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TH Nepolian
Department of Genetics & Plant Breeding, COA, CAU, Imphal, Manipur, India

MS Jeberson
AICRP (MULLaRP), Directorate of Research, CAU, Imphal, Manipur, India

Manish Kumar
Department of Genetics & Plant Breeding, COA, CAU, Imphal, Manipur, India

Singh NB
Department of Genetics & Plant Breeding, COA, CAU, Imphal, Manipur, India

Shashidhar KS
AICRP (MULLaRP), Directorate of Research, CAU, Imphal, Manipur, India

PH Ranjit Sharma
Department of Genetics & Plant Breeding, COA, CAU, Imphal, Manipur, India

Correspondence
Manish Kumar
Department of Genetics & Plant Breeding, COA, CAU, Imphal, Manipur, India

Mutation and variability studies in M_2 generation of field pea (*Pisum sativum*) under foot hills of Manipur

TH Nepolian, MS Jeberson, Manish Kumar, Singh NB, Shashidhar KS and PH Ranjit Sharma

Abstract
Three genotypes of field pea namely Makhyatmubi, Makuchabi and Rachna were used in the present study. A chemical mutagen, sodium azide, a mono-functional alkylating agent was used in three concentration for induction of mutation in each varietyand subjected to 0.1%, 0.3% and 0.5% of Sodium Azide for 6 hours with intermittent shaking at room temperature 25±2 ºC. Among the parameters studied in M_2 generation chlorophyll mutation frequency and spectrum is very important as it is also used to calculate mutagenic effectiveness. The mutagenic effectiveness of Sodium Azide ranged from 7.08 to 11.10, 5.36 to 7.40 and 5.48 to 14.40 was observed in Makhaytmubi, Makuchabi and Rachna respectively. Whereas spectrum of chlorophyll mutation is concerned it increased with increase in the concentration of Sodium Azide in field pea. Altogether four types of chlorophyll mutation in the order chlorina>viridis>xantha>albina, were identified in M_2 generation of present study. The mutagenic effectiveness of Sodium Azide was found to be higher at lower doses. Although when we look at the mean of the three genotypes an increasing trend in the efficiency of Sodium Azide in producing injury, lethality and sterility in the M_1 population of field pea.

Keywords: mutation, generation, *Pisum sativum*, Manipur

Introduction
Among pulses, pea (*Pisum sativum* L.), also known as field pea and garden pea in English, and *Matar* in Hindi is one of the important *rabi* (winter) crops grown in the world and India. Pea (2n=2x=14) belongs to the family leguminosae and genus Pisum. It is an annual herbaceous, self-pollinated crop. Field pea derives from the Middle East and was first cultivated roughly 10,000 years ago (Jing *et al.*, 2010) [9]. According to Blixt (1970) [4], the Mediterranean is the primary centre of diversity with secondary centres in Ethiopia. Field pea is one of the important pulse crop of India, grown in an area of 0.68 million hectares producing 0.62 MT of grain. The average national productivity of field pea is 911 kg/ha. It is consumed as both green immature seeds as well as dry seeds.

Availability of genetic variation is a pre-requisite for any crop improvement programme but pulses like pea generally lack genetic variability due to their autogamous nature. If enough variability does not pre-exist, then genetic variation can be created by several means; among which hybridization and induced mutation is one of the important methods. In pea, creation of variation through hybridization is tedious processes, due to highly self-pollinated, small, fragile flowers, that make it difficult to carry out the process of emasculation. Hence, the classical breeding methods have got limited application in pea and other pulses and as such, mutation breeding appears to play an important role in the improvement of this important pulse. So, as the primary objectives of mutation breeding is to enlarge the frequency and spectrum of mutations as an approach towards directed mutagenesis, the present investigation on study of biological effects of Sodium Azide in M_2 generation with following objectives of estimation of the chlorophyll mutation frequency and spectrum and estimation of the mutagenic effectiveness and efficiency.

Materials and Methods
The experimental field is situated at 24º 51'N latitudes 93º 56'E longitudes and at an altitude of 790 m above mean sea level. The climate of Imphal is sub-tropical with an average annual

rainfall of about 1212 mm which is distributed mainly during the five monsoon months from June to October. The mean annual maximum and minimum temperatures are 35 ºC and 5 ºC respectively. During the most part of the year humidity ranges from 70 to 80 percent with an average annual sunshine of about 2231 hours. The soil of the experimental site is clay in texture with acidic soil reaction. It is high in organic carbon content, medium in available nitrogen and phosphorus with high in available potassium. The pH of soil ranges from 5.60 to 6.80.

Three genotypes of field pea namely Makhyatmubi, Makuchabi and Rachna were used in the present study. A chemical mutagen, sodium azide, a mono-functional alkylating agent was used in three concentration for induction of mutation in each variety. For Sodium Azide treatment, the selected seeds of each variety were divided into 4 lots which contain 375 seeds per lot in cloth bags. Out of 4 lots, one lot of seeds in cloth bag for all the genotypes were kept as control i.e., no treatment. The three remaining lots of each genotypes were used for Sodium Azide treatment. For chemical treatment, the seed lots were pre-soaked in distilled water for 6 hours before the treatment. One lot of pre-soaked seeds from each of the three genotypes was then subjected to 0.1%, 0.3% and 0.5% of Sodium Azide for 6 hours with intermittent shaking at room temperature 25±2 ºC. Treated seeds of three field pea genotypes along with control were planted on raised beds with single seed per hill. The seeds were sown at 3-5 cm depth. Fertilizers were applied at the rate of 20: 40: 20 kg/ha NPK in the form of Urea (46% N), Single Super Phosphate (16% P_2O_5) and Muriate of Potash (60% K_2O) one day prior to planting in raised beds for better initial growth of the plant. After planting, irrigation was provided at proper interval for better establishment of the plants. Proper weeding and plant protection measures were taken up as and when required.

Result and Discussion

Studies in M_2 generation

The M_2 progeny was screened thoroughly and various chlorophyll mutations were scored throughout the crop duration. Mutation frequency was calculated as percentage of mutated progenies to total population. The mutations affecting different morphological features of the plants are grouped and described below.

Spectrum of chlorophyll mutation

The detection of various chlorophyll mutations were made as per classification of Gustaffson (1940) [8] and Blixt (1961) [3]. The seedling was scored for chlorophyll mutation in the field from the first day of emergence to fourth weeks of the M_2 generation. Four types of Chlorophyll mutations were observed. They are chlorina, viridis, xantha and albina. Kartashova *et al*. (1972) [10] studied production of mutations induced by gamma rays treatment in four pea varieties.

Frequency of chlorophyll mutation

The frequency of chlorophyll mutants calculated as percent of M_2 plants screened for mutation. A linear relationship between mutation frequency and the concentration of Sodium Azide was observed as their frequency increased with increasing concentration of Sodium Azide irrespective of the genotypes. Similar result was reported by Khan and Siddiqui (1993) [11] of chlorophyll mutants in M_2 generation of EMS treated two varieties of mungbean, Vanniarajan *et al.* (1996) [15] in two varieties of black gram with gamma rays and EMS, Waghmare and Mehra (2001) [17] in M_2 generation of gamma ray and EMS treated P27 variety of *Lathyrus sativus*, Auti *et al.* (2007) [1] reported in M_2 progenies of EMS treated mungbean, Sharma *et al.* (2010) [13] recorded on pea after seed treatment with gamma ray and EMS, Wani *et al.* (2011) [18] observed in M_2 generation of EMS, HZ and SA treated mungbean variety and Bind *et al.* (2016) [2] found progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. The highest frequency of chlorophyll mutations was observed at 0.5% Sodium Azide (3.54) for Makhyatmubi. No chlorophyll mutations were observed in untreated control. The frequency of various chlorophyll mutation is presented in Table 1.

Mutagenic effectiveness

The result of mutagenic effectiveness calculated as the rate of mutation induced per unit dose of Sodium Azide are presented in Table 2.In the present investigation, effectiveness of mutagenic treatments differed considerably. Overall from Table 2 when we see effectiveness of Sodium Azide in three genotypes of pea as a whole; the lower dose of Sodium Azide was most effective 10.9.Also the effectiveness showed a trend, which was inversely proportional to the increasing concentration of Sodium Azide in pea. The mutagenic effectiveness of Sodium Azide varied from 4.58 to 10.9. Similar result was reported by Velu *et al.* (2008) [16] in M_2 generation of cluster bean with EMS treatment, Girija and Dhanavel (2009) [6] on cowpea with the treatment of EMS, Mahla *et al.* (2010) [12] of EMS and gamma ray, by Sharma (2001) [14] in a field trial study, mutagenicity of a new dimethyl nitroso compound in two pea (*Pisum sativum* L.) cultivars (Early Superb and Mahndofer), Sharma *et al.* (2010) [13] of EMS and gamma irradiation on Arkel and Azad P-1 varieties of garden pea, Wani *et al.* (2011) [18] of EMS, HZ and SA in two varieties of mungbean i.e., PDM-11 and NM-1, Dube *et al.* (2011) [5] of gamma rays and EMS in alone and combination treatments in cluster bean (Sharada) and Girja and Dhanavel (2013) [7] of EMS, DES and SA in cowpea.

Mutagenic efficiency

The mutagenic efficiency for each Genotype at different concentration of Sodium Azide is presented in Table 3.From the table a varied level of Mutagenic effectiveness of Sodium Azideon different genotypes was evident. In genotypes Makhyatmubi, Makuchabi and Rachna the lower concentration (0.1) was found to be most effective in inducing chlorophyll mutation. An increase in mutagenic efficiency was observed with increase in concentration for these three genotypes. In Rachna (14.40), 0.1%Sodium Azide recorded the highest value of effectiveness among all while in case of Makuchabi (5.36), 0.5% Sodium Azide was least effective getting the lowest value of effectiveness among all the three genotypes. The mutagenic effectiveness of Sodium Azide ranged from 7.08 to 11.10, 5.36 to 7.40 and 5.48 to 14.40 was observed in Makhaytmubi, Makuchabi and Rachna respectively.

Table 1: Chlorophyll mutation frequency in the M_2 generation of three field pea genotypes.

Treatment	Number of M_2 seedlings	Chlorina	Viridis	Xantha	Albina	Total number of mutants	Mutation frequency (%)
Makhyatmubi							
Control	285	-	-	-	-	-	-
0.1% NaN_3	270	2	1	-	-	3	1.11
0.3% NaN_3	260	3	1	2	-	6	2.31
0.5% NaN_3	254	4	2	2	1	9	3.54
Makuchabi							
Control	283	-	-	-	-	-	-
0.1% NaN_3	269	1	1	-	-	2	0.74
0.3% NaN_3	271	2	1	3	-	6	2.21
0.5% NaN_3	261	4	2	1	-	7	2.68
Rachna							
Control	284	-	-	-	-	-	-
0.1% NaN_3	277	3	1		-	4	1.44
0.3% NaN_3	260	4	-	2		6	2.30
0.5% NaN_3	255	3	2	1	1	7	2.74

Table 2: Mutagenic effectiveness of NaN_3 in three field pea genotypes.

Treatment	Mutation Frequency (%), Mp				Mutagenic Effectiveness
	Makhyatmubi	Makuchabi	Rachna	Mean	
0.1% NaN_3	1.11	0.74	1.44	1.09	10.9
0.3% NaN_3	2.31	2.21	2.30	2.27	7.56
0.5% NaN_3	3.54	2.68	2.74	2.99	4.58
Mean	2.32	1.87	2.16	-	-

Table 3: Mutagenic efficiency of NaN_3 in three genotypes of field pea.

Treatment	Lethality (L)				Injury (I)				Sterility (S)				Mutagenic efficiency		
	Makhyat-mubi	Makuch-abi	Rachna	Mean	Makhyat-mubi	Makuch-abi	Rachna	Mean	Makhyat-mubi	Makuch-abi	Rachna	Mean	Lethality	Injury	Sterility
0.1%NaN_3	11.37	10.26	0.61	7.41	1.64	0.50	1.17	1.10	3.22	0.40	3.17	2.26	0.41	0.99	0.48
0.3%NaN_3	29.28	11.61	3.02	14.63	2.67	3.00	1.87	2.51	2.22	8.67	4.93	5.27	0.15	0.90	0.43
0.5%NaN_3	38.47	15.31	15.31	23.03	3.22	3.33	3.00	3.18	5.55	12.07	9.17	8.93	0.12	0.94	0.33
Mean	26.37	12.39	6.31	-	2.51	2.27	2.01	-	3.66	7.04	5.75	-	-	-	-

Table 4: Range, mean, standard error (S.E.), genotypic and phenotypic co-efficient of variations, heritability in broad sense and genetic advance for 6 different characters in M_2 generation of field pea.

Character	Mean ± S.E.	Range	Genotypic Variance (σ^2g)	Phenotypic Variance (σ^2p)	Genotypic Coefficient of Variance (GCV)	Phenotypic Coefficient of Variance (PCV)	Heritability in broad sense (h^2)	Genetic Advance (Gs)	Genetic Advance (% of mean)
Seedling height (%)	11.02±0.152	8.75-13.00	2.22	2.43	13.52	14.14	91.35	2.92	26.49
Plant height (cm)	54.56±0.73	49.40-61.73	11.77	16.63	6.28	7.47	70.77	5.88	10.78
Days to 50 percent flowering	77.63±0.70	75.33-78.66	0.15	4.69	0.49	2.79	3.19	0.13	0.16
Days to maturity	115.22±0.77	13.66-17.66	1.52	6.71	1.07	2.24	22.65	1.17	1.01
Number of pods/plant	3.72±0.04	3.26-4.56	0.19	0.21	11.71	12.31	90.47	0.85	22.84

Character	Mean ± S.E.	Range	Genotypic Variance (σ^2g)	Phenotypic Variance (σ^2p)	Genotypic Coefficient of Variance (GCV)	Phenotypic Coefficient of Variance (PCV)	Heritability in broad sense (h^2)	Genetic Advance (Gs)	Genetic Advance (% of mean)
Grain weight (g)	15.83±0.21	13.66-18.33	3.22	3.64	11.33	12.05	88.46	3.46	21.86
Seed yield /plant	28.17±0.0008	0.62-1.03	0.03	0.04	0.61	0.70	75.00	0.44	1.56
Pollen fertility(%)	92.73±0.58	83.76-97.83	16.98	20.05	4.44	4.82	84.68	7.75	8.36
Number of seeds/pod	4.3±0.03	3.06-5.06	0.39	1.34	14.52	26.92	29.10	0.69	16.04
Number of pods/cluster	2.19±0.04	1.73-2.83	0.11	0.12	0.34	0.35	91.66	0.69	31.32

Induced quantitative variations in M_2 generation

The mean, standard error, range, phenotypic co-efficient of variation and genotypic co-efficient of variation of 10 characters are presented in Table 4 as the measures of variability along with heritability in broad sense and genetic advance as the parameters of selection.

The range is the simplest measure of variability which indicates the differences between the lowest and the highest mean values. Among the 10 characters studied for the variability in the M_2 generation of Sodium Azide induced quantitative mutations in field pea, the highest range value when expressed in terms of percentage of the lowest value of the range was recorded from the character seedling height, pods per cluster (63.58) followed by seeds per pod (66.12) and seed yield per plant (65.36%) and so on.

The phenotypic co-efficient of variation (PCV) indicated the total observable variation which consisted of variations attributable to genotypic differences and environmental variation while the genotypic co-efficient of variation (GCV) measures only the genetic variation which was not affected by environment.

In the present study, the highest value for the GCV was recorded by seedling height followed by number of seeds per pod, seedlings height, number of pods per plant, grain weight, plant height, pollen fertility, days to maturity, seed yield per plant and days to 50% flowering showed the lowest GCV value (Table 4.17). In general, the PCV values were higher than the values of GCV suggesting a marked influence of the environment on the expression of the characters.

The highest differences between the GCV and PCV was observed in the characters number of seed per pod (12.40) followed by 50% flowering (2.33) and plant height (1.19)indicating that these characters were influenced by the environment more than the other characters studied.

High heritability estimates are helpful in making selections of superior genotypes on the basis of phenotypic performance for quantitative characters in the present study, although it ranged from 3.19 per cent for days to 50 percent flowering to 191.66 per cent for no. of pod/cluster. Highest heritability value was estimate for by number of pod/cluster (91.66%) followed by seedling height (91.35%), number of pods per plant (90.47%), grain weight (88.46%), pollen fertility (84.68%) and plant height (70.77%). The other characters showed low heritability which was below 30% (Table 4).

Genetic advance as percentage of mean ranged from 0.16 for days to 50 percent flowering to 31.32 for number of pods per cluster. Estimates of genetic advance was high for number of pods per cluster (31.32) followed by seedling height (26.49), number of pods per plant (22.84) and so on.

Conclusion

Among the parameters studied in M_2 generation chlorophyll mutation frequency and spectrum is very important as it is also used to calculate mutagenic effectiveness. The chlorophyll frequency, in the present study increased with the increasing concentration of the Sodium Azide with a maximum at 0.5% Sodium Azide for each genotype. Whereas spectrum of chlorophyll mutation is concerned it increased with increase in the concentration of Sodium Azide in field pea. Altogether four types of chlorophyll mutation in the order chlorina>viridis>xantha>albina, were identified in M_2 generation of present study. The mutagenic effectiveness of Sodium Azide was found to be higher at lower doses. This suggests an inversely proportional relationship between concentration of Sodium Azide and its effectiveness in inducing chlorophyll mutations per unit dose. When we look at the mutagenic efficiency of Sodium Azide with respect injury, lethality and sterility caused in M_1 generation, various degree of efficiency was observed in different genotypes of field pea. Although when we look at the mean of the three genotypes an increasing trend in the efficiency of Sodium Azide in producing injury, lethality and sterility in the M_1 population of field pea. From the analysis of quantitative characters studied in M_1 generation revealed highest induced variation expressed as range, genotypic covariance (GCV) for the character seedling height (cm), plant height (cm), number of pods/plant, grain weight and number of seeds/pod. The lowest genotypic covariance (GCV) was recorded from the character day to 50% flowering, days to maturity, seed yield/plant and pods/cluster. In the present study high heritability along with high genetic advance was recorded from seedling height (cm), plant height (cm), pollen fertility and grain weight, which indicated that simple selection for these characters may be effective. High heritability and low genetic advance, in the present study was estimated from number of pods/plant, seed yield/plant and number of pods/cluster which indicates that selection for this character may not be rewarding. While other characters showed low heritability and low genetic advance.

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