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Genetic variability induced by gamma radiation and ethyl methane sulphonate on quantitative characters in pigeonpea (*Cajanus cajan*)

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Abstract
The pure seeds of pigeonpea ((*Cajanus cajan* (L.) Millspaugh) genotype ICPL-87 and BSMR-736 were subjected to gamma radiation (50, 100, 200 and 300Gy) and Ethyl Methane Sulphonate (5, 10, 20 and 30mM) treatments. The mutations affecting gross morphological changes in growth and yield characters such as growth habit, flowering, plant height, plant maturity and grain yield were scored as quantitative characters. The micro mutations at the population level can be easily detected in the form of increased variations for quantitative traits in the segregation of mutagen treated populations. Micro mutations can alter morph physiological characters hence they are of a particular interest to the plant breeders. Both the mutagens, gamma radiations and EMS proved to be very effective to induce variability in quantitative traits like plant height, days required for first flowering and maturity, number of branches per plant, grain yield per plant and 100 seed weight in M_2 generations for both genotypes. In present investigation positive as well as negative impact on quantitative traits was recorded.

Keywords: Ethyl methane Sulphonate, gamma radiation, mutation, M_1 and M_2 generation, pigeonpea and variability

Introduction
Pigeonpea), belonging to family fabaceae is an important food legume of the semi-arid tropics of Asia and Africa. Pigeonpea ranks sixth in area and production in comparison to other grain legumes such as beans, peas, and chickpeas. Pigeonpea shares 19.11% production in total pulses in India. It is important food legume crop in human diet and is a major source of protein as well as carbohydrates, mineral like calcium, iron, phosphorus etc., iodine, essential amino acids like lycine, cystine and arginine and B complex vitamin (Salunkhe *et al*., 1985) [20]. Pigeonpea contains 20-22% proteins, 1-2% fat, 45-55% carbohydrates, 1-5% fibers, 3-5% soluble sugars, 1.5% water and 16-18% energy (Lawn and Troedson, 1990) [15]. Protein content of the pigeonpea seeds is about 2-3 times more than the cereals. Thus, the food legumes ensure nutritional security to the poor masses of the country (Chaturvedi and Ali, 2002) [4].

Pigeonpea being a leguminous plant is capable of fixing atmospheric nitrogen and thereby restore lot of nitrogen in the soil (Yadav, 1992) [27]. It also has a unique property of maintaining and restoring soil fertility through biological nitrogen fixation and improvement of physical properties of the soil by virtue of its deep root system. It is also consumed as fresh green pods in many Caribbean and Latin American countries and also to some extent in India especially in Gujarat state. Pigeonpea has several advantages over other leguminous crops for broad scale agricultural production. These include drought tolerance, logging and shattering resistance and perenniality, as compared to other legumes which allow the possibility of ratooning.

Mutation breeding is the purposeful application of mutations in plant breeding area. It offers good prospects for the domestication of promising underutilized wild species, for agricultural or horticultural uses as well as for improving adaptation of recently introduced crops to unsuitable environments. All over the world mutation breeding has made significant contribution to crop improvement.

Mutagenesis is efficient tool to create new desirable genetic variability in legumes. There are several steps between the initiation of a lesion in DNA by a mutagen and the ultimate expression of the change in the form of an altered phenotype. Mutation can bring about genetic variability, which is most desirable in crops such as pigeonpea. In the past, several researchers have tried to improve the crop by applying the techniques of mutation breeding. So the present investigation was undertaken to access the spectrum of variability produced for various quantitative and qualitative traits in pigeonpea.

Material and methods

The genetically pure seeds of genotypes BSMR-736 and ICPL-87 of pigeonpea (*Cajanus cajan*) procured from Pulses Research Unit, Dr. Panjabrao Deshmukh Agricultural University, Akola were used in this study. Physical mutagens (gamma rays) and chemical mutagen (Ethyl Methane Sulphonate (EMS)) were used for mutation. Pigeonpea (*Cajanus cajan*) varieties BSMR-736 and ICPL-87 which is having contrasting character were grown in the plot in a single row at spacing of 90/60×30/20(cm) (row to row and plant to plant distance) by dibbing method.

Mutagenic treatment

Healthy and pure seeds of each genotype were treated with respective doses physical mutagen - Gamma rays and chemical mutagen - Ethyl Methane Sulphonate (EMS).

Gamma rays treatment

Dry seeds of both varieties with a moisture level of 12% were irradiated with gamma rays (CO^{60}) at Bhabha Automic Research Centre (BARC), Mumbai. For each treatment 240 seeds were irradiated with different doses of gamma rays i.e. 50Gy, 100Gy, 200Gy and 300Gy. The untreated seeds of same variety served as control.

EMS treatment

For treatment with EMS, 240seeds/treatment were pre-soaked in sterile distilled water for 6 hours, blotted dry and then treated with freshly prepared aqueous solutions of EMS with concentrations of 5mM, 10mM, 20mM and 30mM respectively for 6 hours with intermittent shaking. The treated seeds were rinsed and washed thoroughly in running tap water and two to three times in distilled water, soon after the completion of treatment the traces of chemical mutagen was removed from the seeds and excess of water from the seed was removed with the help of blotting paper and the seeds were dried at room temperature then dried seeds were sown at field immediately. The seeds soaked in distilled water and without treated with EMS were serving as control.

M_1 generation

The mutagen treated i.e. gamma rays and EMS (M_0 material) and control (untreated) seeds of both varieties were sown in the field by using Dibbling method for generation of M_1 population. The plant survival was computed as the percentage of plants surviving till maturity. The biological damage (lethality/injury) was computed as the reduction in plant survival and plant height. Morphological observations viz., Percent germination (%), Plant survival (%), Plant height (cm), days to 1st flowering, number of branches, days to maturity, number of pods, pod weight (gm), Yield (grain weight) per plant (gm), Test weight (gm) were recorded as per standard methodology. Seeds of M_1 harvested plants were bulked per treatment and used for further showing in M_2 generation.

M_2 generation

The seeds of M_1 harvest were bulked treatments wise viz., gamma rays (50Gy, 100Gy, 200Gy and 300Gy) and EMS (5mM,10mM, 20mM and 30mM) and which were sown to raise M_2 in randomized block design with three replications for each treatment.

For every treatment 280 seeds were sown per replication. A block of 14 rows with 20 plants per row was replicated thrice for each treatment under study for both of the genotypes. M_1 plants were advanced to M_2 by sowing seeds. In M_2 generation approximately 14,480 plant population was shown. Methodology given by FAO/IAEA programme was adopted to develop mutation population by plant to progeny method.

Observations of selected plants from each row were recorded on plant basis for the characters *viz.* growth habit, plant height, days to 1st flowering, 50% flowering, number of branches, days to maturity, number of pods, pod weight, grain weight and test weight per plant. Observation for viable mutations were recorded upto harvesting. Mutations affecting diffrent morphological characters were noted. The mutants showing an alternation of plant type and growth habit were recorded with precision.

From M_2 population desirable mutants were isolated on the basis of plant height (cm) early maturity and other desired morphological characters different from parent. There were 27 desirable mutants selected from both genotypes of M_2 population.

Results

In M_2 generation, all the quantitative characters showed the variation trend with different doses of mutagens in all the morphological parameters.

Plant height (cm)

As per result, maximum variation in plant height was observed in T_1 (50Gy) of gamma rays and T_4 (30mM) of EMS treatment for genotype ICPL-87. In case of genotype BSMR-736, T_2 (100Gy and 10mM) recorded maximum variation for both mutagen treatment. The height of mutants in screened M_2 generation comprising of mutants of different treatment ranged between 50cm (50Gy) to 135cm (50Gy and 30mM) for genotype ICPL-87 and between 70cm (300Gy) to 192cm (50Gy) for genotype BSMR-736. The mean value for control plants of ICPL-87 was 108cm and for BSMR-736 was 165cm.

Table 1 it is observed that for genotype ICPL-87 (short type) all the treatments had recorded dwarf plants. The mean height of ICPL-87 wild type was 108cm whereas dwarf plants were recorded with height of 50cm from treatment with 50Gy only. Treatment T_4 (300Gy) also recorded dwarf plant height of 56cm. In case of BSMR-736, the genotype itself is a wild type genotype. Thus in most of the treatment, (T_3, T_4 of gamma rays and T_1, T_2, T_3 and T_4 of EMS) all type of mutants recorded height below the mean height of wild type BSMR-736 and called as control.

From Table 2, the mean plant height was calculated for each treatment on 20 plants per replication. The data recorded that the mean number treatment of gamma rays could surplus the height of type ICPL-87 and BSMR-736. All the treatments reported significantly dwarf plant as compared to for control both of the mutagen.

Days to maturity

As per data recorded in Table 1, maximum variation for days to maturity was observed in T_4 (300Gy) of gamma rays and T_3 (20mM) of EMS treatment for genotype ICPL-87. In case of genotype BSMR-736, T3 (200Gy and 20mM) recorded maximum variation for both mutagen treatment. The mean value for control plants of ICPL-87 was 125 days and for BSMR-736 was 176 days.

From the Table 1 it is observed that for genotype ICPL-87 (early type) all the treatments had recorded early plants which recorded with days to maturity of 105 days from treatment with 200Gy and 20mM of both mutagens. Treatment T_2

(10mM) also recorded early days to maturity of 125 days. In case of BSMR-736, in most of the treatment, (T_1, T_2, T_4 of gamma rays and T_1, T_2, T_3 and T_4 of EMS) all type of mutants recorded maturity below the mean days to maturity of wild type BSMR-736.

From Table 2, the mean days to maturity was calculated for each treatment on 20 plants per replication. The mean performance of plant maturity for ICPL-87 recorded non any treatments significantly early type plants than control for both the mutagen, but in BSMR-736 observed significant difference between control and other treatments of both mutagens.

Table 1: Induced variation in growth and yield parameters in M_2 generation

	Mutagen Type	Treatment	Plant Ht (cm)	Days to Maturity	Days to 1st flowering	Flower Color	No. of Branches	Grain Wt./plant (gm)	100 seed Wt. (gm)
ICPL-87	Control	-	108	125	81	Faint Yellow	13	24.7	8.6
	Gamma	T_1	50-135	119-138	80-93	Y, Y-O, R	6-16	10.9-29	7.89-11.32
		T_2	75-115	116-135	82-91	Y, Y-O	6-12	10.3-30.2	8.11-11.26
		T_3	60-115	105-140	80-93	F Y, Y-O	8-16	9.4-26.5	5.78-10.3
		T_4	56-130	112-149	83-97	F Y, Y-O, R	7-21	4.3-44.4	8.03-11.1
	EMS	T_1	70-110	110-135	82-91	Y, Y-O, R	6-13	6.5-25.6	6.66-10.54
		T_2	72-115	110-135	78-91	Y, Y-O	9-14	3.3-33.8	7.92-11.67
		T_3	65-121	105-146	74-115	F Y, Y-0, R	5-12	7.6-28.9	6.52-10.76
		T_4	60-135	110-138	80-95	Y, Y-O, R	6-13	3.9-22.5	5.98-11.70
BSMR-736	Control	-	165	176	90	Yellow	32	66.5	9.85
	Gamma	T_1	88-192	132-172	79-98	Y, Y-O, R	0-59	1.9-105.9	7.4-13.1
		T_2	76-187	137-168	81-96	Y, Y-O, R	9-68	3.9-131.1	7.47-12.74
		T_3	103-168	127-194	81-109	Y, Y-O, R	12-63	5.2-96.6	8.02-13.6
		T_4	70-158	144-168	84-91	Y, Y-O	5-59	3.1-143.3	6.29-10.16
	EMS	T_1	97-165	127-158	78-92	Y, R	9-45	12.4-119.8	7.8-12.13
		T_2	95-165	125-160	78-98	Y, Y-O, R	8-33	11-85.6	8.44-11.93
		T_3	100-154	132-172	82-106	Yellow	4-34	0.6-48.6	8.24-12.87
		T_4	90-148	137-158	79-94	Yellow	8-35	0.3-41.2	8.45-11.32

Table 2: Effect of mutagen on different characters in M_2 generation

Genotype	Mutagen Type	Treatment	Plant Ht(cm)	Days to Maturity	Days to 1st flowering	No. of Branches	Grain Wt./plant (gm)	100 seed Wt. (gm)
ICPL-87	Gamma	Control	108.60	123.27	79.00	20.33	24.44	9.09
		T_1	95.28	127.12	87.82	24.37	28.65	9.70
		T_2	89.77	121.27	87.28	21.11	25.24	9.44
		T_3	86.53	125.65	87.47	14.77	22.50	9.02
		T_4	96.17	130.67	89.58	22.08	27.96	9.67
	EMS	Control	108.60	123.27	80.00	20.33	24.50	9.09
		T_1	79.95	122.98	85.85	22.60	28.39	9.78
		T_2	93.30	125.97	85.05	21.50	24.43	9.17
		T_3	84.93	119.85	84.67	16.53	21.49	9.02
		T_4	89.55	120.47	85.72	22.17	27.72	9.71
BSMR-736	Gamma	Control	151.73	179.67	92.00	24.40	45.48	10.09
		T_1	141.65	152.52	87.05	25.74	46.55	10.26
		T_2	142.73	153.75	86.20	26.82	49.82	10.44
		T_3	135.20	151.85	85.68	22.45	43.34	9.97
		T_4	119.38	159.42	90.15	29.27	51.43	10.74
	EMS	Control	155.47	178.40	91.13	23.73	45.07	10.18
		T_1	122.52	150.33	88.57	25.42	45.92	10.42
		T_2	134.05	147.42	86.35	26.38	48.52	10.51
		T_3	125.62	154.83	93.85	21.63	42.77	9.81
		T_4	110.85	148.00	87.98	28.35	50.67	10.81
	SE(m)	Factor A	0.847	0.659	0.378	0.190	0.332	0.044
		Factor B	0.847	0.659	0.378	0.190	0.332	0.044
		Factor C	1.339	1.041	0.597	0.300	0.526	0.069
		A*B	1.198	0.993	0.534	0.269	0.470	0.062
		A*C	1.894	1.473	0.845	0.425	0.743	0.097
		B*C	1.894	1.473	0.845	0.425	0.743	0.097
		A*B*C	2.678	2.082	1.195	0.601	1.051	0.138
	CD (5%)	Factor A	2.425	1.886	1.082	0.544	0.952	0.125
		Factor B	2.425	1.866	-	-	-	-
		Factor C	3.834	2.982	1.530	0.860	1.505	0.197
		A*B	-	-	1.711	-	-	-
		A*C	5.423	4.217	2.419	1.216	2.128	0.279
		B*C	5.423	4.217	2.419	-	-	-
		A*B*C	-	5.963	3.421	-	-	-

Days to 1st flowering

In the Table 1 observed that for genotype ICPL-87 some treatments had recorded early flowering plants. Early flowering plants were recorded with days to 1st flowering of 74 days from treatment with 20mM only followed by Treatment T_2 (10mM) also recorded early days to 1st flowering of 78 days. In case of BSMR-736, in all treatments of gamma rays and EMS recorded days to 1st flowering as below the mean days to 1st flowering for BSMR-736.

In table 2, the mean days to 1st flowering was calculated for each treatment on 20 plants per replication. The mean performance for this trait of variety and mutagen on different treatment found significantly late flowering than control in genotype ICPL-87 and I case of BSMR-736, all treatments recorded significantly earliness of this trait except T_3 (20mM) which found late flowering plants.

Number of branches

Maximum variation in number of branches were observed in T_4 (300Gy) of gamma rays for genotype ICPL-87. In case of genotype BSMR-736, T_1 (50Gy) and T_2 (100Gy) from gamma rays and T_1 (5mM) from EMS recorded maximum variation. The number of branches trait in screened M_2 generation comprising of mutants of different treatment ranged between 6 (100Gy) to 14 (300Gy) for genotype ICPL-87 and between 25 (10mM) to 59 (50Gy and 100Gy) for genotype BSMR-736. The mean value for control plants of ICPL-87 was 13 and for BSMR-736 were 59 as per the table 1 and also observed that for genotype ICPL-87 some treatments had recorded high numbers of branched plants. These plants were recorded with numbers of branches of 21 from treatment with 300Gy only followed by Treatment T_1 (50Gy) and T_3 (200Gy) recorded numbers of branches of 16. In case of BSMR-736, all treatments of gamma rays and T_1 (5mM) of EMS recorded high numbers of branches as the mean value for BSMR-736.

Mean number of branches was calculated for each treatment on 20 plants per replication. The data revealed that the mean number of branches per plant significantly high in T_1 (50Gy) of gamma rays. Where, in mean performance of BSMR-736, treatments with (300Gy and 30mM) both mutagens recorded maximum no. of branches (29.27 and 28.35) (Table 2).

Grain yield per plant

From the Table no. 1 it is observed that for genotype ICPL-87 all treatments had recorded high grain yielded plant except T_4 (30mM). These plants were recorded with high grain yield per plant of 44.4 gm from treatment with 300Gy followed by Treatment T_3 (20mM) recorded grain yield per plant of 33.8 gm. In case of BSMR-736, all treatments of gamma rays and T_1 (5mM) and T_2 (10mM) of EMS recorded high grain yield per plant.

Data recorded in Table 2, it concluded that, in genotype ICPL-87, T_1 from both mutagens (50Gy and 5mM) found significantly high grain yield per plant (28.65 gm and 28.39 gm), whereas in BSMR-736 T_4 from gamma rays and EMS treatment recorded high grain yield per plant i. e. 51.43 gm and 50.67 gm per plant than control.

100 seed weight

According to Table no. 1, maximum variation in 100 seed wt. per plant was observed in T_3 (200Gy) of gamma rays and T4 (30mM) of EMS for genotype ICPL-87. In case of genotype BSMR-736, T_1 (50Gy) and from gamma rays and T_3 (20mM) EMS recorded maximum variation. The 100 seed wt. per plant trait in screened M_2 generation comprising of mutants of different treatment ranged between 5.78 gm (200Gy) to 11.67 gm (10mM) for genotype ICPL-87 and between 6.29 gm (300Gy) to 13.6 (200Gy) for genotype BSMR-736. The mean value for control plants of ICPL-87 was 8.6 gm and for BSMR-736 was 9.85 gm.

From the table no. 2 observed that for genotype ICPL-87 all treatments had recorded high 100 seed wt. which were recorded with high 100 seed wt. per plant of 11.7 gm from treatment with 30mM followed by Treatment T_2 (10mM) recorded 100 seed wt. per plant of 11.67 gm. In case of BSMR-736, treatment 200Gy of gamma rays recorded high 100 seed wt. per plant as 13.6 gm followed by treatment 50Gy with 13.1 gm.

The mean performance of variety and mutagen on different treatments found significant variation. From Table no. 2, it revealed that all treatments of both mutagen for genotype ICPL-87 found significant than control. (T_3 from both mutagens found non-significant) and In BSMR-736 all treatments from both mutagens recorded significant except T_3 (for both mutagen) and T_4 found high 100 seed weight or bold seeded plants.

Discussion

Plant height

Maximum variation in plant height such as dwarf and tall mutants were also obtained in the present investigation. Some dwarf mutants were also reported by Jain (1976) [8], Potdukhe and Narkhede (2002) [18] in pigeonpea. Athwal *et al*. (1970) [2] created variability in plant height in chickpea through gamma radiation. Variability in plant height was observed through EMS treatments in *Capsicum annum* (Jabeen and Mirza, 2002) [7] which supports the present study. A talls mutant was obtained by Khan and Veeraswamy (1974) [11], Jain (1976) [8] in pigeonpea, Khan and Wani (2004) [12] in mungbean and Wani and Anis (2001) [26] in chickpea, Solanki and Sharma (2003) [22] in lentil, Kumar *et al*., (2007) [14] in blackgram, Manjaya and Nandanwar (2007) [16] in soybean and Barshile *et al*., (2009) [3] in chickpea. According to Jana (1962) [9] the tallness of tall mutants is fundamentally due to increase in number and length of internodes.

Plant maturity

Early mutants are always preferred for plant breeding strategies in almost all crops. Early mutants induced in the present study showed rapid growth and early maturity. Early maturity might be due to physiological changes in production of flowering hormones caused by the mutagens. Late Maturing mutants were observed in the present study. Late flowering may be due to insufficient production of hormones required for flowering. Late flowering mutants were recorded earlier by Tripathi *et al*., (1975) in pigeonpea, Kothekar and Kothekar (1992) [13] in moth bean, Manjaya and Nandanvar (2007) [16] and Tambe (2009) [23] in soybean.

Days to flowering

Maximum variation in days to 1st flowering was also recorded by Gaikawad *et al*. (2005) [6], Rudraswami *et al*. (2006) [19], Manjaya and Nandanvar (2007) [16], Ahire (2008) [1] and Tambe (2009) [23] in different legumes were supportive to the present findings.

Number of branches

On the basis of number of branches per plant spreading branched mutant or high number of branched mutant are

positively correlated with grain yield. This type of mutant was also recorded by Jain (1976) [8] in pigeonpea; and Kothekar and Kothekar (1992) [13] in moth bean. Such mutations can be considered as evolutionary conversion of the plant habit genes carrying substantial polygenic significance.

Grain yield

Mutant having high grain yield than control plant also recorded earlier by Chopde (1969) [5], Shrivastava *et al*., (1992) [21], Pawar and Wanjari (1994) [17] in pigeonpea, Tickoo and Chandra (1999) [24] in mungbean, Vanniarajan *et al*., (1993) [25] in blackgram, and Tambe (2009) [23] in soybean. Kashid and subhash (2015) [10] indicated an increase in 100 seed weight with higher concentrations of EMS and SA in both cultivars of chickpea in M_2 and M_3 generations. In M_2 generations, the BDN 9-3 cultivar showed a negative shift in mean values of this trait. This revealed that 100 seed weight has shown a very significant increase from the control with most of the treatments of gamma rays and EMS used either singly or in combination in both the varieties of chickpea.

Conclusion

Both the mutagens proved to be very effective to induce variability in quantitative traits like plant height, plant maturity days to first flowering, number branches per plant, grain yield per plant and 100 seed weight in M_2 generation for both genotype but regarding to grain yield per plant genotype BSMR-736 observed higher rate of variability than genotype ICPL-87.

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