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Induced Mutagenesis and Variability Study in the M_2 Generation of Garden Pea (*Pisum sativum* L.)

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ABSTRACT

Background: Mutation induction in garden pea (*Pisum sativum* L.) aimed to study the induced genetic variability and identify desirable mutants in the M_2 generation with two varieties of garden pea, *Kashi Nandini* and *Kashi Uday*.

Methods: The two varieties of garden peas, *Kashi Nandini* and *Kashi Uday*, were subjected to mutagenic treatments with the EMS at 0.05, 0.10, 0.15, 0.20, 0.30 and 0.40%. The M_1 generation was raised in RBD with two replications during *Rabi* 2019-20. Plants from three concentrations, 0.10, 0.15 and 0.20% EMS, were evaluated in M_1 plant progenies (M_2) during *Rabi* 2020-21. The M_2 was raised during *Rabi* 2020-21 in M_1 plant progenies using M_1 plants from three concentrations -0.10, 0.15 and 0.20% EMS of both varieties.

Result: The two varieties that were exposed to EMS responded differently depending on the concentrations. The GCV and PCV were high for seedling height, fertile branches, pods/plant and seeds/pod in both parents at 0.15 and 0.20% EMS. The highest heritability and genetic advance were apparent for seedling height and seeds/pod at 0.10 and 0.20% EMS, while the estimates were moderate for seeds/pod with 0.20% EMS, followed by seeds/pod at 0.10% EMS and seedling height at 0.20% EMS. Desirable plants were visible for various quantitative traits in the M_2 generation. These mutants showed significant differences from the controls of both varieties in the treated populations at higher concentrations of EMS. Desirable micro mutants in M_2 resulted at 0.15 and 0.20% EMS, which need further evaluation in the M_3 for confirmation.

Key words: Garden pea, GCV, Genetic advance, Heritability, Induced genetic variability, Micro mutants, PCV.

INTRODUCTION

Pea (*Pisum sativum* L.) is a protein-rich, self-pollinated nutritious cool-season vegetable grown throughout the world. It can withstand freezing and has a genome size of 5000 Mb. Pea occupies 459 thousand ha area in India and shares 21% of world production. Current annual global production is approximately 22 million tonnes of dry peas and 14.5 million fresh peas (FAOSTAT, 2019). In India, pea is an extensively cultivated crop in Uttar Pradesh, Bihar and Madhya Pradesh. In Assam, it is mainly cultivated in the districts of Darrang, Nagaon and Kamrup. The significant producers of pea in the world are the USA, China, Holland UK, Egypt, Russia, Australia and France. Pea is commercially important crop that belongs to the family Fabaceae, subfamily Papilionoideae. People use peas as a fresh consumption or processed vegetable. Pea is also used for canning, freezing and dehydration (sundried) purposes. Pea seeds are rich in protein (23%) and digestible starch (50%). The genetics of the pea has been an object of study since the early days and the pea was the original model plant used in Mendel's discovery of the laws of inheritance, making it the foundation for modern plant genetics. Yield potential in pea has reached a plateau due to inadequate natural genetic variability in varieties beyond which no further increase in yield potential results through conventional breeding. Plant breeders always look for unique genetic variants to break the yield plateau. Mutation breeding is one of the possible alternatives to conventional breeding in crop improvement. Exposing plant genetic material to mutagens increases the chance of isolating unique genetic material. Induced mutations can rapidly create variability in quantitatively and qualitatively inherited traits in crops (Maluszynski *et al*., 1995; Muduli and Mishra, 2007). Induced mutagenesis has been successful in obtaining direct mutants. These mutants, through hybridization, overcome yield plateaus and generate desirable horticultural traits (Ahloowalia *et al*., 2004). It is well-documented that mutagenesis can alter the genetic parameters like mean, variance, heritability, genetic variability, genetic advance for

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many characters and the character associations in different crops. Thereby, such a situation increases the opportunities of a breeder to control part of the crop's evolution when subjected to selection. Artificially induced mutations augment the available variability and in these studies, the initial emphasis was on the induction of desired genetic changes. Induced mutations play a significant role in meeting world food and nutritional security challenges through mutant germplasm enhancement and their utilization in developing new mutant varieties. Genetic variation is the prerequisite in any crop improvement programme, but pulses like pea generally lack genetic variability due to their autogamous nature. If enough variability does not exist, then several methods of genetic variation can be created, among which hybridization and induced mutations are the essential methods. The success of any mutation breeding programme depends on the developing screening techniques to identify desirable mutants, which occur with a shallow frequency, among many other mutations with little breeding value (Solanki and Sharma, 2002). Hence, the traditional breeding methods have limited application in pea and other pulses mutation breeding appears to play an essential role in improving this vital crop. Therefore, keeping this in view, the present investigation took up to study the induced genetic variability in M_2 generation and identify the desirable mutants.

MATERIALS AND METHODS

Treatment of experimental material

The experiment was conducted at the Field Experimentation Center, Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam, India. Healthy, uniform and dry seeds of two garden pea (*Pisum sativum* L.*)* varieties namely *Kashi Nandini* and *Kashi Uday* were pre-soaked in distilled water for 6 hours. The soaked seeds were treated with different EMS concentrations of 0.05, 0.1, 0.15, 0.20, 0.30 and 0.40% for 6 hours. The treated seeds were washed in running tap water for 2 hours to eliminate the residual effect of EMS. The control sample consisted of an equal number of seeds soaked in distilled water alone under identical condition. One hundred were considered per treatment.

During the *Rabi* season, 2019-20 mutagen treated seeds were sown as M_1 generation and in the second *Rabi* season 2020-21, their progenies were sown as M_2 generation. Seeds were harvested from all the treatments of M_1 generation including controls of both varieties. Seeds from only three doses were selected for growing the M_2 generation in both the varieties and were sown as M_2 families. Observations taken in M_2 generation were seedling height (cm), plant height at maturity (cm), days to flowering, days to maturity, number of fertile branches plant^{-1}, number of pods plant^{-1} and number of seedspod^{-1}. All the M_2 lines in each treatment were used for screening various quantitative traits and observations were recorded on the individual plants of each progeny.

RESULTS AND DISCUSSION

Mean performances in M_2 generation

Seedling height registered the highest value in the treatments having a higher concentration (0.20% EMS) in both the varieties (11.6 cm in *Kashi Nandini* and 11.0 cm in *Kashi Uday*), which were above both controls (11.4 cm and 10.9 cm in *Kashi Nandini* and *Kashi Uday*, respectively and was followed by the treatment of 0.15% (9.0 cm in *Kashi Nandini* and 9.2 cm in *Kashi Uday*). In comparison, the lowest value was at 0.10% EMS (8.8 cm in *Kashi Nandini* and 8.9 cm in *Kashi Uday*). These results were in tune with the earlier findings of Srivastava *et al*. (2008) and Govardhan and Lal (2013). Days to 50% flowering and maturity in M_2 generation showed a delay in all the mutagen treated populations of both varieties (Table 1). The maximum delay in days to 50% flowering and maturity was at 0.20% EMS in both the varieties and the minimum was in controls. The decreasing trend for these two characters with higher concentrations of EMS also agreed with previous findings of Ahire (2008), Singh *et al*. (1999) and Waghmare and Mehra (2000) also reported similar results. Plant height at maturity showed the highest value at a concentration of 0.20% EMS in both the varieties (60.50 cm in *Kashi Nandini* and 60.1 cm in *Kashi Uday*). The lowest plant height was at 0.10 and 0.15% EMS in both varieties (Table 2), suggesting an increase in plant height at higher concentrations, while a decrease at lower concentrations of EMS in M_2 generation, as also reported by Wani and Anis (2001) and S.M. Sangle (2020) in Pigeon pea.

The fertile branches were the highest for 0.2% EMS in both varieties, higher than the controls (3.6 in *Kashi Nandini* and 3.7 in *Kashi Uday,* Table 2 and3), followed by 0.15%

Table 1: Mean performance for the traits observed on plot basis of the two varieties treated with different doses of EMS in M_2 generation.

Dose (%)	Days to 50% flowering	Days to maturity	100-seed weights (g)
Kashi Nandini			
0	35.0	70.0	28.2
0.1	40.0	76.0	30.6
0.15	47.0	80.0	29.2
0.2	50.0	85.0	32.6
Mean	43.0	77.8	30.2
$\pm SE_m$	3.4	3.2	1.0
CV (%)	15.8	8.2	6.3
Kashi Uday			
0	38.0	75.0	28.8
0.1	45.0	78.0	31.6
0.15	50.0	82.0	32.8
0.2	54.0	90.0	34.3
Mean	46.8	81.3	31.9
$\pm SE_m$	3.4	3.3	1.2
CV (%)	14.8	8.0	7.3

EMS (3.5 and 3.6 in *Kashi Nandini* and *Kashi Uday*, respectively). The number of fertile branches/plant was the lowest for 0.10% EMS. The results were similar to Amjad *et al*. (1993) in pea variety Green Feast and Sadashiv and Kondiram (2012) in horse gram and Wani and Anis (2001). The pods/plant and seeds/pod at 0.20% EMS in both varieties significantly differ from the controls in having higher values. The treatments with 0.10 and 0.15% EMS showed low values for these characters. These results were in agreement with Savant (2008) in sesame, Khan and Praveen (2004) and Waghmare and Mehra (2000). Seed yield/plant and 100-seed weights had the highest values at 0.20% EMS in both varieties, followed by 0.10 and 0.15% EMS. These findings were similar to the earlier reports of Wani and Anis (2001), Pawar *et al*. (1979), Singh (1973), Biradar (2004) and Dahiya (1977), Patil and Wakode (2011) and Wani (2011).

Induced genetic variability in M_2 generation

For the improvement of any character, breeders have to select superior individuals based on phenotypic expression. Phenotype based selection may sometimes be misleading because character development is the result of heritable and non-heritable components. The coefficient of variation is the best tool to measure the relative magnitude of variation among the characters and predicts the variability in the population under study, necessitating partitioning the overall variability into its heritable and non-heritable components. The variability parameters GCV, PCV, heritability and genetic advance for yield and its components are essential to know the clear picture of variability created for different characters.

The estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability in a broad sense (h^2_{bs}) and expected genetic advance (GA) as per cent of the mean for all the characters revealed that the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters (Fig 1 and 2). Genotypic coefficient of variation (GCV) provides a means to study the genetic variability generated in quantitative characters (Johnson *et al*., 1955). It indirectly measures the environmental influence on the inheritance pattern of yield attributes. Heritability is a good index of the transmission of characters from parents to their offspring. Genetic advance gives the extent of genetic gain. The genetic advance shows the extent of genetic gain that could be expected through selections in character to be improved (Burton, 1952; Johnson *et al*., 1955).

A comparison of GCV estimates in *Kashi Nandini* and *Kashi Uday* revealed the existence of the highest genetic variation for seedling height, fertile branches/plant and pods/plant at 0.20 and 0.15% EMS (Table 2 and 3), suggesting the scope of selection for these traits for isolating desirable genotypes in garden pea, as also reported by Khan and Wani (2006), Kumar and Dubey (2001) and Kumar (2009) in pea. The results closely agreed with the findings of

Table 2: Simple genetic parameters and mean performance for the traits in M_2 generation for the variety *Kashi Nandini*.

Traits	EMS dose (%)	Mean	SE_m	GCV (%)	PCV (%)	h^2_{bs}	GA (5%), as % of control
Seedling height (cm)	0	11.4	0.3	-	8.7	-	-
	0.10	8.8	0.7	23.4	26.0	80.8	43.3
	0.15	9.0	0.6	18.4	21.5	73.5	32.5
	0.20	11.6	0.5	9.1	12.5	52.5	13.5
Plant height (cm)	0	59.1	0.9	-	4.9	-	-
	0.10	56.1	1.0	2.4	5.7	17.5	2.1
	0.15	57.7	1.3	4.6	6.9	45.6	6.5
	0.20	60.5	1.3	4.7	6.7	48.3	6.7
No. of fertile branches plant^{-1}	0	3.6	0.3	-	29.9	-	-
	0.10	3.3	0.2	-	20.5	-	-
	0.15	3.5	0.3	-	24.3	-	-
	0.20	3.8	0.3	-	24.2	-	-
No. of pods plant^{-1}	0	7.8	0.3	-	11.8	-	-
	0.10	7.5	0.3	4.2	13.0	10.6	2.8
	0.15	7.6	0.3	-	11.1	-	-
	0.20	9.3	0.4	10.4	14.4	52.8	15.6
No. of seeds pod^{-1}	0	6.7	0.1	-	3.4	-	-
	0.10	6.0	0.2	8.2	9.1	82.1	15.3
	0.15	6.1	0.1	2.7	4.6	33.5	3.2
	0.20	6.9	0.1	3.6	4.9	53.9	5.4
Seed yield plant^{-1} (g)	0	16.1	0.3	-	6.7	-	-
	0.10	15.1	0.3	-	6.5	-	-
	0.15	15.5	0.2	-	4.1	-	-
	0.20	16.7	0.4	3.1	7.1	19.0	2.8

Waghmare and Mehra (2000), Muduli and Misra (2008), Mensah and Obadoni (2007), Khan and Wani (2006), Kumar and Dubey (2001) and Kumar (2009) in pea. Similarly, the estimates of PCV were also high for seedling height, fertile branches/plant in *Kashi Nandini*, while in *Kashi Uday*; PCV was high for fertile branches/plant, pods/plant at 0.10 and 0.15% EMS (Table 2 and 3). These findings indicated the role of both the genotype and environment for the expression of these characters. These results aligned with the findings of Meshram *et al.* (2014), Sarma *et al.* (2022), Arul balachandran and Mullainathan (2009). The phenotypic variation was low for the remaining characters (Wani and Anis, 2014; Wani 2011).

The highest heritability estimates in the broad sense (h^2_{bs}) were for seeds/pod and seedling height in *Kashi Nandini* at 0.10% EMS. In *Kashi Uday*, the heritability was

Table 3: Simple genetic parameters and mean performance for the traits in M_2 generation for the variety *Kashi Uday.*

Traits	EMS dose (%)	Mean	SE$_m$	GCV (%)	PCV (%)	h^2_{bs}	GA (5%), as % of control
Seedling height (cm)	0	10.9	1.1	-	32.5	-	-
	0.10	8.9	0.9	-	31.8-	-	
	0.15	9.2	0.8	-	29.2	-	-
	0.20	11.0	0.5	-	13.0	-	-
Plant height (cm)	0	60.1	1.1	-	6.0	-	-
	0.10	56.5	2.5	12.7	14.2	80.0	23.4
	0.15	54.5	2.2	11.1	12.9	74.0	19.7
	0.20	61.0	0.7	-	3.8	-	-
No. of fertile branches Plant^{-1}	0	3.7	0.2	-	18.2	-	-
	0.10	3.5	0.2	-	15.1	-	-
	0.15	3.6	0.3	14.0	23.4	35.9	17.3
	0.20	3.9	0.4	25.4	30.7	68.2	43.1
No. of pods Plant^{-1}	0	8.4	0.4	-	15.1	-	-
	0.10	7.2	0.2	-	11.0	-	-
	0.15	7.8	0.5	14.3	21.6	43.8	19.5
	0.20	9.4	0.8	23.5	27.1	75.3	42.1
No. of seeds Pod^{-1}	0	6.8	0.1	-	2.8	-	-
	0.10	6.3	0.1	4.8	5.7	70.8	8.3
	0.15	6.6	0.1	5.7	6.4	79.0	10.4
	0.20	6.9	0.1	6.2	6.8	83.0	11.6
Seed yield Plant^{-1} (g)	0	17.3	0.3	-	5.2	-	-
	0.10	16.3	0.2	-	3.4	-	-
	0.15	16.5	0.3	-	5.1	-	-
	0.20	17.4	0.2	-	4.3	-	-

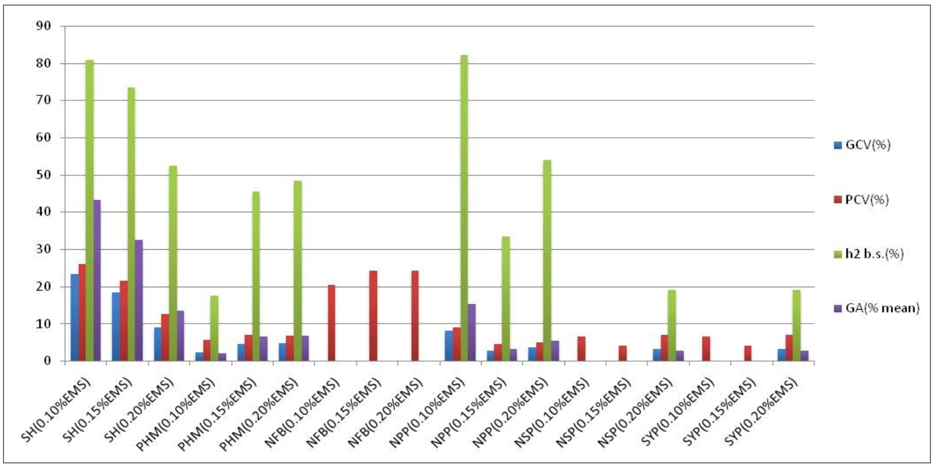

Fig 1: Genetic parameters for the characters in M_2 generation variety *Kashi Nandini.*

high for plant height at maturity at 0.10% EMS and seeds/ pod at 0.20% EMS. Moderate estimates recorded for the seedling height in *Kashi Nandini*, seeds/ pod, pods/plant at 0.10 and 0.15% EMS (Fig 1 and 2) indicated that these characters showed a more significant environmental influence and, thus, a more precise evaluation would be necessary for the improvement of these characters. These results had the support of Kumar (2009). In the present experiment, heritability in a broad sense was high in treated populations than in control in M_2 generation, which aligned with Wani (2011).

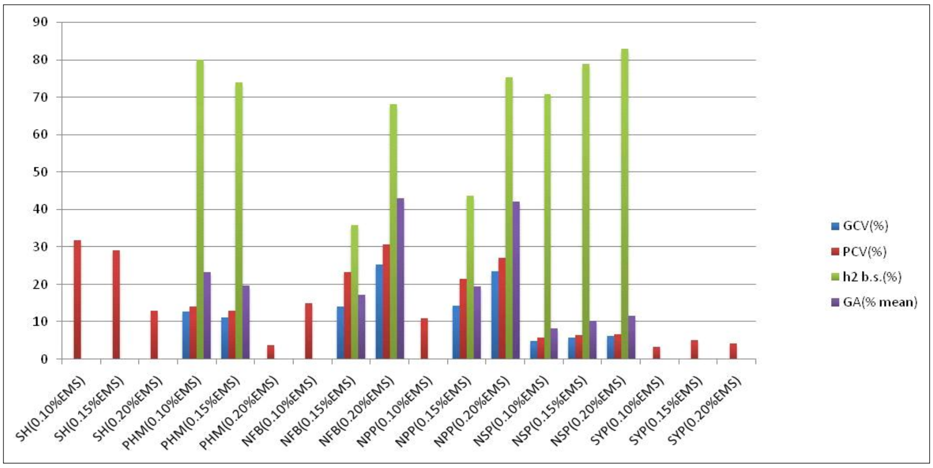

Fig 2: Genetic parameters for the characters in M_2 generation variety *Kashi Uday.*

Table 4: Micro mutants identified in M_2 generation showing superiority over the parent varieties for desirable characteristics.

Mutant	No. of fertile branches/Plant	No. of pods/Plant	No. of seeds/Pod	Seed yield/Plant (g)
		Variety: *Kashi Nandini*		
0.15% EMS-5	5.0*	6.0	5.8	17.7*
LSD (5%)	**0.9**	**0.3**	**0.2**	**0.8**
0.20% EMS-1	4.0	10.0*	6.7	17.8*
0.20% EMS-3	6.0*	8.0*	6.9	14.4
0.20% EMS-5	5.0*	9.0*	7.2*	17.7*
0.20% EMS-6	4.0	9.0*	7.5*	17.9*
0.20% EMS-7	2.0	12.0*	6.8	16.8
0.20% EMS-8	3.0	11.0*	6.8	15.8
0.20% EMS-10	5.0*	10.0*	7.3*	16.4
LSD (5%)	0.9	0.3	0.3	1.1
Kashi Nandini	3.6	7.8	6.7	16.1
		Variety: *Kashi Uday*		
0.15% EMS-3	3.0	6.0	7.2*	16.5
0.15% EMS-7	4.0	10.0*	6.6	15.3
0.15% EMS-8	4.0	11.0*	5.9	16.3
0.15% EMS-10	5.0*	6.0	6.9	18.4*
LSD (5%)	0.7	1.4	0.3	0.8
0.20% EMS-1	4.0	12.0*	6.7	18.5*
0.20% EMS-2	6.0*	9.0	6.8	16.5
0.20% EMS-4	7.0*	12.0*	7.2*	17.8
0.20% EMS-5	3.0	10.0	7.5*	16.9
0.20% EMS-6	3.0	14.0*	6.8	16.7
0.20% EMS-7	2.0	6.0	7.4*	18.5*
0.20% EMS-8	5.0*	8.0	6.8	18.2*
0.20% EMS-9	3.0	7.0	7.5*	17.0
LSD (5%)	0.9	1.9	0.3	0.8
Kashi Uday	3.7	8.4	6.8	17.3

*Significantly different from control at 5% level.

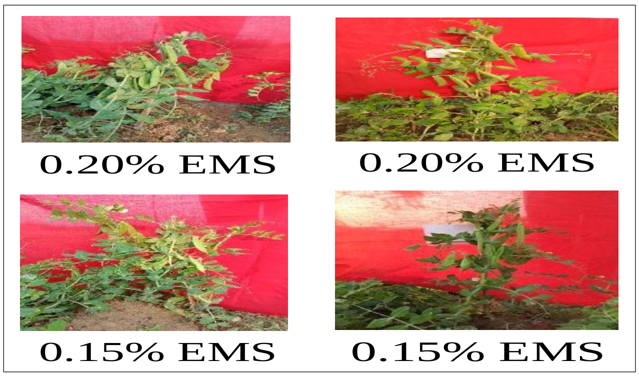

Fig 3: Mutant plants with a greater number of pods/plant variety *Kashi Uday.*

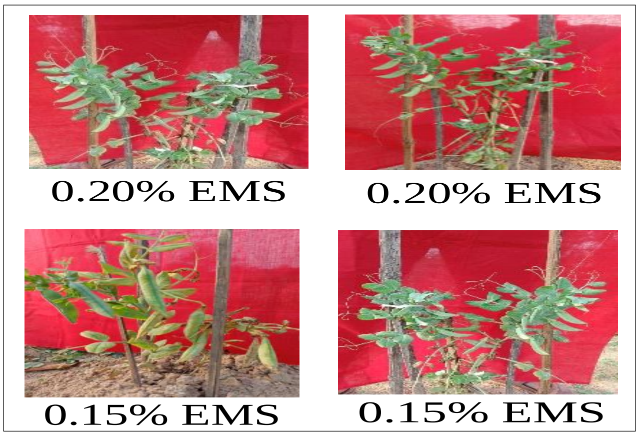

Fig 4: Mutant plants with a greater number of pods/plant *Kashi Nandini.*

As per cent mean, the expected genetic advance is a valuable criterion in a plant breeding programme. Heritability alone is not ideal for predicting the genetic progress that would result from selecting the best individuals from a population. When estimated together, genetic advance and heritability are more helpful in predicting the selection response as high heritability in concurrence with high genetic advance reflects the preponderance of additive gene action (Johnson *et al*., 1955). As per cent of the mean, the genetic advance was the highest for seedling height at 0.10 and 0.15% EMS in *Kashi Nandini*. The estimates were moderate for seeds/pod with 0.20% EMS, followed by seeds/pod at 0.10% EMS and seedling height at 0.20% EMS in *Kashi Nandini* (Table 2). In *Kashi Uday*, the highest genetic advance was for fertile branches at 0.20% EMS, pods/plant at 0.20% EMS and plant height at 0.10% EMS (Table 3). Moderate estimates were recorded for the characters' plant height at maturity, followed by pods/plant and fertile branches at 0.15% EMS. The estimates were low for the remaining characters (Fig 1 and 2). The results conformed to Khan and Praveen (2004) in green gram, Muduli and Misra (2008), Mensah and Obadoni (2007) and Kumar and Dubey (2001) and Wani. A.A. (2011).

Desirable mutants observed in M_2 generation

The present study identified different types of desirable mutants for various quantitative traits in the M_2 generation (Table 4). These mutants showed significant differences over the controls of both the varieties (*Kashi Nandini* and *Kashi Uday,* Fig 3 and 4). Desirable mutant plants showed superiority over the controls for fertile branches, pods/plant, seeds/pod and seed yield/plant in both the varieties, as also reported by Tabti *et al*. (2018), Khan *et al.* (2018), Dhilan *et al.* (2018), Sharma *et al*. (2010).

CONCLUSION

The present experiment allowed the isolation of different desirable plants for essential quantitative traits in the M_2 generation. Superior progenies were identified for fertile branches/plant, pods/ plant, seeds/pod and seed yield/ plant in the treatments having 0.15 and 0.20% EMS concentrations. These plants with altered phenotypes might result in superior yielding mutants in subsequent generations of mutagenized populations. Further, cross-breeding involving these variants of both the varieties might evolve better recombinants with improved yield. Once validated, the mutants need multiplying for preliminary yield test, DUS characterization, quality assessment, pest and disease screening and multilocational trials. The confirmed mutants also need molecular characterization to distinguish them from the parent varieties.

Conflict of interest: None.

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