

## Isolation of mutants from gamma ray-irradiated population of *Solanum melongena* cultivar 'Pusa Purple Long'

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**ABSTRACT:** Three mutants isolated according to differed morphological traits were isolated from the M<sub>2</sub> progeny of gamma ray-irradiated *Solanum melongena* L. var. PPL, viz. PPL-j<sup>3</sup> (5kR), PPL-k<sup>3</sup>-1 (15 kR), and PPL-k<sup>3</sup>-2 (15 kR). The seeds of selected mutants of M<sub>2</sub> generation were collected individually and grown in M<sub>3</sub> generation, separately and data on various quantitative traits, such as plant height (cm), number of branches per plant, number of fruits per plant, fruit weight (g), fruit size (cm), 1000-seed weight (g), and yield (kg) per plant, were recorded for all three mutants. The mutants were morphologically quite distinct, as compared to the control and to each other. The average performance of different quantitative traits was better in a high yielding mutant (PPL-j<sup>3</sup>) as compared to the control, while in other two mutants (PPL-k<sup>3</sup>-1 and PPL-k<sup>3</sup>-2), poor yield was observed. Cytological analysis revealed almost normal chromosomal set in mutant screened from 5kR irradiated population, except for a few chromosomal aberrations. The higher frequency of meiotic aberrations was recorded in mutants isolated from 15 kR irradiated population. However, the frequency of these chromosomal irregularities decreased at anaphase, exhibiting recovery at later stages. A reduction in pollen fertility was also recorded in mutants isolated from 15 kR, indicating low fruit set.

**KEYWORDS:** *Solanum melongena* L., Mutation breeding, Gamma-ray, Chromosomal anomalies

Vegetables play a pivotal role in our diet as they are the main source of some important supplements, especially vitamins and minerals. Most of the fruits of *Solanum* are used as vegetables and the eggplant is one of the most common and popular vegetable grown throughout India. Based on production statistics, eggplant is the third most important crop in the Solanaceae, after potato and tomato (FAO 2011). The greatest eggplant producers at present are: China (32 Mt), India (12.5 Mt), Egypt (1.2 Mt) and Turkey (0.9 Mt) (FAO 2016). India feeds 17.3% of the world's population, which is still growing constantly. Moreover, with 24.5% of the world's undernourished population, India is bearing a huge burden of global food insecurity (WFP-FSI 2017).

Development of new varieties through mutation breeding with higher yield and nutrient content could be particularly beneficial to grower-consumer relationship. The main aim of mutation breeding is to improve one or a few desirable traits in a crop plant without changing the undesired characters by controlling and directing the induced mutation process. In order to enhance the genetic variability and improve the yield potential of eggplant through the use of physical mutagen, an attempt was made to induce genotypic alterations in present study, which

deals with the screening of mutants in the M<sub>2</sub> and M<sub>3</sub> generations of  $\gamma$ -ray irradiated populations.

### MATERIALS AND METHODS

**Plant materials:** Certified seeds of eggplant (*Solanum melongena* L.) var. PPL were procured from the Sale Counter of National Seed Center (NSC), Indian Agriculture Research Institute (IARI; New Delhi, India). Dry and healthy seeds (10%-12% moisture content) were irradiated with different doses of gamma ray (5, 10, and 15 kR) from a <sup>60</sup>Co source at the National Botanical Research Institute (NBRI; Lucknow, India). Thereafter, the treated as well as untreated seeds were sown in earthen pots and the seedlings were raised and transplanted at the stage of four to five leaves to well prepared experimental field in a complete randomized block designs (CRBD) in three replicates. The row-to-row and plant-to-plant distances were maintained at 60 cm and 45 cm, respectively. Seeds from M<sub>1</sub> plants were harvested separately for raising the M<sub>2</sub> generation.

Several mutants for growth and yield studies were isolated from mutagenized populations. Three mutants, viz. PPL-j<sup>3</sup>, PPL-k<sup>3</sup>-1 and PPL-k<sup>3</sup>-2, were isolated from 5 kR and 15 kR in M<sub>2</sub> generation. A set of 60 seeds from each

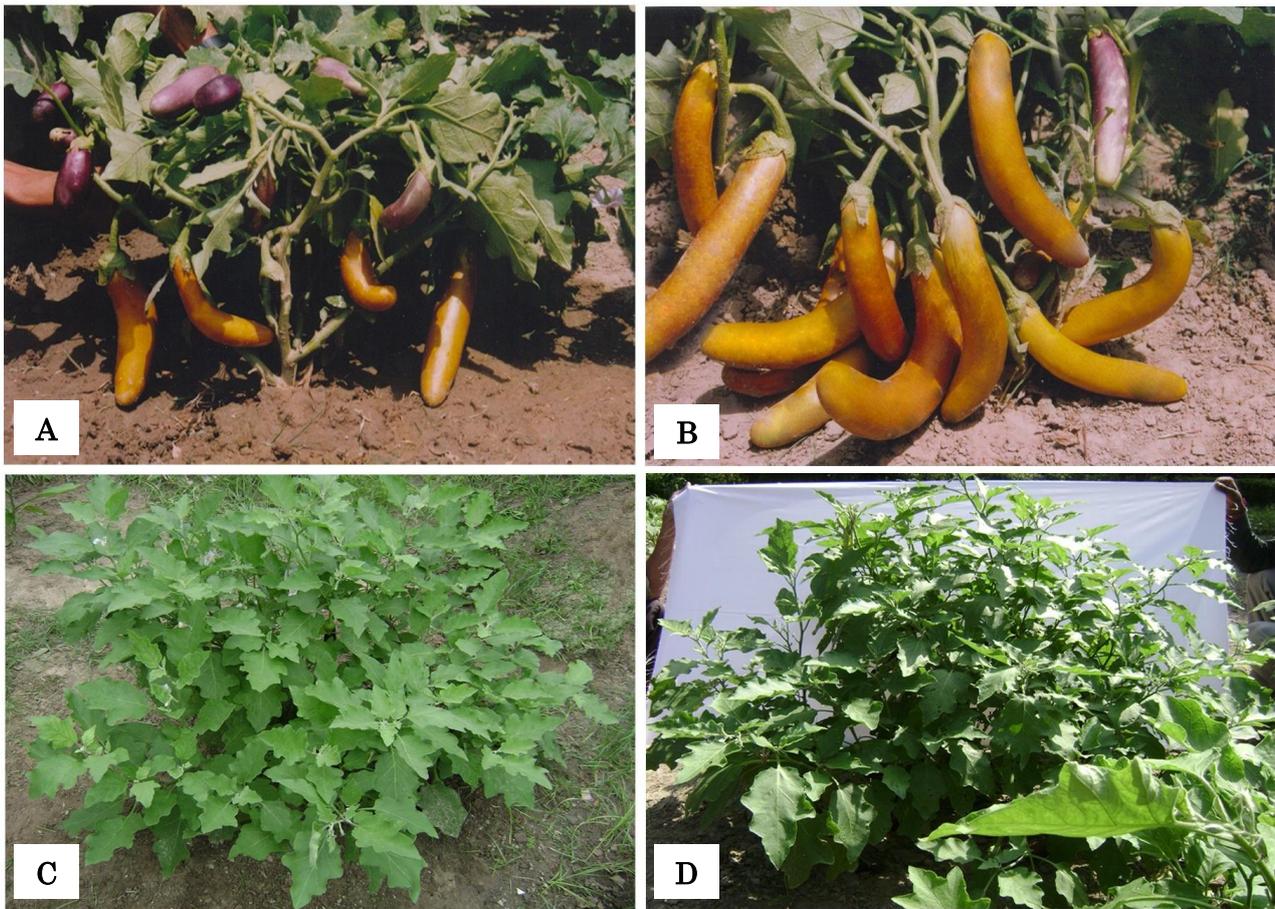


Fig. 1.

(A) Eggplant (Control): normal height, leaves, fruits, normal yield.

(B) Mutant (PPL- $j^3$ ): increased height, number of branches and number of fruits; bigger fruits, improved yield.

(C) Mutant (PPL- $k^3-1$ ): increased height, number of branches; bushy, poor fruiting, poor yield.

(D) Mutant (PPL- $k^3-2$ ): increased height, increased number of branches, bushy, poor fruiting, poor yield.

mutant type was sown in the following year (3 replicates of 20 seeds each) to raise the  $M_3$  generation, with the same row-to-row and plant-to-plant distances as mentioned above. Data for various quantitative traits were recorded from 30 randomly selected plants (10 in each replicate) of each mutant type, as well as the control. For meiotic analysis, young flower buds were collected from 5-10 randomly selected  $M_3$  mutants and fixed in Carnoy's solution (absolute ethanol:chloroform:glacial acetic acid = 6:3:1) for 24 hrs, supplemented with a few crystals of ferric chloride. The buds were, then, stored in 70% ethyl alcohol at room temperature. Slides were prepared by using 1% acetocarmine, using the standard squash technique. The slides were made permanently in NBA series, mounted on DPX and dried at 45°C.

## RESULTS

The data and photograph of the control plant have been given for comparison (Table 1, Fig. 1A). Three mutants have been isolated in  $M_3$  generation, which were inherited

from 5 and 15 kR gamma ray induced mutants in  $M_2$  generation. As yield per plant is the most desirable character in any breeding program, the mutant (PPL- $j^3$ ) that was distinctly superior to untreated population, with regard to yield per plant, was isolated in the  $M_2$  generation (Table 1, PPL- $j^3$ , Fig. 1B). The mutant (PPL- $j^3$ ) was morphologically quite distinct especially with regard to number of branches, number of fruits, fruit weight (g), fruit size (cm), 1000 seeds weight (g) and ultimately yield per plant (kg), as compared to the control. Two mutants were screened from 15 kR gamma ray, in which, the one was tall and bushy bearing normal leaves, increased number of branches, highly decreased number of fruits, giving poor yield (Table 1, PPL- $k^3-1$ , Fig. 1C), while, the other was tall, bushy mutant with larger leaves, increased number of branches but decreased number of fruits and poor yield (Table 1, PPL- $k^3-2$ , Fig. 1D). All these mutants were confirmed as true breeding in the  $M_3$  generation.

PMCs of the mutants were observed and compared with control PMCs. In control plants only a few abnormal PMCs were observed, while, the PMCs of all the three

Table 1. Various quantitative characters of the mutants isolated in *Solanum melongena* L. var. PPL.

Mutant	Plant Height (cm) Mean±SD CV	Number of Branches/Plant Mean±SD CV	Number of Fruits/Plant Mean±SD CV	Fruit Weight (g) Mean±SD CV	Fruit Size (cm)		1000-Seeds Weight (g) Mean±SD CV	Yield/Plant (kg) Mean±SD CV
					Fruit Length (cm) Mean±SD CV	Fruit Diameter (cm) Mean±SD CV		
Control	56.60±2.27 4.01	11.10±1.42 12.79	13.50±1.25 9.26	155.03±1.30 0.84	21.50±1.91 8.88	3.70±0.42 11.35	3.82±0.30 7.85	2.09±0.20 9.57
PPL-j <sup>3</sup>	57.25±2.65 4.63	12.00±1.38 11.50	14.00±1.49 10.64	183.56±2.30 1.25	26.20±2.07 7.90	4.05±0.45 9.32	4.07±0.23 5.65	2.57±0.32 10.89
PPL-k <sup>3</sup> -1	72.00±3.57 4.96	13.00±1.78 13.69	3.00±0.36 12.00	154.24±1.97 1.28	21.25±1.98 9.32	3.70±0.57 15.41	3.75±0.29 7.73	0.46±0.06 13.04
PPL-k <sup>3</sup> -2	98.00±4.65 4.74	15.00±2.10 14.00	4.00±0.45 11.25	156.20±2.36 1.51	21.58±2.19 10.15	3.72±0.54 14.52	3.80±0.27 7.11	0.62±0.08 12.90

Table 2. Frequency of chromosomal abnormalities in the screened mutants of *Solanum melongena* L. var. PPL

Mutant	Total Number of PMCs Observed	Prophase-I (diakinesis)			Metaphase-I/II					Anaphase-I/II			Telophase-I/II				Total Number of Abnormal PMCs Observed	Total % of Abnormal PMCs Observed	Pollen Fertility (%)
		Univalents	Multivalents	Chiasma Frequency	Univalents	Multivalents	Stickiness	Precocious Movement	Stray Chromosomes	Laggards	Bridges	Unsynchronized Movement	Laggards	Bridges	Disturbed Polarity	Tripolar Condition			
Control	218	-	-	21.60	-	-	2	1	1	-	-	1	-	1	1	-	7	3.21	92.50
PPL-j <sup>3</sup>	232	1	1	21.40	1	-	3	2	2	1	-	1	-	-	-	-	12	5.17	91.04
PPL-k <sup>3</sup> -1	214	2	3	18.55	2	2	9	4	3	4	3	3	1	1	-	1	38	17.76	66.50
PPL-k <sup>3</sup> -2	201	1	2	19.85	2	1	7	4	2	4	3	2	-	1	1	1	31	15.42	72.84

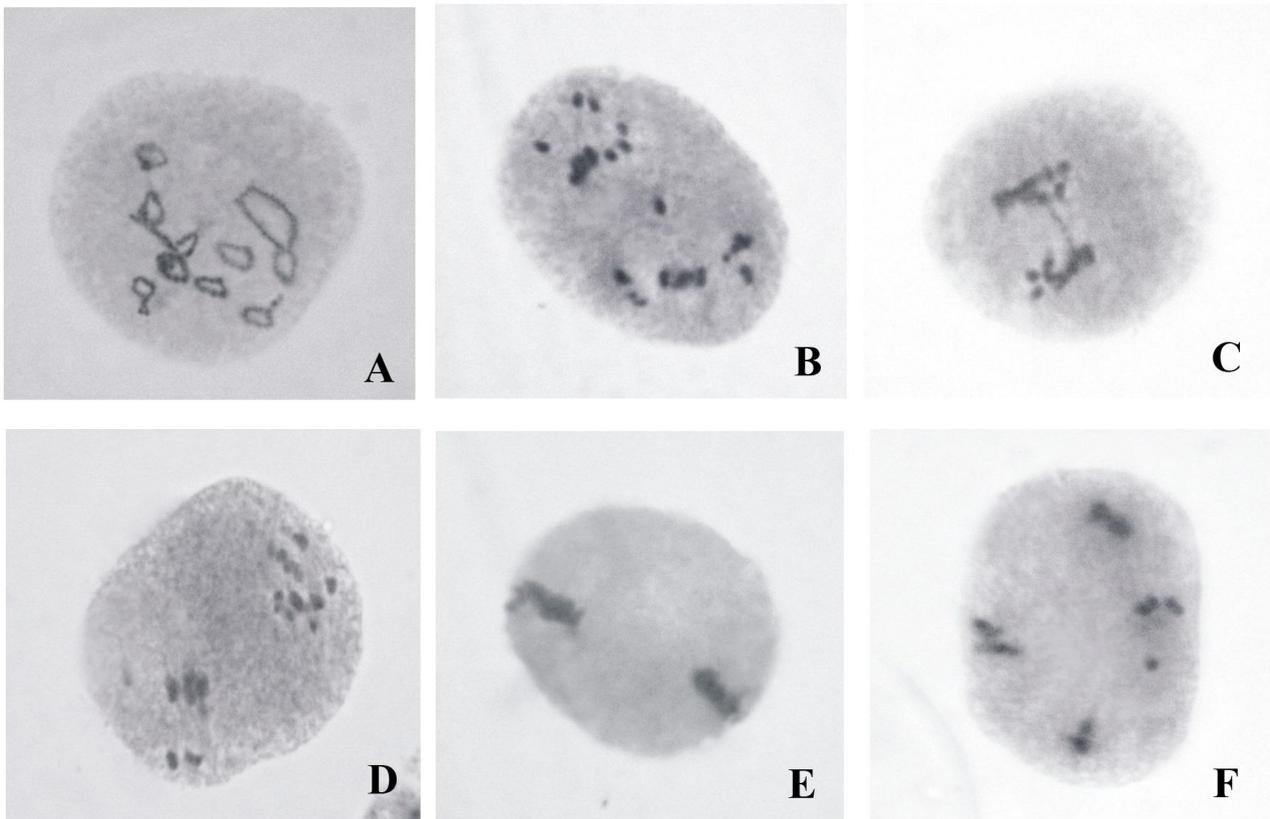


Fig. 2. (A) Prophase I: PMC showing  $1^{IV} + 9\text{-ring}^{II} + 1\text{-rod}^{II}$ ; (diakinesis); (B) Anaphase I: 1-laggard, unsynchronized movement of chromosomes; (C) Anaphase I: single chromatin bridge; (D) Anaphase I: unsynchronized movement of chromosomes; (E) Metaphase II: sticky chromosomes at equator; (F) Telophase II: 1-laggard.

mutants exhibited various chromosomal anomalies, such as multivalent (Fig. 2A) at diakinesis; stickiness of chromosomes (Fig. 2E) at metaphase; laggard (Fig. 2B), unsynchronized movement of chromosomes (Fig. 2B, 2D) single chromatin bridge (Fig. 2C) at anaphase and laggard (Fig. 2F) at telophase stage in varying frequencies. PMCs were found almost normal, although a few meiotic abnormalities (5.17%) were also observed in the mutant PPL-j<sup>3</sup> (Table 2), screened from 5kR, suggesting the absence of any major structural changes at different loci in this mutant. However, the higher frequency of abnormal PMCs was recorded in other two mutants, which was 17.76% in the mutant PPL-k<sup>3</sup>-1 followed by 15.42% in PPL-k<sup>3</sup>-2 (Table 2). Moreover, chiasma frequency per cell at diakinesis was also calculated and was found to be 21.60 at diakinesis in the control (Table 2), while it decreased in all three screened mutants at diakinesis (Table 2).

Pollen fertility was recorded to be 91.04, 66.50 and 72.84% in mutants 1, 2 and 3, respectively, as compared to the control, where it was 92.50% (Table 2).

## DISCUSSION

The main aim of mutation breeding is to create some useful mutations and widen the spectrum of variability in a desired crop plant for the future selection process. The useful mutations are created in any desired crop plant by a plant breeder through the application of mutagens in term of induction of morphological changes as well as the chromosomal aberrations. Ultimately, the occurrence of abnormal PMCs in plants may cause various genetic consequences in the form of altered traits (Al Qurainy and Khan 2009; Sheidai *et al.* 2009; Jafri *et al.* 2012). Physical mutagens predominantly induce chromosome aberrations either through ionization of the target itself or indirectly through mutagenic free radicals as a result of ionization of background components (Lagoda 2012). The present study reveals that the creation of mutations through gamma ray-irradiation could be achieved in eggplant due to the ability of gamma ray to make structural changes in the chromosomal configuration, which might be responsible for alteration in traits of economic importance.

Chiasma formation characterizes the pairing of homologous chromosomes at meiosis and controls the degree of recombination. Chiasma counting is the most straight forward method of scoring the total no. of crossing-over events in the genome (Baptista-Giacomelli *et al.* 2000). Variation in chiasmata number reveals new

forms of recombination which influences variability within natural populations in an adaptive way (Sheidai *et al.* 2006). The chiasma frequency in mutants was found lower than control, apparently, it was caused by increasing frequency of univalents, rod bivalents and multivalent, but particularly due to increased heterology induced by mutagens which is in agreement with the results of Siddique and Ansari (2005), Khan *et al.* (2007a, 2007b) and Gulfishan *et al.* (2011).

Previously it has been reported that the presence of univalents at metaphase I might be due to asynapsis (lack of chromosome pairing during the late prophase I), so that the homologous chromosomes failed to pair (Bione *et al.* 2002) or desynapsis (inability to retain chiasmata in synapsed homologous chromosomes) resulting in premature separation of bivalents (Kumar *et al.* 2006, Gulfishan *et al.* 2013). The multivalent formation was due to the breakage in chromosomes caused by mutagens and their reunion through reciprocal translocations (Khan and Verma 2017). Stickiness could arise due to depolymerization of nucleic acid caused by mutagenic treatment (Jabee *et al.* 2008, 2010) or due to dissociation of nucleoproteins and alteration in their pattern of organization (Kumar and Rai 2007).

The precocious movement of chromosomes may be due to the disturbed homology for chromosome pairing or disturbed spindle mechanism (Agarwal and Ansari 2001), either because of the abnormal spindle activity (Kumar and Gupta 2009) or due to early chiasma terminalization at diakinesis or metaphase I (Khan and Verma 2017). Stray chromosomes at metaphase I also seemed to be caused by spindle dysfunction and clumping of chromosomes (Bhat *et al.* 2007). The disturbed polarity at anaphase and telophase stages could also appear because of spindle disturbances.

Laggards might have appeared due to improper spindle functioning (Kumar and Rai 2007), even the fragments which appeared on the breakage of bridges, as a result of spindle fibres functioning to pull the chromosomes towards the poles, formed laggards (Kumar and Gupta 2009). Gene mutation or direct action of mutagen on the target protein responsible for chiasma terminalization during diakinesis at meiosis-I cause some structural defects in this protein which lead to their improper functioning, thus resulting in bridges (Kumar and Gupta 2009).

The tripolar condition may be attributed to the arrangement of chromosomes in various groups at metaphase, and then their tripolar and multipolar segregation due to abnormal spindle formation which may lead to the formation of polyads during cytokinesis (Kumar and Tripathi 2004).

Moreover, the accumulation of more and more chromosomal abnormalities greatly affected microsporogenesis leading to the formation of non-viable gametes, which considerably reduced plant fertility (Cali 2009). Low chiasma frequency may be one of the causes of low pollen fertility, because chiasmata are responsible for the

maintenance of the bivalents which permit normal chromosome segregation and this process ensures pollen fertility (De Muyt *et al.* 2009). Therefore, it may be assumed that in the irradiated populations of *Solanum melongena* L., the disturbances in chromosomal pairing, other chromosomal anomalies and physiological causes were responsible for the sterility of pollen grains, and ultimately for low fruit set.

The cytological analysis of these mutants might indicate their genetic nature; however, cryptic structural changes in the chromosomes due to the effect of gamma ray cannot be denied as the mutants were isolated in the M<sub>2</sub> generation, they are expected to be recessive in nature. These mutants may be utilized either, in various yield improvement programs as donor parents as per the aim and objective of the breeding programme or, the high yielding mutant could be released as new cultivars after extensive evaluation in future generations.

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