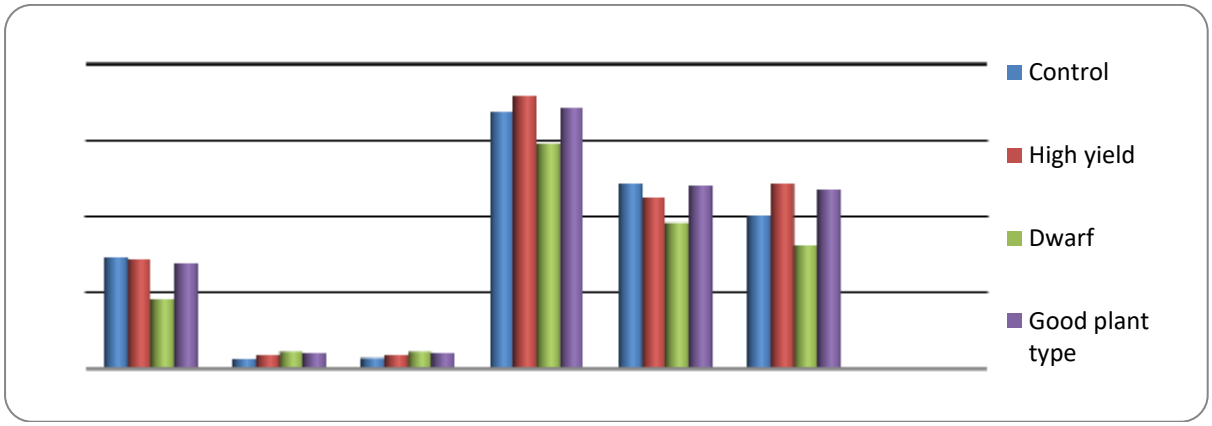


Fig.1.4: Phenotypic characterization of M_3 non-segregating mutants of *Dianthus caryophyllous* L. Var. Chabaud during 2015 – 2016

Lower doses like 240Gyr, 320Gyr and 400Gyr were found to be more effective in increased morphological variations. Begum and Dasgupta (2010) also reported that the use of lower doses of chemical and physical mutagens does not cause drastic chromosomal damage and hence may be more effective in increasing the amount of variability.

CONCLUSIONS:

Gamma doses 240Gyr, 320Gyr and 400Gyr were found to be more prominent and can be used very effectively in inducing desirable variations. Mutants of *Dianthus caryophyllus* var. Chabaud. obtained from M_2 generation viz., Good Plant Type, High Yield and Dwarf can be used for commercial cultivation as a new floriculture crop for this region for farmer.

REFERENCES:

Ahloowalia, B. S., Maluszynki, M. and Nichterlein, K., (2004). Global impact of mutation derived varieties. *Euphytica,* 135:187 – 204.

Bharathi, T., Gnanamurthy, S., Dhanavel, D., Murugan, S. and Ariraman, M. (2013). Induced Physical mutagenesis on seed germination, lethal dosage and morphological mutants of Ashwagandha (*Withania somnifera* (L.) Dunal) International Journal of Advanced Research, 1(5): 136-141.

Begum T. and Dasgupta T.(2010). A comparison of the effects of physical and chemical mutagens in sesame (*Sesamum indicum* L.). *Genet. Mol. Biol. vol.33 no.4 São Paulo 2010 Epub Oct 29, ISSN 1415-4757.*

Bhosale RS and More AD., (2013[b]). Effect of EMS (Ethyl Methane Sulfonate) on Seed Germination, Seedling Height and Seedling Injury in *Withania somnifera,* (L.) Dunal., *Int. J. of Life Sciences*, 1 (2):158-160.

Bhosale, R.S. and More, A.D. (2014). Effect of Gamma radiation on Seed Germination, Seedling caryophyllus L.): A Mutation Breeding Approach, LAP Lambert Academic chlorophyll mutation in mung bean (*Vigna radiata* L. Wilczek). J. Nuclear Agric. Bio., 16(1): 13-17.

Facciola, S.(1990). Cornucopia: A Source Book of Edible Plants. Kampong Publications, Vista, California, pp. 678.

Hughes, S. 1993. Carnations and Pink. The Crowood Press, Marlborough, UK

Ravichandran V. and Jayakumar, **S.(2015)**. Mutagenic effectiveness and efficiency of gamma rays and EMS in sesame (*Sesamum indicum* L.). *Int. J. of Res. in Bot.*, 2319–7854.

Siddiqui, B. A., Khan, S. (1999). Breeding in crop plants:Mutation and In vitro Mutational Breeding.1st Edn. Kalyani Publisher, Ludhiyana.

Sikder, S. Biswas, P. Hazra, P. Akhtar, S. Chattopadhyay, A. Badigannavar, A.M. and D'Souza S.F. 2013. Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. *Indian Journal of Genetics*, 73(4): 392-399.

Staby, G. L., Robert son, J. L., Kiplinger, D. C., and Conover, C. A. 1978. Chain of Life, Ohio Florist's Association, Ohio State University, Columbus.

Tomlekova, N.B. (2010). Induced mutagenesis for crop improvement. *Pl. Mut. Rep. 2, 4-27.*

Jambhulkar,S.J.(2002). An extreme dwarf mutant in sunflower. Scientific Correspondence: *current science, vol.83, no. 2, 25 (116).*

Nasare P. N. (2011). Characterisation of Induced Morphological Mutants in *Ocimumsanctum* Linn. *Online International Interdisciplinary Research Journal,{Bi-Monthly}, ISSN2249-9598, Volume-I, Issue-II, Nov-Dec2011*

Siddiqui, A. M., Khan, A. I. and Khatri, A. (2009) Induced quantitative variability by gamma raysand ethylmethane sulphonate alone and incombination in rapeseed (*Brassica napus* L.). ***Pak. J. Bot*., 41(3): 1189-1195.**

Wongpiyasatid, A., Chotechuen, S., and Hormchan, P. (2000). Induced mutations in mungbean breeding regional yield trail of mungbean mutant lines. Kasetsart J. Nat. Sci. 34, 443-449.

Bharathi, T., Gnanamurthy, S., Dhanavel, D., Murugan, S. and Ariraman, M. (2013). Induced Physical mutagenesis on seed germination, lethal dosage and morphological mutants of Ashwagandha (*Withania somnifera* (L.) Dunal) International Journal of Advanced Research, 1(5): 136-141.

Bhosale RS and More AD., (2013[b]). Effect of EMS (Ethyl Methane Sulfonate) on Seed Germination, Seedling Height and Seedling Injury in *Withania somnifera,* (L.) Dunal., *Int. J. of Life Sciences*, 1 (2):158-160.

Facciola, S.(1990). Cornucopia: A Source Book of Edible Plants. Kampong Publications, Vista, California, pp. 678.

Jambhulkar,S.J.(2002). An extreme dwarf mutant in sunflower. Scientific Correspondence: *current science, vol.83, no. 2, 25 (116).*

Nasare P. N. (2011). Characterisation of Induced Morphological Mutants in *Ocimumsanctum* Linn. *Online International Interdisciplinary Research Journal,{Bi-Monthly}, ISSN2249-9598, Volume-I, Issue-II, Nov-Dec2011*

Siddiqui, A. M., Khan, A. I. and Khatri, A. (2009) Induced quantitative variability by gamma raysand ethylmethane sulphonate alone and incombination in rapeseed (*Brassica napus* L.). ***Pak. J. Bot*., 41(3): 1189-1195.**

Sikder, S. Biswas, P. Hazra, P. Akhtar, S. Chattopadhyay, A. Badigannavar, A.M. and D'Souza S.F. 2013. Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. *Indian Journal of Genetics*, 73(4): 392-399.

Staby, G. L., Robert son, J. L., Kiplinger, D. C., and Conover, C. A. 1978. Chain of Life, Ohio Florist's Association, Ohio State University, Columbus.

SELECTION RESPONSE AND CORRELATION STUDIES FOR AGRONOMIC TRAITS IN MUTANT GENOTYPES OF DIANTHUS CARYOPHYLLOUS L. VAR. CHABAUD

Preprint · January 2018

CITATIONS
0

READS
36

2 authors:

Prashant Deshmukh
Sant Gadge Baba Amravati University
4 PUBLICATIONS **1** CITATION
SEE PROFILE

Satish Malode
Sant Gadge Baba Amravati University, Amravati, India Govt. Vidarbha Institute of …
25 PUBLICATIONS **34** CITATIONS
SEE PROFILE

UGC (CPE) and DST Sponsored

International Conference on Recent Trends in Science and Technology

22-23 March, 2018

PROCEEDINGS

Organized by

Vidya Bharati Shaikshanik Mandal Amravati's

S.S.S.K.R.Innani Mahavidyalaya

Karanja (Lad), Dist. Washim (M.S.)

Reaccredited at level 'A' grade by NAAC (CGPA 3.24)

& Conferred CPE status by UGC,New Delhi.

In collaboration with

Department of Mathematics & IQAC

Sant Gadge Baba Amravati University, Amravati

Vidya Bharati Mahavidyalaya, Amravati

&

Shri Dr.R.G.Rathod Science College, Murtizapur

Venue: S.S.S.K.R. Innani Mahavidyalaya Campus, Karanja (Lad)

Email: icrtst2018@gmail.com Website : www.ssskrimv.org.in Contact No.: 07256-222148/222171

Aayushi International Interdisciplinary Research Journal (AIIRJ)
ISSN 2349-638x Impact Factor 4.574 Special Issue No. 26
UGC Approved Sr.No.64259 **Website :-** www.aiirjournal.com **Email id:-** aiirjpramod@gmail.com

SELECTION RESPONSE AND CORRELATION STUDIES FOR AGRONOMIC TRAITS IN MUTANT GENOTYPES OF *DIANTHUS CARYOPHYLLOUS* L. VAR. CHABAUD

Deshmukh, P.D. & Malode, S.N.*
Cytology and Genetics Laboratory Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati-444604, (M.S), India
satishmalode17@gmail.com

ABSTRACT

The present study was conducted to evaluate mutant genotypes of Carnation (Dianthus caryophyllus L. var. Chabaud) to assess the inheritance pattern and genetic variability of heritable component of variation for selection response in agro metrical traits. Seeds were treated with three different mutagens (EMS, SA and Gamma Radiations) to raise M_1 population. M_1 population was harvested plant wise. M_2 population raise from M_1 seeds and M_2 population was screened to analyze the variation in plant type and isolated mutants or variants from different mutagen treated M2 population were analyzed for different agronomic traits. Four different type of mutant's isolated viz., Bold Seeded (6), Good Plant Type (11), High Yield (4) and Dwarf Plant (4) were recorded with different frequencies. Isolated mutants were characterized for different morphological and yield contributing traits subjected to statistical analysis for phenotypic and genotypic co-efficient of variation (PCV & GCV), Heritability (h^2), Genetic Advance (GA) and Percentage Genetic Gain (GG %). Agro metric traits plant height, number of branches, flower size and shelf life may serve as an effective selection parameter for screening of promising mutant lines on the basis of high GCV and PCV value, high heritability and genetic advance in crop improvement for Bold seed, High Yield, Good Plant Type and Dwarf mutant of Dianthus caryophyllous L. var. Chabaud for future studies.

Key words: *Carnation, heritability, selection response, agro metric traits, mutation, GCV, PCV, Dianthus.*

INTRODUCTION

Induce mutation is highly effective in enhancing natural genetic resources and have been used to develops improved cultivars of cereals, fruits and other crops. Similarly it is simple relatively cheap to perform and equally usable on a small and large scale (Siddiqui and Khan, 1999). A huge range of chemical and physical mutagens have been investigated for their use in crop improvement. Induce mutation breeding by using chemical and physical mutagens, are suitable source of producing variations in crop improvement through mutation breeding (Domingo *et al*., 2007) which may produce several improved mutant varieties with high economic value (Din *et al*., 2004). Induced mutation by using physical and chemical mutagens is a way to produce genetic variation, resulting in the creation of new varieties with better characteristic (Wongpiyasatid, 2000).

Carnation (*Dianthus caryophyllus* L.) 2n = 30 belongs to family Caryophyllaceae, is an important floriculture crop all over the world and ranks just next to rose in popularity in western countries (Staby *et al*., 1978). It is well known cut flower with its variegated petals color, high spicy fragrance and long shelf life of flowers. It is mostly found in temperate climate throughout the world with high worldwide demand. In western countries, such as USA, it ranks next only to rose in popularity. This genus is important due to its pharmacological properties, aromatic things and polymorphism in morphology, genetics and hybridization (Facciola, 1990 and Hughes, 1993). Wide spectrum of genetic variability has been induced in *Dianthus caryophyllus* L. by using both physical and chemical mutagens which help in floricultural improvement and inheritance studies suggested by Patil, 1966; Ashri, 1970; Gowda *et al*., 1996, Roychowdhury, 2011, Roychowdhury and Tah, 2011. Due to all this properties and high worldwide demand throughout the years, cultivation of this crop can improve economical status of farmers from Vidarbha region and stand as a best alternative crop to the routine practices. Present studies induced variation in *Dianthus caryophyllus* L. var., Chabaud with Gamma radiations, EMS and SA treatments to develop mutants more suitable for economical prospective and identify the traits with higher heritability, less influence by environmental condition with much scope in genetic improvement in successive generations.

MATERIALS AND METHODS

Seeds of *Dianthus caryophyllus* L. var., Chabaud were procured from Universal Seed Company, Pune, (M.S.) India. First year initially seeds were multiplied at Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati. Dry and presoaked water seeds were treated with different concentrations/doses of chemical and physical mutagens i.e., (EMS), (SA) and gamma radiations. Dry and pre-soaked water seeds in DNA – synthetic phase were used for the mutagenic treatments. Different concentrations of mutagens were determined on the basis of LD_{50} seed germination studies. Dry Seeds were treated with EMS concentrations of 0 (control), 0.003%, 0.006% and 0.009% (v/v) for 18 hrs. period. Twelve and eighteen hour pre-soaked water seeds were treated with EMS concentration of 0 (control), 0.003%, 0.006% and 0.009% (v/v) solutions respectively for 6 hrs. period. Treatments of sodium azide used were 0 (control), 0.02%, 0.05% and 0.07% (w/v) solutions for dry seeds, 0 (control), 0.02%, 0.05% and 0.07% (w/v) for 6 hrs. period, respectively. Seeds were rinsed for 60 min. with running tap water to completely remove mutagens. Dry seeds were exposed to gamma radiations doses of 80Gy, 160Gy, 240Gy, 320Gy, 400Gy, 500Gy, 600Gy and 700Gy at Sophisticated Instrumentation and Analytical Faculty, RSTM Nagpur University, Nagpur. All the treatments along with control were carried out in triplicates. The experiments were performed in the Cytology and Genetics Laboratory and experimental field of Department of Botany, Govt. Vidarbha Institute of Science And Technology, and Amravati (M.S.).

M_1 seeds were sown and obtained M_2 generation. Desirable mutants were screened from M_2 generation, characterized and selected for raising M_3 generations. Mean data of mutants were subjected to statistical analysis for phenotypic and genotypic co-efficient of variation (PCV & GCV), Heritability (h^2), Genetic Advance (GA) and Percentage Genetic Gain (GG %) for agronomic traits plant height, number of branches per plant, number of flower per bloom, size of flower, weight of flower, shelf life of flower, number of pods per plant and number of seeds per pod. The genotypic and phenotypic coefficients of variation (GCV and PCV) were worked out according to the method given by Singh and Chaudhary (1985). Heritability was worked out according to the method given by Allard (1960). Expected Genetic Advance (GA) of the genotypes were calculated according to Singh and Chaudhary (1985).

RESULTS AND DISCUSSION

M_2 population was screened for promising mutants viz. bold seeds, dwarf plant, good plant type, and high yield mutations. EMS, SA and gamma radiations recorded different mutants from M_2 populations such as Bold seed mutant (6), dwarf mutant (4), good plant type (11) and high yield mutant (4) and its segregation pattern and true breeding nature has been studied in successive M_3 generations (Table. 1). In this investigation, all 11 studied quantitative characters showed significant differences in the mean sum of squares due to genotypes or treatments (Table. 2). Control *Dianthus caryophyllus* var. chaubad had a height of 73.5 cm. No. of branches (6), Total no. of leaves (276), Size of flower 3.2 cm., Weight of flower 1.3 gm. Shelf life of flower 9 days, No. of seeds per pod 122, Total no. of pods 101, Single seed weight 0.0031gm. and 0.3476gm 100 seed weight. Significant variations in these traits were recorded in four isolated mutants which indicate that the mutant genotypes of *Dianthus* cultivar were genetically divergent (Table.2)

Malode and Khalatakar (1994) induced gene block for low glucosinolate in *Brassica juncea* L. CZERN through induced breeding. Different mutants lines were in *Brassicas* have been previously obtained by many workers like Sathawane (1993); Malode, (1995); Landge and Khalatkar (1995); Shelke (2014); Ambavane *et al.* (2015) and More and Malode (2018). Jambhulkar (2002) also reported an extreme dwarf mutant observed in the M_9 generation of the diploid sunflower variety 'Surya' is reported through gamma irradiation. High yielding mutant was obtained in *Ocimum sanctum* Linn. Nasare (2011) through physical mutagen (gamma rays), as well as the chemical mutagens (SA and EMS).

Table1: Showing frequency of segregating and non-segregating mutants of *Dianthus caryophyllous* L. var. Chabaud.

Total No. of M_2 plant screened	Mutants	Treatments	Total No. of Mutants recorded & frequency	Non-segregating M_3 Mutants	Segregating M_3 - mutants
3654 (GR = 1040 + EMS= 1278 + SA = 1336)	**Bold Seed Mutant**	240gyres	04 (0.38 %)	2	2
		18 hrs. Dry 0.003% EMS	02 (0.15%)	1	1
	Dwarf mutant	320gyres	1 (0.096%)	1	0
		12hrs. PSW + 6hrs. 0.006% EMS	2 (0.15%)	0	2
		18hrs. Dry 0.02% SA)	1 (0.07%)	1	0
	Good plant Type	400gyres	6 (0.57%)	2	4
		18hrs PSW + 6hrs 0.003% EMS	3 (0.23%)	2	1
		12hrs. PSW + 6Hrs. 0.05% SA	2 (0.14%)	0	2
	High yield Mutant	(240gyres)	02 (0.19%)	2	0
		12hrs. PSW + 6hrs. 0.006% EMS	1 (0.07%)	1	0
		18Hrs. Dry 0.02% SA	1 (0.07%)	0	1

Table 2: Agronomic characterization in mutants of *Dianthus caryphyllous* L. var. Chabaud in M_2 generation.

Character	Control	Bold Seed	Dwarf	Good plant type	High yield
Plant height	73.5	43.5	45.2	69.3	71.5
No. of branches	6	6	5	9	9
Total no. of leaves	276	276	187	286	295
Total no. of flowers	169	169	118	171	179
Size of flower	3.2	3.2	3.1	3.2	2.9
Weight of flower	1.3	1.3	1.1	1.4	1.3
Shelf life of flower	9	9	8	9	9
No. of seeds per pod	122	122	96	120	112
Total no. of pods	101	101	81	118	121
Single seed weight	0.0031	0.0031	0.0025	0.0026	0.0029
100 seeds weight	0.3476	0.3476	0.2742	0.2854	0.3147

The presence of wide range of variability might be due to diverse source of materials after mutation taken as well as environmental influence affecting the phenotypes. Hence agronomic characterization and estimates of mean (x□), standard error (SE), standard deviation (SD), T-test, Genotypic coefficient variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (*H2%)*, Genetic advance (GA) and Genetic percentage as a percentage (GA%) of mean in mutants of *Dianthus caryophyllous* L. var. Chabaud in M_2 generation was carried out depicted in (Table 3). A wide range of variation was observed among different mutants with regard to

different characters. The analysis of variance showed that genotype mean squares for all traits studied were highly significant. The estimates of phenotypic variances (σ2P), genotypic variances (σ2G), Phenotypic Coefficients of Variation (PCV) and Genotypic Coefficients of Variation (GCV) are varied considerably with respect to control given in Table 3. Whereas Heritability values are helpful in predicting the expected progress to be achieved through selection process and GA with the high genetic advance as per mean is usually more helpful in predicting gain under selection than heritability with change in gene due to mutations (Table 3).

The GCV provides a measure to compare genetic variability present in various quantitative characters. The higher value clearly indicated high degree of genotypic variability in these quantitative traits in *Dianthus caryophyllous***.** Highest GCV (9.017) and PCV (47.82) were recorded for High Yield (number of branches) and Good Plant Type (size of flower) respectively indicting high genetic variability with better improvement scope. Highest GCV value for total branches per plant (Raychoudhary and Tah, 2011), moderate for days to branching and seeds per inflorescence however lowest for total flowers per plant *Dianthus caryophyllous* L. PCV which measure total relative variation was highest for Good Plant Type mutant (47.82) for size of flower followed by Bold mutant (34.7) for flower weight, Dwarf and High yield mutant for plant height (24.27 and 22.59) respectively. Pathania *et al.* (1988) and Raychoudhary and Tah, (2011a) however reported higher PCV for total branches per plant, moderate for seeds per inflorescence, leaf area, days to branching and seed germination, lowest for flower diameter (cm), total no. of flowers per plant and plant height (cm) at 50% flowering phase in *Curcuma longa* L. and *Dianthus caryophyllous* L. respectively. Burton (1951, 1952) suggested that the genetic coefficient of variation together with heritability estimates gave the better picture of the extent of heritable variation. Mutant Good Plant type showed high heritability (32.8) for flower size and high genetic advance (69.5) for plant height indicated less influence by environmental condition with high inheritance ability. Similarly high heritability (16.4) with high genetic advance (72.36) was recorded in Bold Seeded Mutant for trait plant height. Also high heritability (33.8) with low genetic gain (32.3) was noted for trait flower size in Good Plant Type Mutant indicated non-additive gene interaction and can be proved more effective in selection responses. However mutant Good Plant Type showed high heritability (17.2) and moderate genetic advance (56.3) for total number of branches indicating additive gene interaction preferred less in induced breeding.

Table 3: Agronomic characterization and estimates of mean ($\bar{x}$), standard error (SE), standard deviation (SD), T-test, Genotypic coefficient variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (*H2%)*, Genetic advance (GA) and Genetic percentage as a percentage (GA%) of mean in mutants of *Dainthus caryphyllous* L. var. Chabaud in M_2 generation.

	Trait	$\bar{X}$	*SE*	*SD*	*T-test*	*GCV*	*PCV*	*H2%*	*GA*	*GA%*
Control	PH (cm)	73.5	1.54	2.38	0.00	8.671	15.78	3.3	17.4	59.1
	NB	6	1.49	2.23	0.00	8.839	32.14	13.2	97.4	40.8
	F/B	169	1.42	2.02	0.00	8.006	24.61	9.5	75.8	55.9
	SF(cm)	3.2	1.43	2.03	0.00	8.192	22.11	7.3	69.3	52.9
	WF(gm.)	1.3	1.40	1.96	0.00	8.422	12.05	2.0	11.0	35.5
	SLF	9	1.37	1.89	0.00	8.305	21.50	6.7	48.0	44.7
	NP/B	101	1.57	2.45	0.00	7.809	19.38	6.2	61.7	69.1
	NS/P	122	1.49	2.22	0.01	7.785	30.39	15.2	56.6	44.4
Bold seed mutant	PH (cm)	68.5	2.53	6.38	0.02	8.305	33.60	16.4	72.36	80.0
	NB	6	1.52	2.30	0.04	8.248	20.14	6.0	64.3	51.3
	F/B	169	1.66	2.76	0.04	8.111	19.31	5.7	62.0	64.7
	SF	3.2	2.28	5.20	0.03	8.111	28.46	12.3	65.8	75.7
	WF(gm.)	1.3	2.86	8.20	0.04	7.906	34.07	18.6	52.1	32.4
	SLF	9	1.80	3.24	0.08	8.248	22.43	7.4	86.1	65.7

	NP/B	101	1.52	2.31	0.03	7.906	22.35	8.0	69.6	70.2
	NS/P	122	2.57	6.63	0.03	8.006	29.34	13.4	53.9	22.5
Dwarf	PH (cm)	45.2	1.47	2.16	0.03	8.006	24.27	9.2	74.9	51.6
	NB	5	1.41	1.98	0.06	8.192	15.76	3.7	38.0	62.4
	F/B	118	1.35	1.81	0.05	8.220	14.45	3.1	36.2	52.3
	SF(cm)	3.1	1.40	1.95	0.03	8.192	13.47	2.7	35.0	75.6
	WF(gm.)	1.1	1.47	2.15	0.03	8.248	24.55	8.9	75.9	50.6
	SLF	8	1.38	1.89	0.03	9.017	16.94	3.5	41.2	65.6
	NP/B	81	1.53	2.33	0.04	7.881	21.60	7.5	67.6	82.0
	NS/P	96	1.38	1.91	0.02	7.931	16.23	4.2	38.3	68.4
Good Plant Plant	PH (cm)	79.3	1.44	2.07	0.05	8.006	22.27	7.7	69.5	67.6
	NB	9	2.44	5.93	0.65	7.931	32.93	17.2	56.3	71.7
	F/B	171	2.90	8.40	0.99	8.192	41.14	25.2	44.6	75.5
	SF(cm)	3.2	3.41	11.61	0.83	8.305	47.82	33.8	33.2	67.4
	WF(gm.)	1.4	1.54	2.38	0.04	7.981	20.59	6.7	65.1	69.4
	SLF	9	2.13	4.54	0.4	8.111	28.02	11.9	50.6	49.4
	NP/B	118	1.76	3.09	0.07	8.220	32.37	15.5	119.4	82.5
	NS/P	132	1.47	2.16	0.04	7.956	19.95	6.3	63.4	43.1
High yield	PH (cm)	81.5	1.46	2.14	0.09	8.276	22.59	7.5	70.7	67.1
	NB	9	1.50	2.24	0.01	9.017	21.18	5.5	68.2	82.5
	F/B	179	1.49	2.23	0.01	8.276	17.76	4.6	58.5	78.5
	SF(cm)	2.9	1.42	2.02	0.05	7.956	15.78	3.9	53.2	64.5
	WF(gm.)	1.3	1.42	2.02	0.01	8.220	22.14	7.3	69.5	72.8
	SLF	9	1.49	2.23	0.13	8.248	18.93	5.3	61.3	59.5
	NP/B	121	1.41	1.98	0.04	8.085	18.42	5.2	42.2	86.4
	NS/P	128	1.57	2.45	0.18	7.809	19.38	6.2	61.7	49.1

Note: PH: plant height (cm), **NB:** Number of blooms, F**B:** Flowers per blooms, **SF:** size of flower (cm), **WF:** Weight of flower, **SLF:** Shelf life of flower, **NP/B:** Number of pods per bloom, **NS/P:** Number of seeds per bloom.

CONCLUSIONS

The knowledge on heritability of traits is helpful to decide the selection procedure to be followed to improve the traits with respect to mutations. Higher estimate of heritability with higher genetic gain as percent of mean was observed for flower size and plant height indicating the presence of additive gene action and so selection can be easily done for these traits. The trait which expressed high heritability (flower size) and low genetic gain (Plant height) showed non additive gene interaction, hence Heterosis breeding would be recommended for that trait. Highest GCV value showed high genetic variability and huge scope of genetic improvement as recorded for shelf life and number of branches per plant. Thus traits plant height, number of branches, flower size and shelf life may serve as an effective selection parameter in developing promising mutant lines in crop improvement for Bold, High Yield, Good Plant Type and Dwarf mutant of *Dianthus caryophyllous* L. var. Chabaud for future studies.

REFERENCES

1. **Allard, R. W. (1960).** Principles of plant breeding. pp. 89-98. John Wiley and Sons, New York.
2. **Ambavane, A.R., Sawardekar, S.V., Sawantdesai, S.A. and Gokhale, N.B. (2015).** Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn) Journal of Radiation Research and Applied Sciences. **8:** 2 - 2 5.
3. **Ashri, A., (1970).** A dominant mutation with variable penetrance and expressivity induced by diethyl sulfate in peanuts (*Arachis hypogaea* Linn). *Mutation Res.*, 9: 473-480.
4. **Burton, G.W. (1951).** Quantitative inheritance in Pearl millet (*P.glaucum*). *Agron. J.* **43,** 409-417.

5. **Burton, G.W. (1952).** Quantitative inheritance in grasses. *6th Int.Grassland Congress* **1**, 277-283.
6. **Din, R. Qasim, M. and Ahmad, K. (2004.)** Radio - sensitivity of various wheat genotypes in M_1 generation. *Int. J. Agri. Biol.* 6, 898-900.
7. **Domingo, C., Andres, F. and Talon, M. (2007).** Rice cv. Bahia mutagenized population: a new resource for rice breeding in the Mediterranean basin. *Span. J. Agric. Res.*5, 341-347.
8. **Facciola, S. (1990).** Cornucopia: A Source Book of Edible Plants. Kampong Publications, Vista, California, pp. 678
9. **Gowda, M.V.C., Nadaf, H.L. and Sheshagiri, R. (1996).**The role of mutation in intraspecific differentiation of groundnut (*Arachis hypogaea* L.). *Euphytica* 90, 105-113.
10. **Hughes, S. (1993).** Carnations and Pink. The Crowood Press, Marlborough, UK.
11. **Jambhulkar, S. J. (2002).** An extreme dwarf mutant in sunflower. Scientific Correspondence: *current science, vol.83, no. 2, 25 (116).*
12. **Landge, S. P. and A. S. Khalatkar (1995).** Early flowering induced mutation in *B. napus* cv. Westar GCIRC 9th International Rapeseed Congress Cambridge, UK **3:** 742-744.
13. **Malode, S. N. (1995).** Cytogenetic studies in *Brassica carinata* A. Braun. Ph. D. thesis. Nagpur University, Nagpur.
14. **Malode, S. N. and Khalatkar, A. S. (1994).**Possible induction of gene block for low glucosinolate through induced mutation in *Brassica juncea* L.CZERN.
15. **More, U. A and S.N. Malode (2018).** Ethyl methane sulphonate induced early flowering mutations in Brassica napus L. cv. Excel. Multidisciplinary International Conference on Green Earth: A Paronaomic View. *Proceedings.* 201- 207.
16. **Nasare P. N. (2011).** Characterisation of Induced Morphological Mutants in *Ocimumsanctum* Linn. *Online International Interdisciplinary Research Journal,{Bi-Monthly}, ISSN2249-9598, Volume-I, Issue-II, Nov-Dec2011.*
17. **Pathania, N., Singh, M. and Arya, P.S. (1988).** Genetic evaluation of some economic traits in turmeric (*Curcuma longa* L.). *Himachal Journal of Agricultural Research* **14 (1)**, 38-43.
18. **Patil, S. H., (1966).** Mutation induction in groundnuts by X- rays. *Ind. J .Genet.,26 A: 334-348.*
19. **Roychowdhury, R. (2011).** Effect of Chemical Mutagens on Carnation (*Dianthus caryophyllus* L.): A Mutation Breeding Approach, LAP Lambert Academic Publishing,Germany.
20. **Roychowdhury, R. and Tah, J. (2011).** Mutation breeding in *Dianthus caryophyllous* for economic traits. *Elec. J. Plt. Breed.* 2(2), 282-286.
21. **Roychowdhury, R. and Tah, J. (2011a).** Chemical mutagenic action on seed germination and related agrometrical traits in M1 *Dianthus* generation. *Current Botany*,2(8), 19-23.
22. **Sathawane, K. N.** (1993). Production of improved 'Varuna' variety of *Brassica juncea* (L.) Coss. and Czern. Through induced mutagenesis. Ph. D. thesis, Nagpur University, Nagpur.
23. **Shelke, Prerna B (2014).** Incorporation of qualitative and quantitative characteristics in *Brassica campestris* L. through induced mutation. Ph.D. thesis, S.G.B. Amravati University, Amravati.
24. **Siddiqui, B. A., Khan, S. (1999).** Breeding in crop plants: Mutation and In vitro Mutational Breeding.1st Edn. Kalyani Publisher, Ludhiyana.
25. **Singh, R.K. and Chaudhary, B.D. (1985).**Biometrical Methods in Quantitative Genetic Analysis. pp. 318. Kalyani Pub., Ludhiana.
26. **Staby, G. L., Robert son, J. L., Kiplinger, D. C., and Conover, C. A. (1978).** Chain of Life, Ohio Florist's Association, Ohio State University, Columbus.
27. **Wongpiyasatid, A., Chotechuen, S., and Hormchan, P. (2000).** Induced mutations in mungbean breeding regional yield trail of mungbean mutant lines. Kasetsart J. Nat. Sci. 34, 443-449.

Impact Factor –6.261 Study of Different Mitotic Abnormalities Induce by Ems in Dianthus Caryophyllus Var. Chabaud

Conference Paper · February 2019

CITATIONS
0

READS
92

2 authors:

Prashant Deshmukh
Sant Gadge Baba Amravati University
4 PUBLICATIONS **1** CITATION
SEE PROFILE

Satish Malode
Sant Gadge Baba Amravati University, Amravati, India Govt. Vidarbha Institute of Sci…
25 PUBLICATIONS **34** CITATIONS
SEE PROFILE

INTERNATIONAL RESEARCH FELLOWS ASSOCIATION

Impact Factor – 6.261 ISSN – 2348-7143

INTERNATIONAL RESEARCH FELLOWS ASSOCIATION'S

RESEARCH JOURNEY

International E-Research Journal

PEER REFREED & INDEXED JOURNAL

February-2019 Special Issue – 110 (E)

BOTONY

This Journal is indexed in :
- University Grants Commission (UGC)
- Scientific Journal Impact Factor (SJIF)
- Cosmoc Impact Factor (CIF)
- Global Impact Factor (GIF)
- International Impact Factor Services (IIFS)

For Details Visit To : www.researchjourney.net

SWATIDHAN PUBLICATIONS

INTERNATIONAL RESEARCH FELLOWS ASSOCIATION

'RESEARCH JOURNEY' International E- Research Journal
Impact Factor - (SJIF) – 6.261, (CIF) - 3.452(2015), (GIF)–0.676 (2013)
Special Issue 110 (E) - Botony
UGC Approved Journal
ISSN : 2348-7143
February-2019

Study of Different Mitotic Abnormalities Induce by Ems in Dianthus Caryophyllus Var. Chabaud

Deshmukh P.D. And S.N. Malode *
Cytology and Genetics Laboratory,
P.G. Department of Botany,
Govt. Vidarbha Institute of Science and Humanities,
Amravati – 444604,(M.S), India.
Email: satishmalode17@gmail.com

Abstract:

Present study was formulated to evaluate the efficiency and effectivity of EMS in floriculture plant Dianthus caryophyllus var. Chabaud. Seeds of Chabaud were treated with three different treatments of EMS viz., 18Hrs. Dry, 12Hrs. PSW + 6Hrs. and 18Hrs. PSW + 6Hrs for 0.003%, 0.006% and 0.009% doses along with control. Seeds were sown in petriplate with three replicas and fixed after 6-7 days in Carnoys – I. Fixed roots were then transfer in 70% alcohol and utilized for mitotic studies. All type of mitotic abnormalities viz., Sticky chromosomes, fragments, laggard, clumped metaphase, bridges and precious chromosomes were reported in all the treatments for all the doses except control. Bridges showed maximum mitotic abnormality percentage 5.53 followed by fragments 5.51,clumped metaphase 5.37, precocious chromosome 4.72, sticky chromosome 4.46 and least for laggard 4.31. Dose 0.009% of 18Hrs. PSW + 6Hrs. EMS noted for maximum abnormality (4.47%) over all the treatments. Total mitotic abnormality induced by EMS was reported to 29.75 over control. Among the treatments used, maximum mitotic abnormality reported by 18Hrs.PSW + 6Hrs. (10.76) and decreased in 12Hrs.PSW + 6Hrs. (9.80) with least for 18Hrs.Dry (9.21). Hence pre - soaked treatments with higher doses was found to be more promising in inducing mutation and can be used more effectively to gain promising mutants.

Keywords: Carnation, Mutagens, EMS, Mitotic Abnormality, Bridges, Laggards, effectivety.

Introductions :

Carnation (Dianthus caryophyllus L.) 2n = 30 belongs to the angiospermic family Caryophyllaceae, is an important floriculture crop all over the world and ranks just next to rose in popularity in western countries (Laurie et al., 1968; Staby et al., 1978). It is well known cut flower with its variegated petals colour, high spicy fragrance and long shelf life of flowers. It is mostly found in temperate climate throughout the world with high worldwide demand. In western countries, such as USA, it ranks next only to rose in popularity (Laurie et al., 1968). This genus is important due to its pharmacological properties, aromatic things and polymorphism in morphology, genetics and hybridization (Facciola, 1990; Hughes, 1993; Su Yeons, 2002; Lee et at., 2005 and Mc George and Hammett, 2008).

Any sudden change either in the amount or in the arrangement of structure of DNA of a living being is called mutation. The concept of mutation was first of all proposed by Dutch Botanist Hugo de Veries in 1901, following his work on inheritance in the evening Primrose Oenothera lamarckiana. As the frequency of natural mutation is very low in nature, mutation can also be artificially induced for bringing the desired attributes in living organisms. Such mutation is called as induced mutation. Many physical and chemical agents have been used successively for inducing mutations in plants and animals.

Induced mutation breeding offer possibility for the induction of desired changes in various attributes which can be exploited as such or through recombination breeding i.e. hybridization (Akbar and Manzoor, 2003). A huge range of chemical and physical mutagens have been investigated for their use in crop improvement. Physical mutagens especially the ionizing radiation i.e. Gamma rays, have been widely and routinely used to generate variability in various crop species including pulses (Tomlekova, 2010).

Induce mutation is highly effective in enhancing natural genetic resources and have been used to develops improved cultivars of cereals, fruits and other crops (Lee et al., 2006) Similarly it is simple, relatively cheap to perform and equally usable on a small and large scale (Siddiqui and Khan, 1999). Mutagenesis has been widely used as a potent method of Producing and enhancing variability for crop improvement (Singh and Singh, 2001). Induced mutation by using physical and chemical mutagens is a way to produce genetic variation, resulting in the creation of new verities with better characteristic (Wongpiyasatid, 2000). Any mutagen that induces single base pair mutations or small deletions or insertion is an effective for genetic modification (Bhosale and More, 2013b).

Induced mutational breeding is a powerful and effective tool in the hand of plant breeders especially for autogamous crops having narrow genetic make up (Micke, 1988). Wide spectrum of genetic variability has been induced in Dianthus caryophyllus L. by using both physical and chemical mutagens which help in floricultural improvement and inheritance studies (Patil, 1966; Ashri, 1970; Gowda et al., 1996). Gamma radiations have also been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants with respect to doses (Ashraf et al., 2003).Development of Dianthus cultivars with more desirable floral characteristics and higher productivity can be produce by mutation (Roychowdhury and Tah, 2011a; Roychowdhury, 2011).

Cytological abnormalities considered as an indexes to determine the effectivity among different mutagens. Doses with highest efficiency for induced breeding can also be determined with the help of study of mitotic and meiotic behavior of chromosomes.

Material And Method:

Seeds of Dianthus caryophyllus L. var., Chabaud were collected from Universal Seed Company, Pune, (M.S.) India. These seeds were treated with chemical mutagens Ethane Methyl Sulphonate with three treatments Viz., 18Hrs. Dry, 12Hrs. PSW + 6Hrs. and 18Hrs. PSW + 6Hrs. in three replicas along with control. The experiments were performed in the Cytology and Genetics Laboratory, Department of Botany, Govt. Vidarbha Institute of Science And Technology, Amravati (M.S.).

In 18Hrs. Dry treatments, 300 seeds were kept in three different concentrations (0.003%, 0.006% and 0.009%) of EMS for 18 Hrs. Out of 300 seeds, 75 seeds were sown in three replicas each with 25 seeds (in three different glass) Petri-dish. Petri-dishes as well as germination slots was kept at 21 0 C with 90% moisture. The rhythmic light of 9Hrs was provided by using 40watts tungsten bulb. In each Petri- dish 25 seeds were sown in triplicates, observed for germination percentage and also fixed for mitotic studies.

After germination, roots (6- 7days germinating seeds) were fixed in Carnoys - I fixative for 24Hrs. Then roots were transfer in 70% alcohol and used for mitotic study. Squash was made according to the method prescribed by Darlington and LaCour (1962). Photographs of various

phases were captured under Photographs were taken by CMOS Camera on Zeiss microscope at 10X x 100X magnification.

Remaining 75 seeds were used to study root shoot length and hence allow growing in comb with moist blotting paper. The slots (15 cm X 4 cm) were prepared from blotting papers and seeds of equal numbers (10 seeds / slot) were allowed and observed for cytological variations at seedling stages (after 14 days).

Last 150 seeds were directly sown in the field for study of agronomic characteristics. Same procedure was repeated for 12Hrs. PSW + 6Hrs. and 18Hrs. PSW + 6Hrs. for 0.003%, 0.006% and 0.009% concentrations. Data for all the abnormalities was taken from all the replicas for different treatments and put forth for further analysis.

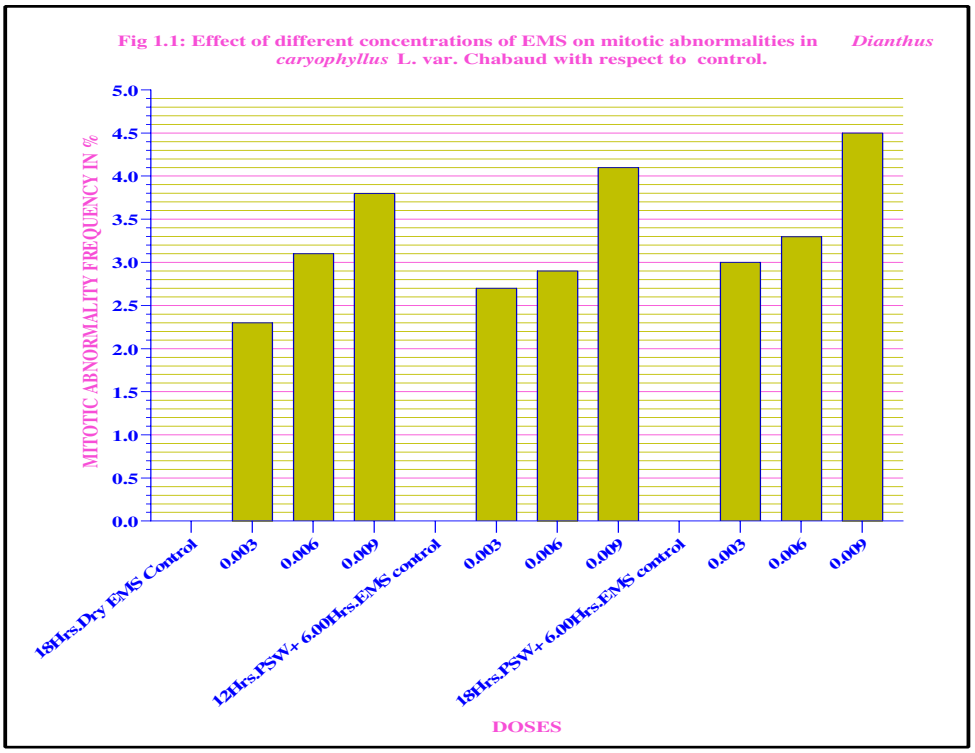
Results

In EMS 18Hrs. Dry treatment, dose 0.003% showed 2.3% mitotic abnormality followed by increased in 0.006% (3.1%) and 0.009% (3.8%). In 12Hrs.PSW + 6Hrs.Ems, doses 0.003%, 0.006% and 0.006% showed 2.7%, 2.9% and 4.1% mitotic abnormality respectively. However dose 0.003% in 18Hrs.PSW +6Hrs.EMS showed 3.0% followed 0.006% (4.2%) and highest in 0.009% (4.5%) meiotic abnormity. In all the treatments of EMS, minimum mitotic abnormality was recorded for dose 0.003% (2.3%) in 18Hrs. Dry EMS and maximum in dose 0.009% (4.5%) as tabulated in (Table 1 and Fig 1.1).

In 18Hrs. Dry EMS, Bridges was recorded as significant mitotic abnormality (2.00%) followed by fragment (1.60%), sticky chromosome (1.52%), clump metaphase and precocious chromosome (1.44%) and lower for laggard (1.20). However 12Hrs. PSW + 6Hrs. EMS , clump metaphase was significant and showed (2.07%) abnormality followed by fragment (1.97), precocious chromosome and laggard (1.58%), sticky chromosome (1.41%) and lower for bridge (1.35%).Bridges were recorded as significant mitotic abnormality (highest among all the doses of EMS, 2.18%) in 18Hrs. PSW + 6Hrs. EMS treatment followed by fragments (1.95%), clump metaphase (1.86%), precocious chromosome (1.70), sticky

Table 1: Effect of different concentrations of 18Hrs.Dry EMS, 12Hrs. PSW + 6Hrs. EMS and 18Hrs. PSW + 6Hrs.EMS treatments on mitotic chromosomes in Dianthus caryophyllus L. var. Chabaud.

Treatments (EMS in %)	Total no. of mitotic Cells observed	Mitotic abnormality in percentage (%)						Total Mitotic abnormality (%)	't' value
		B	CM	F	L	PC	SC		
18Hrs.Dry EMS									
Control	1200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
0.003	1200	0.50	0.42	0.25	0.25	0.17	0.25	2.34	0.17
0.006	1200	0.67	0.42	0.16	0.50	0.17	0.16	3.11	0.21
0.009	1200	0.67	0.33	0.20	0.67	0.67	0.33	3.36	0.18
12Hrs.PSW+6Hrs.EMS									
Control	1200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
0.003	1200	0.67	0.67	0.49	0.41	0.17	0.25	2.71	0.19
0.006	1200	0.83	0.42	0.25	0.42	0.33	0.25	2.95	0.20
0.009	1200	0.67	0.58	0.50	0.33	0.67	0.50	3.31	0.21
18Hrs.PSW+6Hrs.EMS									
Control	1200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
0.003	1200	0.67	0.42	0.25	0.25	0.17	0.42	3.03	0.22
0.006	1200	0.67	0.42	0.17	0.42	0.17	0.42	3.26	0.20
0.009	1200	0.67	0.58	0.50	0.33	0.67	0.50	3.47	0.23
Total Abnormality (%)	15078	5.53	5.37	5.51	4.31	4.72	4.46	29.75	
Average abnormality (%)	1257	0.46	0.44	0.46	0.36	0.39	0.37	2.47	



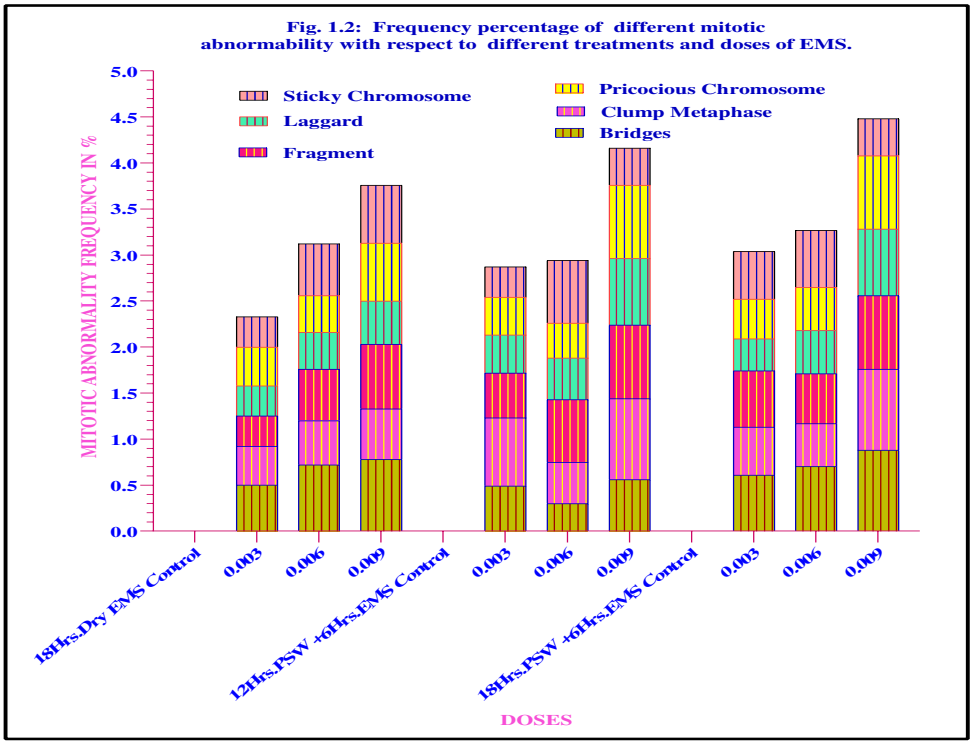


Fig 1.3: Percentage of different mitotic abnormality induced by three different treatments of EMS in Dianthus caryophyllu var. Chabaud

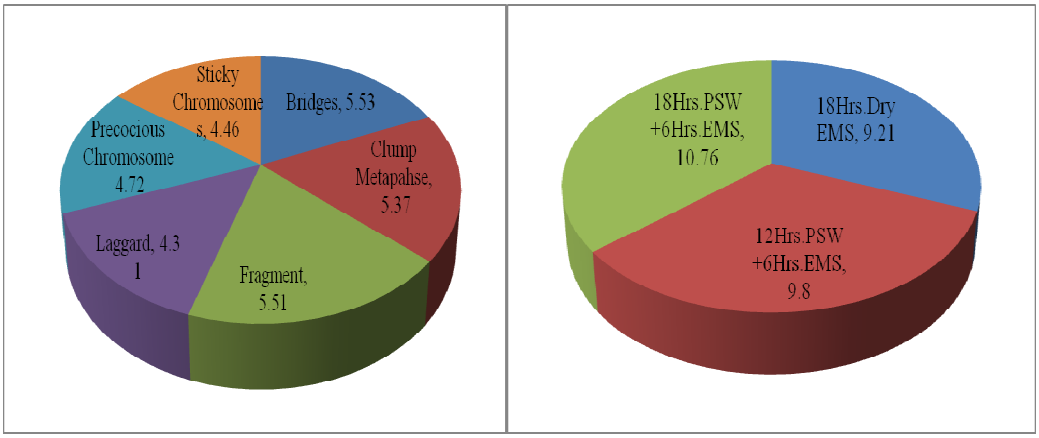


Fig 1.4: Percentage of total different mitotic abnormality induced by three different treatments of EMS in Dianthus caryophyllu var. Chabaud

Table 2: Effect of different treatment OF EMS on mitotic chromosomes in Dianthus caryophyllus L. var. Chabaud

Treatments	Total no. of mitotic Cells observed	Mitotic abnormal cells (%)						Mitotic abnormality (%)
		B	CM	F	L	PC	SC	
18Hrs.Dry EMS	5032	2.00	1.44	1.60	1.20	1.44	1.52	**9.20**
12Hrs.PSW +6Hrs.EMS	5117	1.35	2.07	1.97	1.58	1.58	1.41	**9.79**
18Hrs.PSW +6Hrs.EMS	4929	2.18	1.86	1.95	1.53	1.70	1.54	**10.75**
EMS	15078	5.53	5.37	5.51	4.31	4.72	4.46	**29.75**

chromosome (1.54) and finally laggard with (1.53 %). Total mitotic abnormality for 18Hrs. Dry EMS, 12Hrs. PSW + 6Hrs. and 18Hrs. PSW + 6Hrs. EMS was recorded .

18Hrs Dry, 12Hrs. PSW + 6Hrs. and 18Hrs. PSW + 6Hrs.EMS treatments reported 9.20%, 9.79% and 10.75% respectively with total 29.75% mitotic abnormality (Table 2; Fig. 1. 2 and 1.3).

Discussion:

Differential response for mutagenic abnormalities was reported with respect to different treatments and doses in EMS (Fig. 1.1). Kharkwal (1998) also noted that mutagenic effectivety and efficiency showed differential behavior with respect to doses of mutagens and depends upon varietal type used for experiments. However John (1999) reported mix trend of mutagenic effectivety and efficiency with respect to doses concentration in Black gram, Malode (1995) in

Brassica canola, Shelke (2014) in Brassica compestris, Wadanker (2014) in Dehlia, More (2016) in Brassica napus.

A higher concentration of EMS mutagen was reported for high rate of cytological abnormalities and decreased with decreased in doses concentrations. However Kavithamani et al. (2008), Mundhe (2008), Tambe (2009), Pavadai et al. (2009) and Khan and Tyagi (2010) in Glycine max L. merrill reported higher mutagenic effectiveness at lower concentrations / dose of EMS and gamma rays in soybean.

The decrease in effectiveness with increasing concentrations/dose of mutagen has been reported by several authors Sassi Kumar et al.(2003) in limabean, Sharma et al. (2006) in urdbean, Badere and Choudhary (2007) in Linseed, Dhanavel et al. (2008) Girija and Dhanvel (2009), Ashok kumar et al. (2009) in cowpea, Bhosle and kothekar (2010) in cluster bean.

As the concentration of all these three treatments increases, percentage abnormalities were also increased. Means mitotic abnormality increases with increased in doses concentrations for all the treatments of EMS mutagens (Fig. 1.1). Dose dependent increase mitotic aberration following mutagenic treatment has been also reported in faba bean by Vandana and Dubey, (1992 a, b), Malode (1995) in Brassica carinata , Mitra and Bhowmik, (1996), Inceer et al. (2000), Kumar et al. (2003), Khan et al. (2009), Sirsat et al. (2010) in horsegram, Green et al. (2012), El – Adi et al. (2013) in Onion (Allium cepa, L.), Shelke (2014) in Brassica copestris, Mendhulkar et al. (2015) Glycine max Linn. (Merr), More (2016) in Brassica napus cv. Excel.

Ethyle methane sulphonate (EMS) is one of these mutagenic and carcinogenic agents very closed to the nitrogen base guanine Swann (1990).Thus it produce unknown mutations as a result of the random nucleotide substitution of guanine. The dangerous effect of EMS appeared in mitosis resulted from its interference with DNA replication which appears as different chromosomal aberrations (Sultan and Celik, (2009).

However different worker revealed different finding regarding with higher effectivety and efficiency of mutagens. Mutagenic potentiality of combined treatment of SA and gamma rays has been reported as more efficient than their individual ones in cowpea (Kumar and Verma, 2011). Hassan and Ahmad (2000) found that although all types of chemical mutagens were effective for the induction of chromosomal aberrations, but chromosomal aberrations were increased due to the effect of EMS in comparison to the other chemical mutagens.

Patial et al. (2013) reported that EMS was almost four times more effective and two times more efficient than gamma-rays and both mutagenic effectiveness and efficiency decreased with an increasing doses/ concentrations of mutagens. Higher effect of combine treatment of SA and Gamma rays has been also reported in wild chickpea (Kamble and Patil, 2014). However high mutagenic aberration was reported by Kamble and Petkar (2014) in Wild Chickpea (Cicer reticulatum) L noted effectivety trend as X - ray > SA > SA + X - ray.

Different treatments of mutagen showed different mitotic abnormalities percentage (Fig. 1.4). Same were also reported by Thengane and Tengane (1986) in Cassia aungustifolia, Jayabalan and Rao (1987) in Lycopersium esculentum Mill. Var. Pusa Ruby, Malode (1995) in Brassica canola, Maraj and Ali (2011), Kamble and Petkar (2012) in wild Cheak pea Cicer reticulatum, Shelke (2014) in Brassica copestris, Wadanker (2014) in Dehlia, Ramesh and Verma (2015) in Plox drummandii, More (2016) in Brassica napus cv. Excel and Ninave and Malode (2018) in Brassica juncia. ,

Treatment 18Hrs.PSW + 6Hrs. has found to be most effective than 12Hrs. PSW + 6Hrs. and finally in 16Hrs. Dry EMS. Among all the dose, dose 0.009% in 18Hrs. PSW was found to more potent than any other dose from the same or different treatments. Variations in mitotic abnormality with respect to dry and presoaked treatments was also reported by Malode (1995) in Brassica canola, Shelke (2014) in Brassica compestris, Wadanker (2014) in Dehlia, More (2016) in Brassica napus and Ninave and Malode (2018) in Brassica juncia. While Mendhulkar et al. (2015) Glycine max Linn. (Merr) reported high motitic abnormality in presoaked treatments than dry dose.

Different types of abnormalities viz., Bridges (single, double or multiple), Clump metaphase (CM), Fragments (F), Laggard (L), Precocious chromosomes (PC) and Sticky chromosome (SC) were also reported for all the treatment and doses of EMS (Table 1 and 2; Fig 1.2 and 1.3). Similarly different mitotic abnormality was also reported by using different doses of EMS by many worker viz. , Meenakshi and Subramanium, (1960), Tarar and Dnyansagar (1980a), , Malode (1995), Kumar et al. (2003), Sirsat et al. (2010), Patial, et. al. (2013),Shelke (2014), and Gadhi et al. (2015). Mitra and Bhowmik, (1996), Inceer et al. (2000), Adi et al. (2013) in Onion (Allium cepa, L.) and Mendhulkar et al. (2015) Glycine max Linn. (Merr)

EMS caused disrupted chromosomes in anaphase Grundmann (1979) and normal course of mitosis may be disrupted by various pathological processes. Gisselsson et al. (2002) found that chromatin bridges may be implied as a diagnostic marker for cancer. Gaulden, mary (1989) who found that chromosomal aberrations caused by the physical stretching and breaking of chromatids at the sticky sites. Abo El Khier and Abo El Khier (1992),

During abnormal chromosomal behavior of mitosis, spindle fiber can not to attract one chromosome , this chromosome remains near the middle of the cells leads to lagging chromosome and resulted genome aneuploidy 2n-1 Aydemir et al . (2008).

Overall in EMS, bridges were recorded as maximum abnormality (5.53%) followed by fragments (5.51), clump metaphase (5.37%), precocious chromosomes (4.72%), sticky chromosome (4.46) with lowest noted for laggard (4.31%) over control (Table 1 and 2; Fig. 1.2 and 1.3). Similar results of higher percentage of anaphasic bridges were observed more frequent at the higher doses. Bridges are formed generally due to stickiness of the chromosomes at metaphase or breakage and reunion of chromosome as reported by Kaur and Grover (1986a), Singh and Khanna (1986) in wheat, triticale and rye, Ahamad and Yasmin (1992) in Allium cepa. Singh and Khanna (1988) considered that anaphase bridges may be formed due to unequal exchange or dicentric chromosomes.

Single and multiple chromosome bridges possibly due to the occurrence of dicentric chromosomes formed as a result of breakage and fusion bridge cycles (McClintok, 1941; Kumar and Singh, 2002). Precocious movement of chromosomes may be due to the dysfunction of spindle fibers. Kumar and Rai (2007) noted that precocious chromosome migration to the poles may be due to the formation of univalent chromosome at the end of prophase I or precocious chaisma terminalization at diakinesis or metaphase. Un-orientation at metaphase and scattering of chromosome may be due to inhibition of spindle formation or damage of spindle fiber formed Kumar and Rai (2007). Laggards observed during the meiotic studies might have originated due to delayed terminalization, stickiness of chromosomal ends or because of failure of the chromosomal movements (Jayabalan and Rao, 1987; Soheir et al., 1989). Disturbed polarity observed during anaphase may be due to the disturbance in spindle formation Bhat et al. (2007).

Bridges are formed generally due to stickiness of the chromosomes at metaphase or breakage and reunion of chromosome as reported by Kaur and Grover (1986a), Singh and Khanna (1986) in wheat, triticale and rye, Ahamad and Yasmin (1992) in Allium cepa. Singh and Khanna (1988) considered that anaphase bridges may be formed due to unequal exchange or dicentric chromosomes.

Stickiness has been attributed to the entanglement of inter chromosomal chromatin fibers that leads to sub-chromatid connection between chromosomes (Klasterka, 1972) but was considered by increased with increasing doses of mutagen. Patil and Bhat (1992) reported that it's a type physical adhesion involving mainly the proteinacious matrix of chromatin. Gaulden, (1987) postulated that stickiness may result from defective functioning of one or two types of specific non-histone proteins involved in chromosomal separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutation in structural genes coding for them (hereditary stickiness) or by the action of mutagens (induced stickiness).

According to Achkar et al. (1989) sticky chromosomes are formed when breaks in the double strands of DNA are introduced while the intra- chromatid links initiate their formation during chromosome condensation, determined by chromosome protein bond as histones. Thus chemical mutagen EMS was found to be more effective with 18Hrs. PSW + 6Hrs. 0.009% dose treatments and can be used to induce desired and beneficial mutants / variants with economic characteristics in crop as well as in ornamental plants.

References:

1. Abo E. K., zakia, G. M. and Abo, E. K. (1992). Cytological Effects of Certain Active Constituents of Peganum harmol and Harmine Alkaloids on Mitosis of Allium cepa , L . J.King Saud Univ ., 4, Science (1) : 37- 45
2. Achkar A. l. W., Sabatier, L. and Dutrillaux, B. (1989). How are sticky chromosomes formed? Ann Genet. 32: 10-15.
3. Ahamad S. and Yasmin R. (1992). Effect of parathion and trimiltox on the mitosis of Allium cepa. Cytologia 57:155-160.
4. Ashok Kumar, V., Usha Kumari, R. Amutha, R., Siva Kumar, T., Juliet Hepziba, S. and Anand Kumar, C. R. (2009).Effect of chemical mutagen on expression of characters in arid legume pulse cowpea (Vigna unguiculata (L.) Walp.). Res. j. Agric. Biol. Sci. 5(6):1115-1120.
5. Ashraf, M., Cheema, A., Rashid, A. and Zia-ul-Qamar, M. (2003). Effectof gamma rays on M1 generation in basmati rice. Pak. J. Bot. 35(5), 791-795.
6. Aydemir, N. E. Summak, S. and Yilmaz, D. (2008). Evaluation of Clastogenicity of 4, 6 - Dinitro –o- cresol (DNOC) in Allium Root Tip Test. J. Biol Environ. Sci. 2(5): 59-63.
7. Badere, R. S., and Choudhry, A. D. (2007). Effectivity and Efficiency of Gamma rays, Sodium azide and Ethyl Methane sulphonate in Linseed. Bioinfolet. 4: 181-187.
8. Badr A. (1883). Cytogenetic activities of triazine herbicide in root tips of Allium cepa and Vicia faba. Mutation Research 117:173-182.
9. Bhosle S. S. and Kothekar V. S. (2010). Mutagenic efficiency and effectiveness in cluster bean (Cyamopsis tetragonoloba L. Taub). J. Phytology.2: 21 27.
10. Darlington C.D. and La Lour (1962). The handling of chromosomes. Fillen and Univer.Ltd. London, 43-49.
11. Dhanavel, D. P., Pavada, L., Mullainathan, D., Mohana, G., Raju, M., Girija and Thilagavathi, C.(2008).Effectiveness and efficiency of chemical mutagens in cowpea (Vigna unguiculata L. Walp. African Journal of Biotechnology.7: 4116 - 4117.

12. Facciola, S. (1990). Cornucopia: A Source Book of Edible Plants. Kampong Publications, Vista, California, pp. 678.
13. Gaulden, M. E. (1987) Hypothesis: Some mutagens directly alter specific chromosomal proteins to produce chromosome stickness. Mutagenesis 2: 357-365,
14. Giri, S. P. and Apparao, B. J. (2011). Studies on effectiveness and efficiency of EMS in pegionpea (Cajanus cajan L.). Bioscience Discovery.2:29-3
15. Girija, M. and Dhanvel D. (2009). Mutagenic Effectiveness and Efficiency of Gamma rays, EMS and their combined treatments in cowpea Vigna unguiculata L. Walp).Global J. Mol. Sci.4: 68-75.
16. Gowda, M.V.C., Nadaf, H. L. and Sheshagiri, R. (1996).The role of mutation in
17. intraspecific differentiation of groundnut (Arachis hypogaea L.). Euphytica 90, 105-113.
18. Green L. C., Kalitsis, P. M., Cipetic, M., Kim, J. Marshall, O. and Hudson, D. F (2012). Contrasting roles of condensin I and condensin II in mitotic chromosome formation . Cell Sci., 125: 1591-1604.
19. Grundmann, E. (1979). In Handbuch der allgemeinen Pathologi c. 2 (1). Edited by H. Altman. Berlin – Heidelberg - New York. Pages: 282 – 479
20. Hassan, L. and Ahmad S. D. (2000). Chromosomal Aberrations Induced by Chemical Mutagens in Allium cepa. Pakistan Journal of Biological Sciences, 3: 1186 – 1189.
21. Hughes, S. (1993). Carnations and Pink. The Crowood Press, Marlborough, UK.

22. Ignacimuthu S., Babu C.R. (1983) Induced chromosomal abnormality and pollen sterility in wild and cultivated urd and mungbean. Cytologia 54: 159-167, (1989).
23. Inceer, H, O. Beyazoglu, O. and Ergul, H. A. (2000) . Cytogenetic Effects of Wastes of Copper Mine on Root Tip Cells of Allium cepa L. Pakistan Journal of Biological Sciences, 3: 1186 – 1189.
24. Jayabalan, N and Rao, G. R. (1957). Gamma radiation induced cytological abnormalities in Lycopersium esculentum Mill. Var. Pusa Ruby, Cytologia, 52, 1987, pp.1-4.
25. Kamble, G. C and Petkar, H. J. (2014). Comparative mutagenice effects of sodium azide and X-ray in the Wild Chickpea Cicer reticulatum L. IJISET - International Journal of Innovative Science, Engineering & Technology, ISSN 2348 – 7968, Vol. 1 (9), 410 - 415.
26. Kavithamani, D. Kalamani, A. C. Vanniarajan, C. and Uma, D. (2008). Mutagenic effectiveness and efficiency of gamma rays and EMS in soybean (Glycine max (L.) merrill). Madras Agric. J. 95(7-12): 448-451.
27. Khan M. H. and Tyagi S. D. (2010). Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soybean Glycine max L. Merrill. J. Plant Breeding Crop Sci. 2 (3): 055 - 058.
28. Kharkwal, M.C. (1998). Induced mutations in chickpea (Cicer arietinum L.). I. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. Indian Journal of Genetics and Plant Breeding, 58: 159 - 167.
29. Klasterka I., Natarajan A.T., Ramel C. (1976) An interpretation of the origin of subchramatid aberrations and chromosome stickiness on a category of chromatid aberration. Hereditas 83: 153-162.
30. Kumar, G. and Rai, P. (2007). EMS Induced Karyomorphological Variations in Maize (Zea mays L.) Inbreds. Turk. Jour. Biol., Vol.31, pp. 187-195.
31. Kumar, G. and Singh, V. (2002). EMS induced chromosomal stickiness in Hordeum valgare. The Nucleus, 45(3): 139-142.
32. Kumar, G.and Avisha,G. (2002). Comparative mitotoxicity and mutagenicity of two heavy metals in Allium cepa L., J. Cytol. Genet.,Vol. 4 , , pp. 169-173.
33. Laurie, A., Kiplinger, D. C. and Nelson, K. S. (1968) .Commercial Flower Forcing (7th Ed.). McGraw-Hill Book Company, New York.72-76, 94- 96, 105,116, 117, 284, 285 p.

34. Lee, S. Y., Yae, B.W. and Kim, K. S. (2005). Segregation patterns of several morphological characters and RAPD markers in interspecific hybrids between Dianthus giganteus and D. carthusianorum. Scientia Horticulturae. 105: 53-64.
35. Malode, S. N. (1995). Cytogenetic studies in Brassica carinata A. Braun. Ph.D. thesis. Nagpur University, Nagpur.
36. Maraj, U. and Niamat, A. (2011). Genotoxic effect of ethyl methane sulphonate (EMS) in Rattus norvegicus . 6 (2) January – February, Article-013.
37. Meenakshi G., Subramanium M. K.. (1960). Studies on the nucleous II. The persisting nucleoli of Cicer arietinum Lin. Proc. Ind. Acd. Sci. B.57: 73-82.
38. Mendhulkar V. D., Tejprakash B. and Kharat S. N. (2015). Cytogenetical and Morphological Variations in EMS treated Glycine max Linn. (Merr.). Research in Biotechnology, 6(4): 19-26. Also available at www.researchinbiotechnology.com
39. Mitra P. K and Bhowmik G. (1996). Studies in the frequency and segregation of induced chlorophyll mutation in Nigella sativa L. Adv. Plant Sci. 12: 125-129.
40. More U. A. (2016). Induced mutation studies for development of new improved Brassica napus L. Cv. Excel. Ph.D. Thesis, Sant Gadge Amravati University, Amravati.
41. Mundhe, B. F. (2008). Gamma rays and Ethyl methane sulphonate (EMS) induced mutation studies in soybean Glycine max (L.) Merill. Ph. D. Thesis, University of Pune, Pune (M.S.).
42. Patil, B. C. and Bhat G. I. (1992). A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in Clitoria ternatea L. Cytologia 57: 259 – 264
43. Pavadai, P., Girija, M., Dhanavel, D. (2009).Effectiveness efficiency and biochemical content of physical and chemical mutagens in soybean (Glycine max (L.) merrill). J. Phytol.1:444 – 447.
44. Ramesh, A. and Verma, R. C .(2015). Effect of gamma radiation on chromosome at mitotic division in phlox Drummondii. International Research Journal of Biological Sciences, Vol. 4(1), 82-85. ISSN 2278 - 3202. Also availabe on: http:// www.isca.in, www.isca.me
45. Roychowdhury, R. (2011). Effect of Chemical Mutagens on Carnation (Dianthus caryophyllus L.): A Mutation Breeding Approach, LAP Lambert Academic Publishing,Germany.
46. Roychowdhury, R. and Tah, J. (2011a). Chemical mutagenic action on seed germination and related agrometrical traits in M1 Dianthus generation. Current Botany, 2 (8), 19-23.
47. Sassi Kumar, D., Nepolean, T. and Gopalan, A. (2003).Effectiveness and Efficiency of the mutagens gamma rays and EMS on lima bean (Phaseolus lunatus L.). Indain J. Agric. Res. 37: 115-119
48. Sharma, A. K., Singh, V. P. and Singh, R. M. (2006): Efficiency and effectiveness of the gamma rays, EMS and their combinations in urdbean; Indian J. Pulses Res.19: 111-112.
49. Shelke, Prerna B (2014). Incorporation of qualitative and quantitative characteristics in Brassica campestris L. through induced mutation. Ph.D. thesis, S.G.B. Amravati University, Amravati.
50. Siddiqui, B. A. and Khan, S. (1999). Breeding in crop plants: Mutation and In vitro Mutational Breeding.1st Edn. Kalyani Publisher, Ludhiyana.
51. Singh G. M and Khanna V. K. (1988). Effect of gamma radiations on the cross ability of wheat, triticale and rye and meiosis, pollen grain germination and pollen tube growth. Cytology 53: 123-130
52. Singh, R. K. and Chaudhary, B.D. (1985). Biometrical Methods in Quantitative Genetic Analysis. pp. 318. Kalyani Pub., Ludhiana.

53. Sirsat, R. K., Mohrir, Kore, M. A. and Bhuktar, A. S. (2010) Induced Mutation in Horsegram: Mutagenic efficiency and effectiveness., Recent Research in Science and Technology, 2 (7), pp. 20-23.
54. Soheir., E. Antooinette, H and Arif, H. (1989): Cytological effect of herbicide Garlon-4 on root mitosis of Allium cepa. Cytologia, 54:465-472.
55. Staby, G. L., Robert son, J. L., Kiplinger, D. C., and Conover, C. A. (1978). Chain of Life, Ohio Florist's Association, Ohio State University, ColumbusRoychowdhury, R. and Tah, J. (2011a). Chemical mutagenic action on seed germination and related agrometrical traits in M1 Dianthus generation. Current Botany, 2 (8), 19-23..
56. Staby, G. L., Robert son, J. L., Kiplinger, D. C. and Conover, C. A. (1978). Chain of Life, Ohio Florist's Association, Ohio State University, Columbus.
57. Stadlerl, J. (1930). Some genetic effects of X-rays in plants. - J. Heredity 22:3-19.
58. Su Yeons, K. (2002). Genetic relationship among Korean Dianthus species based on morphological characteristics and RAPD Analysis
59. Sultan, A. O. and T. A Celik (2009). Genotoxic and Antimutagenic Effects of Capparis spinosa L. on the Allium cepa L. Root Tip Meristem Cells, Caryologia , 62 (2) : 114-123.
60. Swann P. F (1990). Why do O6 - alkylguanine and O4-alkylthymine miscode? The relationship between the structure of DNA containing O6 - alkylguanine and O4 - alkylthymine and the mutagenic properties of these bases. Mutat Res, 233 (1 -2):81-94.
61. Tambe, A.B. (2009). Induction of genetic variability in Soybean Glycine max (L.) merrill for yield contributing traits. Ph. D. Thesis, University of Pune, Pune (M.S).
62. Tarar, J.T. and Dnyansagar, V. R. (1980a) Comparison of ethyl methane sulphonate and radiation induced meiotic abnormalities in Turnera ulmifolia Linn var. Angustifolia wild., Cytologia, 45, pp. 221-231.
63. Thengane S. and Tengane R. J. (1986). Nucleolar behaviour during mitosis of Cassia aungustifolia. Effect of gamma radiation on nucleolar persistence. J. Cytol. Genetics 21: 77-81.
64. Tomlekova, N.B. (2010). Induced mutagenesis for crop improvement. Pl. Mut. Rep. 2, 4-27.
65. Vandana, and Dubey D. K. (1292), Heritability and genetic advance in induced mutants of faba bean (Vicia faba L.), J. Genet.Breeding, Vol. 46, pp. 143-146.
66. Vandana, and D. K. Dubey (19992). Mitotic anamlies induced by EMS and DES in Vicia faba L., J. Indian Bot. Sci., Vol. 71, , pp. 179-184.
67. Wadanker G. W. (2014). In vitro mutation studies in Dehlia cav. With special reference to quality improvements. Ph.D. Thesis, Sant Gadge Amravati University, Amravati.
68. Wongpiyasatid, A., Chotechuen, S. and Hormchan, P. (2000). Induced mutations in mungbean breeding regional yield trail of mungbean mutant lines. Kasetsart J. Nat. Sci. 34, 443-449.

EFFECT OF ETHYL METHYL SUPPHONATE (EMS) ON SEED GERMINATION IN *DIANTHUS CARYOPHYLLUS* L .VAR. CHABAUD

DESHMUKH, P. D.

Department of Botany,

Shri Shivaji College of Arts Commerce and Science,

Akola – 444001, (M.S.), INDIA

Email: prashantdeshmukh2008@gmail.com

Abstract

The present investigation was carried out to evaluate the effect of different concentration of EMS on seed germination in *Dianthus caryophyllus* L. var. Chabaud. Seeds were treated with three different treatments viz., 12 Hrs. Dry, 12 Hrs. pre-soaked + 6 Hrs. EMS and 18 Hrs. pre - soaked + 6Hrs. EMS. Different doses of EMS were selected on the basis of LD_{50} and doses 0.003%, 0.006% and 0.009% were used for experimentation along with control. Germination was carried out in Petri - plates in controlled condition in three replicas. The study revealed that increased doses concentration results into corresponding decreased in germination percentage for all the three treatments. Treatment 18Hrs. PSW + 6Hrs. EMS shows better germination percentage followed by 12Hrs. PSW + 6Hrs. EMS and 18Hrs. Dry. 18Hrs. Dry EMS showed 76% germination at 0.003% dose, 68% for 0.006% dose and 59% for 0.009% dose as compared to control (81% germination). Slight increase in germination percentage was recorded in 12Hrs. PSW + 6Hrs. treatment. Doses 0.003%, 0.006% and 0.009% showed 79%, 69%, 63% germination in 12Hrs. PSW + 6Hrs. treatment of EMS with control (83%). Highest germination percentage was recorded for 18Hrs. PSW + 6Hrs. treatment in dose 0.003% with 81% germination. It was followed by 0.006% with 73% germination and 0.009% with 65% germination. However control recorded maximum (84%) germination in 18Hrs. PSW + 6Hrs. treatment.

Key words: Dianthus caryophyllus, Chabaud, germination, Ethyl Methyl sulphonate (EMS)

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) 2n = 30 belongs to the angiospermic family Caryophyllaceae and it is well known cut flower with its variegated petals colour, high spicy fragrance and long shelf life of flowers **(Deshmukh and Malode, 2018)**. It is an important floriculture crop all over the world and ranks just next to rose in popularity in western countries **(Staby *et al.*, 1978).** It is mostly found in temperate climate throughout the world with high worldwide demand due to its pharmacological properties, aromatic things and polymorphism in morphology, genetics and hybridization **(Facciola, 1990 and Hughes, 1993).**

As the frequency of natural mutation is very low in nature, mutation can also be artificially induced for bringing the desired attributes. Any sudden change either in the amount or in the arrangement of structure of DNA of a living being is called mutation. Induced mutation by using physical and chemical mutagens is a way to produce genetic variation, resulting in the creation of new verities with better characteristic (Wongpiyasatid, 2000). EMS as most effective and powerful mutagen was earlier reported by **Van Harten, (1998)** and **Khatri *et al*. (2005)** due to high frequency of gene mutations.

REVIEW OF LITERATURE

On accounts of its excellent keeping quality, wide range of form, colors and ability to withstand long distance transportation they are excellent for bedding, pots, borders, edging and rock garden along with commercial importance. They also have particular demand for Mother's day, wedding, Christmas, valentine day and to pay attributes to one of their beloved god. Carnations are associated with some sentiments and symbols **(Jawaharlal *et al.*, 2005)**. In Korea, red and pink carnations are used in expressing love and gratitude toward parents on parent's day. In French culture, carnation symbolized misfortune and bad luck. It is a traditional funeral flower in France, given in condolence for the death of a loved one (**Jawaharlal *et al.*, 2005).**

In traditional European Herbal medicine, it is prescribed to treat coronary, nervous disorders and also used as Alexi telic, antispasmodic, cardio tonic, diaphoretic and nerving **(Shiragur *et al*., 2004)**. Whole plant has been use as a vermin-fuse in China and as an animal feed in Spain. Due to all these mentioned commercial importance, these plants stand preferentially first in selection and hence mutational studies in these plants definitely will open various aspects in future generations. Also as this plant is the plant of temperate region (**Shiragur *et al.*, 2004)**, this limits can also be overcome by developing mutants with worldwide distributions.

MATERIAL AND METHOD

The present work was carried out to developed suitable cultivar of *Dianthus caryophyllus* L. variety Chabaud for (open field) cultivation in Vidarbha region through induced mutation. The experiments were performed in post graduate laboratory of cytology and genetics, Department of Botany, at Govt. Vidarbha Institute of Science And Humanity, Amravati, and Maharashtra, India. The experiment included six standard Mix Chabaud varieties *viz.*, Black King, Pink Rose, Purple (Violet), Milky White and White, seeds of which were collected from Universal Seeds Company, Pune. The seeds can also obtain from Namdhari Seeds Company, Bangalore.

EMS (Ethyl Methyl Sulphonate) mutagen **(Plate 2)** in three different concentrations (0.003%, 0.006% and 0.009%) for dry, 12 hours presoaked and 18 hours presoaked along with control were used in experimentation. Doses were determined on the basis of Lethal dose (LD_{50}) value. In dry seed treatments, the seeds are directly treated with mutagens

without any prior procedure. In Pre-soaked water treatment, seeds were immersed in water for different hours before treated with mutagens. In 12hr pre-soaked treatments, 50 seeds in three replicas were kept in distilled water for 12 hrs and then shifted for 6 hours treatment with respective doses of EMS. Similarly in 18hrs pre-soaked treatments, 50 seeds in three replicas were kept in distilled water for 18 hrs and then shifted for 6 hours treatment with respective doses of EMS **(Plate 2).**

50 sees were allowed to grow over moist blotting paper kept in Petri plate and analyzed the result of germination in three replica for each concentration. Cut the blotting paper cut according to the size of floor of Petri-plate and wetted well through distilled water and seeds were equally place over the blotting paper **(Plate 2)**. Close the Petri-plate tightly and kept it in well moistened 21^0 C controlled conditions. Seeds shows germination after 72 hours and recorded after fifth day (110 hours). A diffuse light (20 volt bulb with Tug stance coil) was provided after 72 hours for a span of 4-6 hours per day. Averages of all three replicas were considered for further analysis. Germination percentage was calculated by the below mentioned formula.

$$\text{Seed germinations (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}}$$

RESULT AND DISSCUSSION

Germination is the process by which a seed initiates growth after a period of quiescence and it starts with the uptake of water by imbibitions through the dry seed, followed by embryo expansion **(Bewley and Black, 1994).** Once the radical has emerged germination get finished. It requires seed imbibitions and is defined as the process leading to emergence of the radical through the testa **(Bewley, 1997 and Koornneef *et. al*., 2002)**.

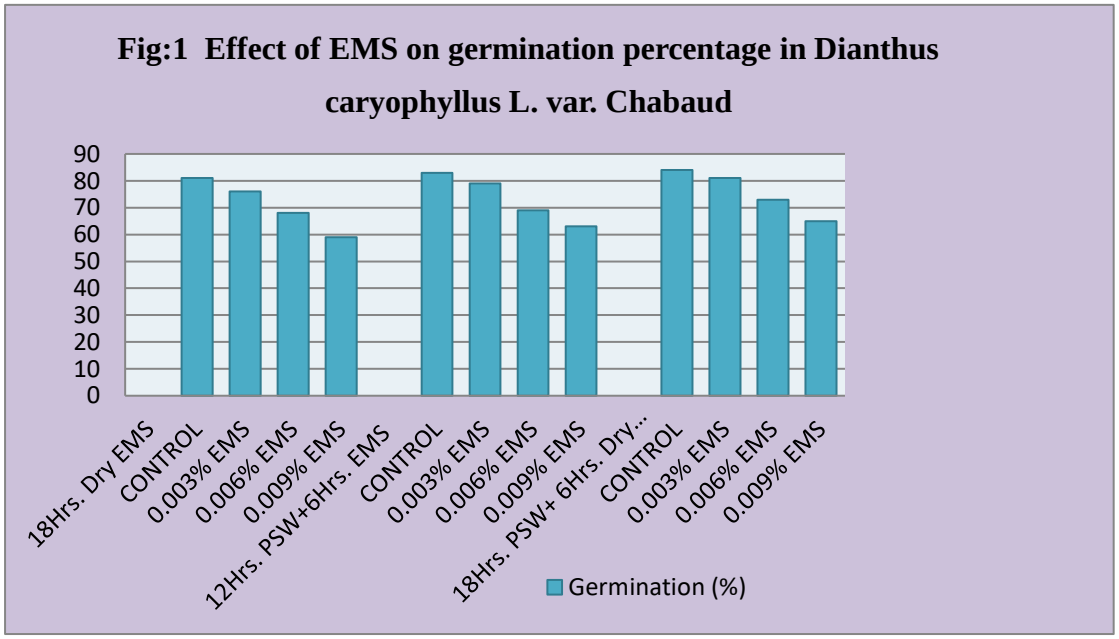
18Hrs. Dry EMS showed 76% germination at 0.003% dose, 68% for 0.006% dose and 59% for 0.009% dose as compared to control (81% germination). Slight increase in germination percentage was recorded in 12Hrs. PSW + 6Hrs. treatment. Doses 0.003%, 0.006% and 0.009% showed 79%, 69%, 63% germination in 12Hrs. PSW + 6Hrs. treatment of EMS with control (83%). Highest germination percentage was recorded for 18Hrs. PSW + 6Hrs. treatment in dose 0.003% with 81% germination. It was followed by 0.006% with 73% germination and 0.009% with 65% germination. However control recorded maximum (84%) germination in 18Hrs. PSW + 6Hrs. treatment.

It was observed that increased in doses concentration results into corresponding decreased in germination percentage (Table:1 and Fig.1). Similar findings were also reported by **Mahna *et al*. (1989**) in *Vigna mungo*, **Potdukhe and Narkhede (2002)** in pigeon pea and **Datir *et al.* (2007)** in horsegram, **Bhosale *et al.* (2013)** in urdbean (*Vigna mungo* L. Hepper), forsk., **Sarada *et al*. (2015)** in Coriander (*Coriandrum sativum* L.), **Deshmukh and**

Malode (2018) in *Dianthus caryophyllus* var. Chabaud, **Ninawe *et al*. (2018)** in *Brassica juncea* (L) Czern and Coss. However no any fixed pattern and doses relation with seed germination have been noticed by **Ambavane *et al*. (2015)** while studying both the variety of in *Eleusine coracana* L. Gaertn.

Table 1: Effect of EMS on germination percentage in *Dianthus caryophyllus* L. var. Chabaud

Treatments	Germination (%)
18Hrs. Dry EMS	
CONTROL	81 ± 0.04
0.003% EMS	76 ± 0.03
0.006% EMS	68 ± 0.02
0.009% EMS	59 ± 0.03
12Hrs. PSW+6Hrs. EMS	
CONTROL	83 ± 0.04
0.003% EMS	79 ± 0.02
0.006% EMS	69 ± 0.01
0.009% EMS	63 ± 0.02
18Hrs. PSW+ 6Hrs.**EMS**	
CONTROL	84 ± 0.04
0.003% EMS	81 ± 0.03
0.006% EMS	73 ± 0.01
0.009% EMS	65 ± 0.02



It was also noted that lower doses were found to be more effective and beneficial for all the three mutagens studied **(Fig 1).** EMS with doses 0.003% and 0.006% were found to be more beneficial and useful. Similar result was also revealed by **Konzak *et al*. (1965),**

Mensah *et al.* (2005) in cowpea (*Vigna uguiculata*), **Dhakshanamoorthy *et al*., (2010)** in *J. curcas*, **Warghat *et al.* (2011)** in muska ora (*Abelmoschus moschatus)*, **Emrani *et al*. (2011)** in *Brassica napus* L, **Raychoudhary and Tah, (2011)** in *Dianthus caryophyllus* and Mshembula (2012) in Cowpea.

Treatment 18Hrs. PSW + 6Hrs. EMS shows better germination percentage followed by 12Hrs. PSW + 6Hrs. EMS and 18Hrs. Dry 18Hrs. **(Table 1).** Similar finding were reported by **Malode (1995)** in *Brassica carinata*., **More (2016)** in *Brassica napus* L. cv. Excel. However **Shelke (2014)** in *Brassica compestris* and **Elfky, *et al*. (2014)** in *Helianthus annus* reported reverse finding and observed that the increased concentrations of sodium azide and time of soaking had a negative effect on the percentage of germination.

The above results could be attributed to chromosomal aberrations induced enzyme activity such as catalase and lipase and hormonal activity resulted in reduced germination **(Ananthaswamy *et al*., 1971)**. However **Dhakshanamoorthy *et al*. (2010)** recorded in *J. curcas* that the stimulatory effect at a lower dose is due to the fact that mutagens at lower concentrations stimulate the role of enzyme and growth hormone responsible for growth and yield while the inhibitory effect is due to the fact that biological damage increased at a faster rate in higher concentrations of mutagens.

CONCLUSION

All the treatments were found to affect germination percentage with respect to control. Dose 0.003% 18Hrs. PSW + 6Hrs. EMS, 12Hrs. PSW + 6Hrs.EMS and Dry 18 Hrs. showed higher germination percentage with respect to other doses of the same treatments. However germination percentage for all the treatments shows lower value compare to control. Selection of lower doses with enhance period of pre-soaked found to be more fruitful in inducing higher mutagenic effect by any chemical mutagens.

REFERENCES

Ambavane, A. R, Sawardekar, S.V. Sawantdesai, S.A. and Gokhale, N. B. (2015). Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L.Gaertn). ***Journal of Radiation Research and applied Sciences*, 8**, 120-125.

Ananthswami H. N., Vikil, V. K. and Sreenivasan, A.(1971). Biochemical and physiological changes in gamma irradiated wheat during germination. ***Rad. Bot. 11*,** 1-2.

Bewley, J. D. and Black, M. (1994). Physiology and Biochemistry of Seeds inrelation to germination. 2. Viability, dormancy and environmental control***, Berlin, Springer-Verlag*.

Bewley, J. D. (1997). Seed germination and dormancy.***The plant cell***. ***American Soc. of Plant Physiol*. 9,** 1055-1066.

Bhosale1, U. P. Hallale, B. V and Dubhashi, S. V. (2013).M_1 generation studies in urdbean (*Vigna mungo* (L.) Hepper. Pelagia Research Library, ***Advances in Applied Science Research,* 4** (3), 95-97**. *Available online at* www.pelagiaresearchlibrary.com**

Datir, S. S., Dhumal, K .N., Pandey, R. N. (2007). Gamma radiation and EMS induced variation in seed germination and plant survival in Horse gram **(*Macrotyloma uniflorum* Lam. Verdc). *J. Arid Legumes* 4**(1), 15-17**.**

Deshmukh, P. D. and Malode, S. N. (2018).Effects of gamma radiation on seed germination, plant survival and growth characteristics in *Dianthus caryophyllus* L. var. Chabaud. *Journal of Global Biosciences*, 7 (3), 5403-5410. ISSN 2320 – 1355.

Dhakshanamoorthy, D., Selvaraj, R., Chidambaram R. (2010). Physical and chemical mutagenesis in *jatropha curcas* L. to induce variability in seed Germination, growth and yield traits. ***Rom. J. Biol. – Plant Biol*., 55,** (2), 113–125.

Elfeky, S. Hamad, S. and Allah, K. M. (2014). Physiological impact of sodium azide on *Helianthus annus* seedlings. ***International Journal of Agronomy and Agricultural Research*, 4** (5), 102 - 109**. ISSN: 2223 – 7054 (Print), 2225 - 3610 (Online). Also available at: http://www.innspub.net**

Emrani, S. N.,Arzani, A. and Saeidi, G. (2011). Seed viability, germination and seedling growth of canola (*Brassica napus* L.) as influenced by chemical mutagens. ***African Journal of Biotechnology, Vol.10,*** (59), 12602-12613**.**

Facciola, S.(1990). Cornucopia: A Source Book of Edible Plants. Kampong Publications, Vista, California, pp. 678.

Hughes, S. 1993. Carnations and Pink.The Crowood Press, Marlborough, UK

Jawaherlal, M., Padmadevi, M. Jagdeshwari, R. and Rarthikeyan, S.(2005).*A technical guide on carnation*.Department of Horticulture and Landscaping, **Horticultural College of Research Institute,** TNAV, Coimbatore, India.

Khatri, A., Khan,I. A., Siddiqui, M. A., Raza, S. and Nizamani, G. S. (2005). Evaluation of high yielding mutants of *Brassica juncea* cv. S - 9 developed through gamma rays and EMS. ***Pak. J. Bot.*, 37** (2), 279 - 284.

Konzak C. F., Nilan, R. A. Wagner, J. and Foster, R. J. (1965). Efficient chemical mutagenesis in the use of induced mutations in plant breeding.Radiation Botany (suppl.), 5, 49 - 70.

Koornneef, M., Bentsink, L. and Hilhorst, H.(2002). Seed dormancy and germination.***Current Opinion and Plant Biol., 5* (1)**, 33-36.

Mahna, S. $., Garg, R. and Parvateesam, M. (1989). Mutagenic effects of Sodium azide in Black gram. ***Curt. Scr., 58,*** 582 - 584.

Malode, S. N. (1995). Cytogenetic studies in *Brassica carinata* A. Braun. ***Ph. D. thesis. Nagpur University, Nagpur*.**

Mensah, J. K., Akomeah, P. A. and Ekpekurede, E. O. (2005). Gamma irradiation induced variation of yield parameters in cowpea (*Vigna uguiculata* L. Walp). ***Global Journal of Pure and Applied Science*, 11**(3), 327-330.

More, U. A. (2016). Induced mutation studies for development of new improved *Brassica napus* l. cv. excel. ***Ph.D. thesis, Sant Gadge Baba Amravati University***,Amravati.

Ninawe, P. J., Malode, S. N. and Jambhulkar, S. J. (2018). Sodium azide induced yellow seed coat colour mutant in *Brassica juncea* (L.) Czern and coss cv.Bio-902.***Aayushi International Interdisciplinary Research Journal (AIIRJ),* special Issue No. 25**, ISSN 2349-638x.

Potdukhe, N. R. and Narkhede, M. N. (2002). Induced mutation in pigeon pea (*Cajanus cajan* L. Millsp.). ***J. Nucl. Agric. Biol. 31***(1), 41-46.

Roychowdhury, R. and Tah, J. (2011). Chemical mutagenic action on seed germination and related agro - metrical traits in M1 *Dianthus* generation. ***Current Botany*, 2** (8): 19-23. ISSN: 2220 - 4822. Also available on: http://currentbotany.org.in

Sarada, C., Srinivasa Rao, V. Umajyothi, K. and Reddy, P. V. (2015). Induced polygenic variability in coriander (*Coriandrum sativum* L.) and scope of selection. Int. ***J. Pure App. Biosci.*, 3** (2), 440 - 444. ISSN: 2320 – 7051.Available online at: http://www.ijpab.com

Shelke, P. B. (2014). Incorporation of qualitative and quantitative characteristics In *Brassica campestris* L.through induced mutation.***Ph.D. thesis Sant Gadge Baba Amravati University, Amravati.***

Shiragur, M., Shirol, A. M., Reddy, B. S., Kulkarni, B. S.(2004). Performance of standard Carnation (*Dianthus caryophyllus* L.) cultivars under protected cultivation for vegetative characters. ***Journal of Ornamental Horticulture,* 7**, 212-216.

Staby, G. L., Robert son, J. L., Kiplinger, D. C., and Conover, C. A. 1978. Chain of Life, Ohio Florist's Association, Ohio State University, Columbus.

Van Harten, A. M. (1998).*Nature and Types of mutations. Mutation breeding.****Theory and practical applications. Cambridge University Press***, 64 - 110.

Warghat, A. R. Rampure, N. H. and Wagh, P, (2011). Effect of Sodium azide and Gamma rays treatments on percentage germination, survival, morphological variation and chlorophyll mutation in Musk okra,(*Abelmoschus moschatus* L.). ***International Journal of Pharmacy and Pharmaceutical Sciences, 3*** (5), 483-486.