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Isolation, Selection And Characterization Of Parthenocarpic Fruit Somaclonal Variant In Brinjal (*Solanum Melongena* L. Var. PPL)

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Introduction

Brinjal (*Solanum melongena* L.) also known as eggplant (England) or Aubergine (France) belonging to the Solanaceae family is one of the most important vegetable crops of India (Daunay, 2008). Important brinjal growing countries are India, Japan, Indonesia, China, Bulgaria and many African countries (Vavilov, 1928). In India, brinjal is referred by various names viz., Baigan (Hindi), Badanekai (Kannada), Vangi (Marathi), Katharikai (Tamil), Vankai (Telugu) (De Candolle,1883). Brinjal fruits are a good source of calcium, phosphorus, iron and vitamins (Singh & Kumar 2006). Its green leaves are the main source of vitamin C (Gurbuza *et.al*., 2018). Brinjal has got much potential as raw material in pickle making. (Asaolu & Asaolu 2002). The dark purple brinjal are stated to be rich in vitamin C in comparison with white brinjal (Tabing & Tiwari, 2018).
The presence of seeds in brinjal fruit is undesirable to consumers and seedless fruits are therefore in demand due to improved flesh quality and suitability for processing (Denna, 1973; Varoquaux *et.al*., 2000; Yin *et.al*., 2006; Rajender *et.al.*, 2016). The negative effects associated with the presence of seeds in brinjal are browning of flesh upon cutting, increase in saponin and solasodin compounds which cause bitter taste (Aubert *et.al*., 1989) and harder flesh. Plant growth regulators play important role in fruit development leading to seed seedlessness (Nothmann and Koller, 1975; Olympios,1976).With the development of parthenocarpic brinjal, it can now be possible to grow under the protected conditions which ensure safer production of brinjal without using harmful chemicals (Acciarri *et.al.*, 2002).This trait is very useful to develop fruits in a particular environment (greenhouse cultivation) which are unfavourable for successful pollination and fertilization. So, one approach of producing seedless brinjal is parthenocarpy brinjal that can reduce kidney problems in humans. A few lines of parthenocarpic brinjal have been developed through interspecific crossing (Acciarri *et.al.*, 2002).

MATERIALS AND METHODS

Plant material
The seeds of *Solanum melongena* L. var. PPL were collected from the Telangana State Seeds Development Corporation, Hyderabad (TSSDC).

Explants
Mature zygotic embryos, hypocotyl and cotyledon were used as explants.

Preparation of MS medium
Murashige and Skoog medium (1962) was used in the present experiment.

Surface sterilization: Seeds

Mature zygotic embryos

The seeds of *S. melongena* L. var. PPL were thoroughly washed in running tap water and then surface sterilized with 0.1 % Mercuric chloride ($HgCl_2$) for 2 minutes, followed by 2-3 times rinsing in sterile distilled water to remove traces of ($HgCl_2$). The surface sterilized seeds were excised using a sterile blade (10 No.) and intact mature zygotic embryos were inoculated on MS+NAA (1.0 – 10.0 mg/l).

Hypocotyl and cotyledon explants

Fresh seeds of *S. melongena* L. var. PPL were inoculated on MS basal medium to raise axenic cultures. Hypocotyl (1.0 cm in length) and cotyledon (1.0 cm c.a.) were inoculated on MS medium supplemented with various concentrations and combinations of IAA, NAA & IBA (1.0-10.0 mg/l) and BAP & Kn (1.0-10.0 mg/l).

Incubation

Culture tubes were incubated in culture room for 4 weeks under 16/8 hrs photoperiod at 25±2^{0}C and relative humidity of 75%.

Acclimatization

Complete R_o plantlets were transferred to earthen pots for 4 weeks until acclimatization in green house.

Morphological characteristics

Morphological characters like 1. Plant height(cm), 2. Number of branches 3. Number of fruits per plant, 4. Days to flowers, 5. Number of seeds per fruit, 6. Weight of 100 seeds (g) and 7. Length of fruits (cm) of R_1 plantlets derived was recorded.

Mutagens:

Oxadiazole Treatment:

Seeds of *Solanum melongena* L var PPL were treated with Oxadiazole (Collected from the Department of Chemistry, Chaitanya (Deemed to be University, Telangana, India). The concentrations were 0.0, 0.05, 0.1, 0.2 & 0.5 %. The seeds were treated for 24 hrs and washed under running tap water to free the adherent oxadiazole compound and inoculated on MS basal medium. Some of these seeds were excised and zygotic embryo was inoculated on MS+NAA medium. 4 weeks old seeds were used for hypocotyl and cotyledon explants, the studies were carried up to R_1 generations.

γ -Irradiation:

Soaked (24 hrs) seeds of *Solanum melongena* L var PPL were irradiated with γ-rays. The doses were 0.0,0.5,1.0,1.5 & 2.0 Kr. The source of γ-irradiation was Co^{60}, delivering 100 rads/min dose rate from the γ-unit, installed at Mahatma Gandhi Memorial Hospital (MGM), Hanamkonda, Telangana, India. Some of these seeds were excised and zygotic embryo was inoculated on MS+NAA medium. 4 weeks old seeds were used for hypocotyl and cotyledon explants, the studies were carried up to R_1 generations.

Molecular Analysis:

***iaa*M-PCR analysis**

*iaa*M-PCR analysis of R1 plantlets was performed by following the method Carmen Bianco *et.al.*, (2014).

Statistical Analysis:

Statistical analysis of R_1 plantlets was performed ANOVA.

Results & Discussion:

Somatic Embryogenesis:

Embryogenic callus was induced in zygotic embryos, hypocotyl and cotyledon explants inoculated on MS+NAA (1012 mg/l), after 2-3 weeks of culture. Embryogenic callus proliferated from the entire zygotic embryos, while it proliferated from cut ends of hypocotyl and cotyledon explants. Zygotic embryos cultured on MS+ NAA (2-12 mg/l) produced faint to intense green spots after 2 weeks of culture while hypocotyl and cotyledon explants cultured on MS + NAA (4-11 mg/l) produced green spots after 3 weeks of culture. The green spots turned into somatic embryoids upon culture on MS basal medium. NAA at 10 mg/l produced maximum number of somatic embryoids and also R_1 plantlets in zygotic embryos (Table 1). NAA at 10 mg/l produced maximum number of somatic embryoids and also R_1 plantlets in hypocotyl and cotyledon explants, respectively (Tables 2 & 3) (Figure 1)

Oxadiazole Treatment:

The number of green spots and somatic embryoids increase over control in 0.1 % Oxadiazole treatment, while at 0.2 % Oxadiazole, the number of green spots and somatic embryoids decreased significantly over control. Some of the R_1 plantlets obtained via oxadiazole treatment showed variations in leaf size (Table 4).

γ -Irradiation:

The number of green spots and somatic embryoids decreased over control at 0.5 to 2.0 kr (Table 5).

R1 plantlets

The 92 R1 plantlets obtained from mutation breeding experiments were screened for somaclonal variations at morphological, biochemical and molecular levels.

Morphological level:

About 9 R1 plantlets of *S. melongena* plantlets showed changes or variations in floral characters viz., (i) white flower, (ii) Under developed anther with narrow apex for restricted dissemination of pollen grain, (iii) Low pollen fertility (iv) Under developed stigma papillae, (v) Large ovary and (vi) Fruits with seeds but non-viable.

Molecular level:

Two R1 plantlets showed *iaaM* DNA bands suggesting parthenocarpy.

Comparison of quantitative characters in R_1 generation:

Morphological variations:

The objective of the present study was to statistically analyse 7 quantitative characters, namely (i) Plant height, (ii) Number of branches, (iii) Number of days to flowers, (iv) Number of fruits per plant, (v) Fruit length, (vi) Number Seeds per fruit and (vii) Weight of 100 seeds in 92 R_1 generation obtained from NAA, NAA+2,4-D+BAP treatments. We observed that variability in these morphological characters was significant in somaclonal variants obtained from NAA treatment (Table 6 & 7).

Isolation of somaclonal variants in R_1 generation:

We have isolated the following variants in R1 generation viz.(i) Tall, (ii) Dwarf, (iii) Spiny leaf, (iv) Long fruit, (v) Single plantlet with two different fruit colours and (vi) White flower variants. (Table 8) (Figure 2).

Identification of putative parthenocarpic somaclonal variants:

Morphological characters

We identified white flower somaclonal variants in *S.melongena* var PPL that possessed

(i) Under developed anther with narrow apex for restricted dissemination of pollen grain,
(ii) Pollen viability (22%)
(iii) Stigma above the anthers with under developed papillae,
(iv) Small sized ovary and
(v) Fruits with non-viable seeds.

Identification of putative parthenocarpic fruit mutants:

A plant is considered to be parthenocarpic when its fruits are completely devoid of seeds, contain a much-reduced number of seeds or possess aborted seeds (Pandolfini,2009). In our study, we identified small fruit mutant as putative parthenocarpic fruit mutant based on the following characters. (i)Under developed anthers with narrow ape for restricted disseminations of pollen grain, (ii) Large sized ovary and (iii) Fruit wad devoid of seeds. Facultative parthenocarpic genotype was obtained through induced mutations (Restaino *et.al*.,1992).

Molecular Analysis:

***iaaM*-PCR amplification:**

In our study we observed that in small fruit mutants anthers were under developed with narrow apex for restricted dissemination of pollen grain. Bianchi & Soressi (1969), Phiuouze & peuact, (1986), also reported that the androecium of pat flowers in tomato had typically formed short irregular and unfused anthers, dehiscence was preferentially external and had with reduced number of pollen grains.

The ovary size of small fruit mutant was larger than the control and other M2 mutants. This correlate with the precocious on set of cell division in the pericarp which lead to higher auxin, gibberellins and DNA content in the ovaries (Mapelli *et.al.,* 1978). It is well know that the *iaaM* gene from Pseudomonas syringae pv. savatonoi, codes for tryptophan monooxygenase which produced indole acetamide that in turn, is either chemically or enzymatically converted to the auxin indole-3-acetic acid driven by *Defh9* promoter from Antirrhinum majus.

In small fruit mutant, *iaaM* specific primers PCR product size ranged between 1.5 to 2.0 Kb, which corresponds to 1.67 Kb gene size of *iaaM* (Fig-3)isolated from Pseudomonas syringae pv.savastanoi (GenBank accession No-AY530536)

Acciarri *et.al*., (2002), determined the expression of *Defh9-iaaM* gene in different stages of fruit development until it attained 28 cm length by RT-PCR technique and observed the presence of 161 bp amplicon which corresponds to splice *Defh9-iaaM* mRNA

The small fruit mutant we have obtained in M2 generation was a putative parthenocarpic fruit mutant and it was devoid of seed remnants, Acciarri *et.al.*, (2002), reported that *Defh9-iaaM* gene was expressed in the placenta, ovules and young fruit and as a consequence they became rich source of auxin leading to seedlessness.

The *iaaM* gene from *Pseudomonas syringae* pv.*savatonoi*, codes for tryptophan monooxygenase which produces indole acetamide that in turn, is either chemically or enzymatically converted to the auxin indole-3-acetic acid driven by Defh9 promoter from antirrhinum majus. Expression of *Defh9-iaaM* gene takes place in the placenta and ovules resulting in the development of parthenocarpic fruits. In our study we observed that the genomic DNA extracted from ovary tissue of white flower variant was PCR amplified with iaaM specific primer while in other variants PCR product was not observed. Therefore, white flower variant was identified as putative parthenocarpy fruit variant. To date the *Defh9-iaaM* chimeric gene as been shown to cause parthenocarpic fruit development in brinjal (Rotino1999, Rotino *et.al.*,1997, Acciarri *et.al*.,2002), Tomato (Ficcadenti *et.al*.,1999; Panalf ini *et.al*.,2002), Capsicum (Rajender *et.al*.,2016)

Table: 1 Induction of somatic embryoids and R1 plantlet conversion in zygotic embryo of *S.melongena* var. PPL. Mean ± SE

Mean number of somatic embryoids /Zygotic embryo

MS+NAA (mg/L)	No of green spots in embryogenic callus	No of somatic embryoids	No of plantlets (R1)
1	--	--	--
2	1.19±0.41	--	--
3	3.10±0.55	--	--
4	4.64±0.61	1.10±0.34	--
5	7.45±0.07	3.18±0.56	1.36±0.46
6	4.55±0.84	4.96±0.69	2.03±0.28
7	13.01±0.78	5.19±0.63	2.02±0.35
8	11.26±0.88	7.84±0.54	5.89±0.49
9	16.39±0.82	8.80±0.42	7.01±0.47
10	18.25±0.96	10.17±0.61	8.85±0.44
11	5.09±0.59	5.03±0.61	--
12	1.93±0.30	--	--

Table: 2 Induction of somatic embryoids and plantlet conversion in hypocotyl explants of *S.melongena* var. PPL .

MS+ NAA (mg/L)	No of green spots in embryogenic callus	No of somatic embryoids	No of plantlets (R_1)
1	--	--	--
2	--	--	--
3	--	--	--
4	1.11±0.59	--	--
5	1.79±0.38	1.10±0.59	--
6	3.81±0.38	1.84±0.57	1.40±0.41
7	6.95±0.53	2.74±0.52	1.12±0.36
8	10.12±0.47	5.98±0.53	2.71±0.40
9	14.93±0.51	9.05±0.74	4.81±0.44
10	18.35±0.71	8.75±0.72	5.80±0.60
11	5.98±0.53	1.95±0.49	1.20±0.53
12	1.84±0.57	1.88±0.52	--

Table: 3 Induction of somatic embryoids and plantlet conversion in cotyledon explants of *S.melongena* var. PPL

NAA (mg/L)	No of green spots in embryogenic callus	No of somatic embryoids	No of plantlets (R1)
1	--	--	--
2	--	--	--
3	--	--	--
4	--	--	--
5	1.74±0.43	1.12±0.40	--
6	3.76±0.40	1.87±0.35	1.16±0.42
7	6.95±0.57	3.79±0.51	1.80±0.30
8	9.61±0.58	6.89±0.57	3.76±0.46
9	15.18±0.62	7.88±0.62	3.80±0.49
10	17.91±0.47	8.75±0.72	4.56±0.42
11	5.80±0.60	2.94±0.60	1.20±0.45
12	1.81±0.45	--	--

Table: 4 Induction of somatic embryoids and plantlet conversion in hypocotyl, Zygotic embryo and cotyledon in Oxadiazole treated explants of *S.melongena* var. PPL .

Oxadiazole **No of Somatic Embryoids**			
Conc.	**Zygotic Embryo**	**Hypocotyl**	**Cotyledon**
0.0	18.25±0.96	18.35±0.71	17.91±0.47
0.05	4.11±0.60	3.30±0.52	3.01±0.78
0.1	3.78±0.22	1.70±0.48	1.76±0.45
0.2	2.90±0.57	1.95±0.47	1.12±0.44
0.5	1.45±0.34	1.33±0.56	1.24±0.21

Table: 5 Induction of somatic embryoids and plantlet conversion in hypocotyl, Zygotic embryo and cotyledon in γ-ray irradiation explants of *S.melongena* var. PPL.

γ-ray irradiated seeds **No of Somatic Embryoids**			
Conc.	**Zygotic Embryo**	**Hypocotyl**	**Cotyledon**
0.0	20.19±0.62	19.78±0.62	18.39±0.68
0.5	19.11±0.34	19.29±0.44	16.9±0.62
1.0	18.56±0.22	15.09±0.65	15.19±0.43
1.5	18.14±0.33	12.19±0.62	14.19±0.52
2.0	17.12±0.19	10.19±0.62	12.19±0.60

Table: 6 Comparisons of 07 quantitative characters in **Oxadiazole** R1 plantlets of *S.melongena* L var. PPL

Characters	**Control**	**0.05**	**0.1**	**0.2**	**0.5**
Plant height(cm)	52.31±0.53	50.61±0.18	50.11±0.22	49.01±0.34	48.11±0.67
Number of branches	07.23±0.41	07.09±0.46	08.56±0.78	07.43±0.82	06.33±0.52
Number of fruits per plant	16.21±0.37	20.38±0.52	18.42±0.49	17.67±0.89	16.43±0.88
Days to flower	40.26±0.49	44.27±0.41	42.37±0.42	41.11±0.47	41.07±0.33
Number of seeds per fruit	58.34±0.38	62.34±0.39	61.31±0.55	60.33±0.52	60.21±0.44
Weight of 100 seeds(g)	0.20±0.44	0.32±0.40	0.31±0.42	0.30±0.22	0.30±0.12

Length of fruits(cm)	4.32±0.52	3.26±0.47	3.12±0.36	3.11±0.42	3.01±0.23

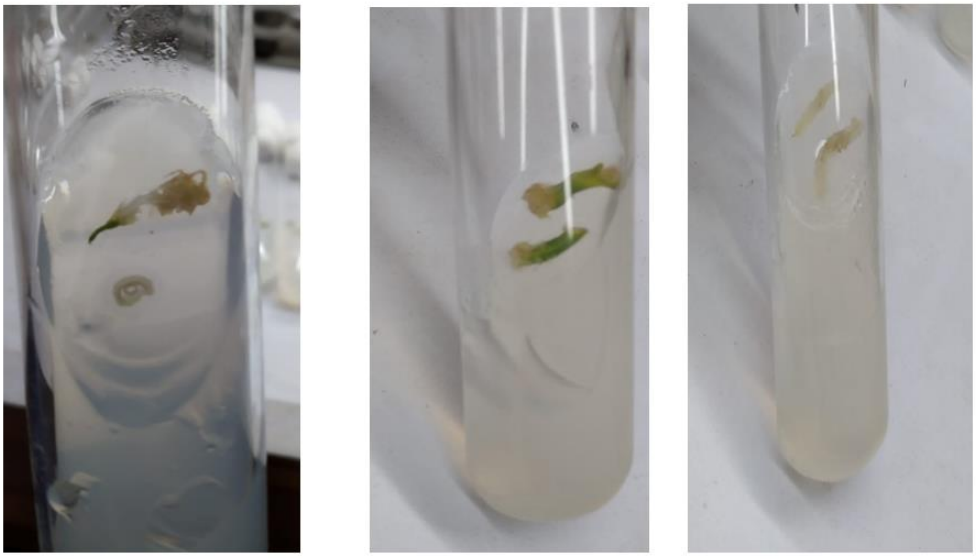
Table:7 Comparisons of 07 quantitative characters in **γ-irradiated** R1 plantlets of *S.melongena* L var. PPL

Characters	**Control**	**0.5Kr**	**1.0Kr**	**1.5Kr**	**2.0Kr**
Plant height(cm)	51.32±0.23	47.31±0.24	44.25±0.36	43.31±0.24	40.25±0.36
Number of branches	08.03±0.18	07.28±0.23	07.26±0.42	06.28±0.23	06.22±0.11
Number of fruits per plant	17.31±0.28	16.34±0.38	15.42±0.37	15.22±0.38	14.42±0.37
Days to flower	41.26±0.42	40.43±0.41	40.29±0.44	39.43±0.41	38.29±0.44
Number of seeds per fruit	50.34±0.17	49.44±0.36	48.34±0.23	47.44±0.36	46.34±0.34
Weight of 100 seeds(g)	0.40±0.36	0.22±0.30	0.30±0.33	0.22±0.30	0.30±0.33
Length of fruits(cm)	3.37±0.52	3.22±0.35	3.12±0.33	3.11±0.35	3.10±0.16

Table: 8 Spectrum and frequency of somaclonal variants obtained from NAA, NAA+2,4-D & NAA+2,4-D+BAP treatments in *S.melongena* var. PPL

Variant	***NAA**	****NAA (10.0 mg/L) + 2,4-D (2.5 mg/L)**	****NAA (9.0 mg/L)+2,4-D (2.5 mg/L)+BAP(1.5 mg/L)**
Long fruit	2.38	3.44	--
A single plantlet with different fruit colours	2.38	--	--
Dwarf	--	3.44	--
Tall	2.38	--	--
Spiny leaf	--	--	9.5
White flower	2.38	--	--

Figure 1: *In vitro* embryogenic callus induction in zygotic embryos, cotyledon, hypocotyl explants cultured on MS medium in *S.melongena* var. PPL



A. Zygotic Embryos **B. Cotyledon** **C. Hypocotyl**

Figure 2: White flower somaclonal variant isolated in R_1 generation of *S. melongena* var PPL.

A. Control (Left) & White flower variant (Right), B. Parthenocarpy fruit,

C. Control fruit, D. Viable seeds (Left), Non - Viable seeds (Right)

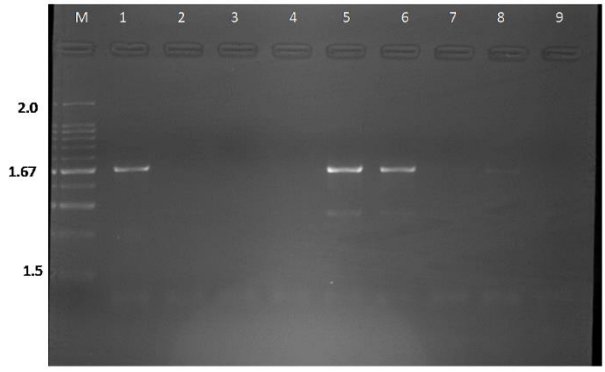


Fig: 3 Molecular identifications of selected somaclonal.

Conclusion:

Present study reveals 7 quantitative characters, namely (i) Plant height, (ii) Number of branches, (iii) Number of days to flowers, (iv) Number of fruits per plant, (v) Fruit length, (vi) Number Seeds per fruit and (vii) Weight of 100 seeds in 92 R_1 generation obtained from NAA, NAA+2,4-D+BAP treatments. We observed that variability in these morphological characters was significant in somaclonal variants obtained from NAA treatment. In our study we observed that in small fruit mutants anthers were under developed with narrow apex for restricted dissemination of pollen grain. Bianchi & Soressi (1969), Phiuouze & peuact, (1986), also reported that the androecium of pat flowers in tomato had typically formed short irregular and unfused anthers, dehiscence was preferentially external and had with reduced number of pollen grains.

The ovary size of small fruit mutant was larger than the control and other M2 mutants. This correlate with the precocious on set of cell division in the pericarp which lead to higher auxin, gibberellins and DNA content in the ovaries (Mapelli *et.al.,* 1978). It is well known that the *iaaM* gene from Pseudomonas syringae pv. savatonoi, codes for tryptophan monooxygenase which produced indole acetamide that in turn, is either chemically or enzymatically converted to the auxin indole-3-acetic acid driven by *Defh9* promoter from Antirrhinum majus. The *iaaM* gene from *Pseudomonas syringae* pv.*savatonoi*, codes for tryptophan monooxygenase which produces indole acetamide that in turn, is either chemically or enzymatically converted to the auxin indole-3-acetic acid driven by Defh9 promoter from antirrhinum majus. Expression of *Defh9-iaaM* gene takes place in the placenta and ovules resulting in the development of parthenocarpic fruits. In our study we observed that the genomic DNA extracted from ovary tissue of white flower variant was PCR amplified with iaaM (Fig-3)specific primer while in other variants PCR product was not observed. In small fruit mutant, *iaaM* specific primers PCR product size ranged between 1.5 to 2.0 Kb, which corresponds to 1.67 Kb gene size of *iaaM* (Fig-3)isolated from Pseudomonas syringae pv.savastanoi (GenBank accession No-AY530536) Therefore, white flower variant was identified as putative parthenocarpy fruit variant.

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