In-vitro Callus Induction and Regeneration of Brinjal (*Solanum melongena* L.) through Cotyledon

Shreedhar Ganapati Bhat, G. Arulananthu, N. Ramesh

**ABSTRACT**

Brinjal is one of the most popular, nutritional and vegetable crops in the world. It plays a vital role in the national economy as a cash crop. Tissue culture techniques used for in-vitro plant regeneration through cotyledon explants of brinjal (*Solanum melongena* L.) with different combinations of plant growth hormones BAP (4.44, 6.66, 8.88, 11.10 and 13.32µM) and IAA (0.57, 1.14 and 1.71µM) used for in-vitro regeneration of brinjal. The cotyledon explants used in this study, the highest callus induction found on BAP 8.88 µM and IAA 1.14 µM. The callus induction occurred after 15 days from initiation, shoot induction occurred after 30 days from initiation and shoot elongation was carried out on the same medium, shoot elongation occurred after 45 days from initiation. MS hormone-free medium found best for root regeneration, the elongated shoots were selected and transferred to a test tube containing MS hormone-free rooting medium and the elongated shoots produce roots after 15 days. Then the rooted plantlets were transferred to poly-cup with a pre-sterilized mixture of coco peat for primary hardening under poly-tunnel for 10 days. Subsequently, there generated plantlets acclimatized under the greenhouse. Then, hardened plants transferred to the open field for further development. This plant regeneration method can be useful for the production of the disease-free plant.

**Key words:** *Solanum melongena* L., Callus induction, Shoot induction, Shoot elongation.

**INTRODUCTION**

The brinjal (*Solanum melongena* L.), commonly known as eggplant under the family Solanaceae, is an economic importance vegetable crop of tropical and subtropical regions of world and is mostly grown in Asian subtropical regions (94% of world production). All over the world, there are about 25 refined species of a genus *Solanum*, which includes the potato, tomato and various eggplant species (Samuels, 2009). Brinjal is considered as King of Vegetables. It is a commercially important vegetable, as well as cash crop. In India nearly 40 eggplant varieties cultivated with an estimated area of 730.40 thousand hectare and the total production is 12800.8 thousand Metric tons in 2017-18 (Anonymous, 2018). The major producing states of brinjal are West Bengal, Odisha, Gujarat, Madhya Pradesh, Bihar, Maharashtra, Karnataka, Uttar Pradesh and Andhra Pradesh. According to the Food and Agriculture Organisation of the United Nations (FAO, 2015), the world’s largest eggplant producers are China and India with production of 28 Mt and 13 Mt per year respectively. The eggplant mainly cultivated in Turkey (827,000 tons), Italy (220,000 tons), Spain (206,000 tons) and Romania (123,000 tons/year) in Europe. The FAO (2015) has reported that both Ukraine (96,000 tons in 2013) and Lithuania (2,000 tons in 2013) now also grow this crop.

As compared to other crop plants like tomato, it is rich in vitamins and minerals that increase its total nutritional value (Kalloo, 1993). The eggplant consist of high soluble fiber and mineral contents as calcium, iron, potassium and phosphorus. Vitamins such as vitamin B-6, vitamin K, vitamin C, folate and choline are also considerably high in the fruits, making it beneficial to human health (Bhatti et al., 2013).

Brinjal has rich in antioxidant compounds, which leads to hepatoprotective properties (Concannon et al., 2012). The crop is susceptible to several diseases like Damping-off, Phomopsis blight, fruit rot, Little leaf of brinjal, Bacterial wilt, Leaf spot, Late blight, Collar rot, etc. (Bhupendra Kumar Singh et al., 2014) and pests, causing massive yield losses. Biotic stress has become a significant risk of infections for the cultivation of brinjal (Krishnaiah, 1980).

The regeneration ability of brinjal has allowed the application of somaclonal variation, haploidy, hybridization and genetic transformation (Collonier et al., 2001). It is also a sound system for *in vitro* studies because plant regeneration can be achieved via the organogenic pathway from different explants. Organogenesis from hypocotyl (Magioli et al., 1998; Dobariya and Kachhadiya, 2004), root (Franklin et al., 2004) explants have been reported. Eggplant has been regenerated via somatic embryogenesis from leaf and cotyledon (Rao and Singh, 1991) and hypocotyl
In-vitro Callus Induction and Regeneration of Brinjal (Solanum melongena L.) through Cotyledon

(Matsuoka and Hinata, 1979) explants. The main objectives of this study are mass production of brinjal cultivar Arka Shirish and standardization of in vitro protocol for the Agrobacterium-mediated transformation with the hevein gene against fungal disease.

**MATERIALS AND METHODS**

**Plant material**

The seeds of Brinjal cultivar Arka Shirish obtained from the Indian Institute of Horticulture Research (IIHR- ICAR) Bangalore. Seeds sowed on 96-well nursery pro-tray and sprinkled the water once in a day up to 12 days. The seeds started germination from the fifth day on wards. Cotyledon excised from the 10-12 days old seedlings.

**Culture media preparation**

The MS medium (the composition of micronutrient, macronutrient, vitamins, amino acids and hormones (Himedia) adjusted to the pH 5.8 and 0.8% agar was added (Murashige and Skoog, 1962). The medium-boiled up to milky appearance for dissolving agar in the medium. Nearly 50 ml of medium dispersed in each culture bottle, 12 ml of the medium was dispersed in each culture tube and sealed with a clean wrap cover before autoclaving. After cooling in the culture room until use. Under laminar airflow cabinet, the surface-sterilized explants were inoculated aseptically in the MS medium supplemented with various concentrations of phytohormones combinations (4.44, 6.66, 8.88, 11.10, 13.32 µM of 6-Benzylaminopurine (BAP) and 0.57, 1.14, 1.71 µM of Indole-3-acetic acid (IAA) which is used for callus and shoot development, likewise induction of root in hormone-free medium.

**Sterilization of explants and inoculation**

The cotyledon excised from germinated seedlings and clean thoroughly under tap water for 10 min, then washed in an agitated solution of 2% Tween 20 for 10 minutes and washed with tap water. Then the explants were treated with 0.5% of bavistin for 30 min. Finally, the explants cleaned with double distilled water for clearing the fungicide on the explant. The treated explants transferred into the horizontal laminar airflow chamber (Sunrise enterprises, Bangalore) for further sterilization. Then the explants were submerged in sodium hypochlorite (2.0%) for 10min then 70% ethanol for 30 seconds, followed by thorough shaking in HgCl₂ (0.1%) for 1.5 minutes and washed thrice with sterile distilled water. After surface sterilization, both ends of the explant were cut and trimmed to 1 cm size.

**Callus, shoot induction and regeneration**

The 20 cotyledon explants inoculated into the MS media supplemented with different concentrations of BAP (4.44, 6.66, 8.88, 11.10 and 13.32 µM) and IAA (0.57, 1.14, 1.71 µM) on culture bottle. The cultures were incubated under the fluorescent lights with 1500-2000 lux for 10 hours per day. The culture room maintained with a temperature of 25 ± 1°C and 65±10 relative humidity during callus induction. The well-developed callus induces shoot and shoots elongation. The data recorded on the 15th, 30th and 45th days of incubation. Each of the experiments repeated thrice; the data recorded for callus formation, shoot initiation and elongation.

**Rooting and hardening**

For root induction, a 2-3 cm size shoot selected and transferred to test tubes containing rooting media without exogenous hormones (Pratap et al., 2011; Chen et al., 1995). The root induction data recorded on the 15th, 30th and 45th days of incubation. After root induction, rooted plantlets were taken out from the culture tubes and washed to remove adhered agar and traces of medium then washed with 20 ppm of bavistin solution for 10min to avoid microbial infection. Plantlet transferred to an 8.0 cm diameter poly cup containing sterilized Coco peat, 1% neem cake and sand mixture. These plants were maintained inside the growth chamber set at 26±1°C and 75-80% relative humidity were maintained by covering the plant with polythene cover for two weeks and irrigated gently every alternate days for acclimatization. After that, the plants were transferred to pots containing organic manure, garden soil and sand (1:1:1) and maintained in a greenhouse. Then, the plants transplanted to the field.

**Statistical analysis**

Tissue culture data were subjected to analysis of variance by One-Way ANOVA to detect the significant differences among the treatment means using Duncan’s Multiple Range Test at P<0.05.

**RESULTS AND DISCUSSION**

**Seed germination**

Seeds of Brinjal Arka Shirish were sown on nursery pro-tray and sprinkled with water once a day, it germinated well. 73% of seed germination observed (Table 1; Fig 1A).

The sterilized cotyledonal explants (Fig 1B) inoculated in the various concentration of BAP (4.44-13.32 µM) combine with IAA (0.57-1.71) supplemented MS medium for callus initiation, shoot induction and also multiple shoot formation. The data observed on the 15th for callus, 30th for the shoot, 45th day for multiple shoots.

**Callus induction**

The combination of BAP and IAA initially induce the callus. The variation of callus induction observed on the 15th day of

| Cultivar name | No of seeds | No of days | Germinated seeds | Germination Percentage |
|---------------|-------------|------------|------------------|------------------------|
| Arka Shirish  | 100         | 10-12      | 73±0.00          | 73                     |
observation. First, the formation of a compact callus later is formed friable callus (Fig 1C). The highest callus induction 18.00±0.00 observed on the MS medium fortified with 8.88 µM of BAP and 1.14 µM of IAA followed by the 8.88 µM of BAP and 0.57 µM of IAA fortified medium (Table 2; Fig 1C). The lowest callus induction 11.33±0.57 observed on the medium fortified with 4.44 µM of BAP and 0.57 µM of IAA.

In the previous study, 100% callus induction observed on the MS media fortified with 8.88µM of BAP and 0.27µM of NAA from cotyledon explants of eggplant (Huda et al., 2007) and callus induction in cotyledon (90.0%) and hypocotyls (63.3%) observed in BAP (2.22 µM) and Kinetin (9.29 µM) combination (Zayova et al., 2008). Similar observation also observed on cotyledon and hypocotyl explants with BAP and IAA fortified MS medium but low frequency of callus induction found on root explants (Mir et al., 2011).

### Shoot initiation and multiple shoot induction

In the 30th day of observation, 12.00±0.00 compact callus are loosely formed friable callus. Later the friable callus initiate shoots out of 14.00±0.00 callus in the MS medium supplemented with 8.88 µM of BAP and 1.14 µM of IAA (Fig 1D). 60.00% (12.00±0.00) of multiple shoots observed on the 45th days of observation. A maximum of 3 multiple shoots are observed in the explant (Table 3; Fig 1E). Pei Ching Foo (2018) reported 2.0 mg/l kinetin + 8.88 µM BAP combinations induce an average number of 0.80 ± 0.25 multiple shoots per explant. But in our study, the low concentration of IAA (1.14 µM) combine with BAP (8.88 µM) induced a better number of shoots. Jamil et al. (2013) reported that kinetin is necessary to be included together with other plant growth regulators in inducing shoots for eggplant, but in this study, both BAP and IAA hormonal combination induced callus and shoots. The cotyledon explants showed a high frequency of shoot regeneration as compared to hypocotyl explants in eggplant (Bardhan et al., 2012) and in Vigna mungo (Anandan et al., 2019). Ani Rani Borah et al. (2019) reported the maximum five shoots was achieved on 4.44 µM of BAP and 2.32 µM of kinetin supplemented MS medium in Coccinia indica.

### Table 2: Induction of callus rate from different combination of media.

| Hormones (µM) | BAP (µM) | 4.44 | 6.66 | 8.88 | 11.10 | 13.32 |
|--------------|---------|------|------|------|-------|-------|
| IAA          | 0.57    | +    | +    | +    | ++    | ++    |
|              | 1.14    | +    | +    | +++  | ++    | ++    |
|              | 1.71    | +    | +    | ++    | ++    | ++    |

Note: Diameter of callus mass: + (0.0-0.5 cm) - slight; ++ (0.5-1.0 cm) - moderate; +++ (1.0-2.0) – massive.

### Table 3: Callus induction after 15 days, shoot induction after 30 days, both multiple shoot induction and shoot elongation after 45 days inoculation of cultivar Arka Shirish.

| BAP (µM) | IAA (µM) | No of explants inoculated | No of explants induced callus - 15th day | Percent | No of explants induced shoots - 30th day | Percent | No of explants induced multiple shoots - 45th day | Percent | No of shoots elongated (2-3 cm height) | Percent | No of shoots elongated | Percent |
|----------|----------|--------------------------|----------------------------------------|--------|----------------------------------------|--------|-----------------------------------------------|--------|-------------------------------|--------|--------------------------|--------|
| 4.44     | 0.57     | 20                       | 11.33±0.57                             | 56.66  | 07.33±0.57                             | 36.66  | 05.33±0.57                                   | 26.66  | 03.33±0.57                                 | 16.66  |                           |        |
| 4.44     | 1.14     | 20                       | 12.66±0.57                             | 63.33  | 08.66±0.57                             | 43.33  | 06.66±0.57                                   | 33.33  | 04.66±0.57                                 | 23.33  |                           |        |
| 4.44     | 1.71     | 20                       | 11.66±0.57                             | 58.33  | 07.66±0.57                             | 38.33  | 05.66±0.57                                   | 28.33  | 03.66±0.57                                 | 18.33  |                           |        |
| 6.66     | 0.57     | 20                       | 13.33±0.57                             | 66.66  | 09.33±0.57                             | 46.66  | 07.33±0.57                                   | 36.66  | 05.33±0.57                                 | 26.66  |                           |        |
| 6.66     | 1.14     | 20                       | 14.66±0.57                             | 73.33  | 10.66±0.57                             | 53.33  | 08.66±0.57                                   | 43.33  | 06.66±0.57                                 | 33.33  |                           |        |
| 6.66     | 1.71     | 20                       | 13.66±0.57                             | 68.33  | 09.66±0.57                             | 48.33  | 07.66±0.57                                   | 38.33  | 05.66±0.57                                 | 28.33  |                           |        |
| 8.88     | 0.57     | 20                       | 15.66±0.57                             | 78.33  | 11.66±0.57                             | 58.33  | 09.66±0.57                                   | 48.33  | 07.66±0.57                                 | 38.33  |                           |        |
| 8.88     | 1.14     | 20                       | 18.00±0.00                             | 90.00  | 14.00±0.00                             | 70.00  | 12.00±0.00                                   | 60.00  | 10.00±0.00                                 | 50.00  |                           |        |
| 8.88     | 1.71     | 20                       | 16.00±0.00                             | 80.00  | 12.00±0.00                             | 60.00  | 10.00±0.00                                   | 50.00  | 08.00±0.00                                 | 40.00  |                           |        |
| 11.10    | 0.57     | 20                       | 14.00±0.00                             | 70.00  | 10.00±0.00                             | 50.00  | 08.00±0.00                                   | 40.00  | 06.00±0.00                                 | 30.00  |                           |        |
| 11.10    | 1.14     | 20                       | 15.00±0.00                             | 75.00  | 11.00±0.00                             | 55.00  | 09.00±0.00                                   | 45.00  | 07.00±0.00                                 | 35.00  |                           |        |
| 11.10    | 1.71     | 20                       | 14.33±0.57                             | 71.66  | 10.33±0.57                             | 51.66  | 08.33±0.57                                   | 41.66  | 06.33±0.57                                 | 31.66  |                           |        |
| 13.32    | 0.57     | 20                       | 12.00±0.00                             | 60.00  | 08.00±0.00                             | 40.00  | 06.00±0.00                                   | 30.00  | 04.00±0.00                                 | 20.00  |                           |        |
| 13.32    | 1.14     | 20                       | 13.00±0.00                             | 65.00  | 09.00±0.00                             | 45.00  | 07.00±0.00                                   | 35.00  | 05.00±0.00                                 | 25.00  |                           |        |
| 13.32    | 1.71     | 20                       | 12.33±0.57                             | 61.66  | 08.33±0.57                             | 41.66  | 06.33±0.57                                   | 31.66  | 04.33±0.57                                 | 21.66  |                           |        |

Note: Each experiment repeated thrice (3-replication), per experiment 15-combinations adopted (15-treatment) and per combination 20 explants inoculated.
**In-vitro Callus Induction and Regeneration of Brinjal \( (Solanum melongena \text{ L.}) \) through Cotyledon**

**Shoot elongation**

In the 45\(^{th}\) day of observation, above 3 cm height of the plant is considered as elongated shoot. In this study, 10.00±0.00 shoots lengths are more than 3 cm height grow on MS medium supplemented with 8.88 µM of BAP and 1.14 µM of IAA (Table 3). The 03.33±0.57 shoots lengths are more than 3 cm height on the 1 µM of BAP and 0.57 µM of IAA supplemented with MS medium (Fig 1E). The high concentration of BAP (8.88 µM) with a low concentration of IAA (1.14 µM) responsible for callus induction initially on the explant. The shoot initiation and multiple shoot formation on the subsequent days. These concentrations of the hormones are suitable for shoot elongation to compare among the tested BAP and IAA combination. In the present study, the culturing of cotyledon with an inverted position (a ventral portion of the cotyledon) touches the medium responds to the callus induction. It may occur due to the rapid meristematic activity that occurs in the ventral region, which is a direct contract with the nutrient medium under the influence of hormones reported by Padma Mallaya and Ravishankar (2013) in hypocotyl region of \( S. melongena \) and Kumar et al. (2005) in \( Capsicum annuum \). The continuous incubation of explants in the same culture medium, which is responsible for inducing the multiple shoots and shoot elongation. The few numbers of elongated shoots observed on BAP and IAA combination medium in eggplant. A similar observation was observed in \( Stevia rebaudiana \) (Tajo Abraham and Smrithi, 2016).

This study is controversy to the earlier report in \( S. melongina \) require multiple shoot initiation culture in TDZ media (Padma Mallaya and Ravishankar, 2013), combination of GA3 and TIBA for elongation of shoot (Cambecedes et al., 1991) in \( Lonicera nitida \).

**Root induction and hardening**

The elongated shoots excised and transferred in MS hormone-free medium for root induction (Fig 1F). The 17.66±0.57 shoots are response for roots induction out of 20 inoculated shoots. The average of 05.44±0.52 roots and 07.44±0.52 leaves per shoot on the 15\(^{th}\) day of observation (Table 4; Fig 1G). Padma Mallaya and Ravishankar, (2013) reported that IBA (4.92 µM/l) need for root induction from the elongated shoots, which produce 4±0.7 roots per explants in eggplant. A similar type of results for the formation of the root in plant growth hormone-free medium has been described in \( S. melongena \) (Sarker et al., 2006) and \( Elaeis guineensis \) (Sparjanbabu et al., 2019). Magioli et al. (1998)

**Table 4:** Shoots response, leaves/plant and roots/plant from the ex-agar plants.

| No of Shoots inoculated | No of shoots responded | No of leaves/plant | No of roots/plant |
|-------------------------|------------------------|--------------------|------------------|
| 20                      | 17.66±0.57             | 07.44±0.52         | 05.44±0.52       |

Note: Each experiment repeated thrice (3-replication), per experiment 20 shoots inoculated.

**Fig 1:** Brinjal cultivar Arka Shirish shows different regeneration stages: (A) Germination of seeds on 10\(^{th}\) day from sowing. (B) Cotyledon explants inoculated on regeneration medium, (C) Callus induction on 15\(^{th}\) day of inoculation. (D) Shoot induction on 30\(^{th}\) day of inoculation, (E) Shoot elongation and multiple shoot induction on 45\(^{th}\) day of inoculation, (F) Elongated shoot inoculated on root induction medium (G) Root induction on 15\(^{th}\) day of transfer to rooting medium (H) Hardened plant in poly-cup on 30\(^{th}\) day of hardening, (I) Field plant on 15\(^{th}\) day of planting on-field.
reported the induction of roots using half strength of MS supplemented with 0.6µM IAA. The rooted plantlets was transferred to 8.0 cm diameter poly cup containing sterilized Coco peat and 1% neem cake sand mixture (Fig 1H). After that, the plants were transferred to pots containing organic manure, garden soil and sand (1:1:1) and maintained in a greenhouse. Then, the plants were transplanted to the field (Fig 1l).

**CONCLUSION**

The 10-12 days old cotyledon explants used in this study, the best callusing and shoot regeneration found regeneration medium supplemented with BAP 8.88µM and IAA 1.14µM. This study reveals that the higher performance of callus induction 90.00%, 70.00% of shoot induction, 60.00% of multiple shoot initiation and 50.00% of shoot elongation observed on medium supplemented within BAP 8.88µM and IAA 1.14µM. In the present study concluded that the combination of BAP at high concentration with a low concentration of IAA supplemented MS medium responsible for callus induction on the cotyledonary explants initially, latter these hormones induced shoot initiation and elongation of shoots. The root induction occurred from elongated shoots on the MS hormone-free rooting medium. This protocol has efficient in being used for the development of desired types of disease-resistant eggplant plants following future genetic transformation.

**REFERENCES**

Anonymous. (2018). Horticultural Statistics at a Glance Ministry of Agriculture and Farmers’ Welfare. Government of India. PP. 148.

Anandan R, Deenathayalan T, Bhuvaneshwari R, Merlin Monisha M and Prakash M. (2019). An efficient protocol for rapid plant regeneration from deembryonated cotyledons of a black gram [Vigna mungo (L.) Hepper]. Indian J. Agric. Res. 53(5): 589-593.

Ani Rani Borah, Anbumalmathri J and Aruna Sharmaji S. (2019). In vitro propagation of Coccinia indica (L.) Voigt. from internodal segments. Indian J. Agric. Res. 53(2):202-207.

Barbhana S K, Sharma C and Srivastava D K. (2012). In vitro plant regeneration studies in brinjal. Journal of Cell Tissue Research. 12(2): 3213-3218.

Bhatti K H, Kausar N, Rashid U, Hussain K, Nawaz K and Siddiqi E H. (2013). Effects of biotic stresses on eggplant (Solanum melongena L.). World Applied ScienceJournal. 26(3): 302-311.

Bhupendra Kumar Singh, Saurabh Singh, Bijendra Kumar Singh and Sanwar Mal Yadav. (2014). Some Important Plant Pathogenic Disease of Brinjal (Solanum melongena L.) and their Management. Plant Pathology Journal. 13: 208-213.

Chen Q, Jelenkovic G, Chinn C K, Billings S, Eherhardt J, Goldberg J C and Day P. (1995). Transfer and transcriptional expression of the Coleopteran CylidBendotoxin gene of Bacillus thuringiensis in eggplant. Journal of the American Society of the Horticultural Science. 120(6): 921-927.

Coltonier C, Fock I, Kashyap V, Rotino G L, Dunay M C, Lian Y, Mariska I K, Rajam M V, Servaes A, Ducreeux G and Sihachakr D. (2001). Application of biotechnology in eggplant. Plant cell Tissue and Organ Culture. 65: 91-107.

Cambuces J, Duron M and Decourtye L. (1991). Adventitious bud regeneration from leaf explants of shrubby ornamental honeysuckle Lonicerar nitida Wils cv. Maigrin: effects of thidiazuron and 2,3,5 tri-iodobenzoic acid. Plant Cell Rep 10:471-474

Concellan A, Zaro M J, Chaves A R and Vicente A R. (2012). Changes in quality and phenolic antioxidants in dark purple American eggplant [Solanum melongena (L.) cv. Lucia] as affected by storage at 0°C and 10°C. Postharvest Biology and Technology. 66:35-41.

Dobaria K L and Kachchadiya J R. (2004). Role of genotype explant and culture medium on invitro morphogenesis in brinjal. Orissa Journal of Horticulture. 32: 90-97.

FAO (Food and Agriculture Organization). (2015). FAO Yearbook Production. Food and Agriculture Organization of the United Nations, Rome, Italy.

Franklin G, Sheeba C J and Sita G L. (2004). Regeneration of eggplants (Solanum melongena L.) from root explants. Plant Cell Reports. 40: 188-91.

Huda A, Bari M A, Rahman M and Nahar N. (2007). Somatic embryogenesis in two varieties of eggplant (Solanum melongena L.). Research Journal of Botany. 20: 195-201.

Jamil M D, Parvaiz M, Tufail M, Arshad J, Hussain S and Imliaz S. (2013). Callogenesis, regeneration of shoot and root of brinjal (Solanum melongena L.). World Applied Science Journal. 26: 1039-1045.

Kaloo G. (1993). Eggplant (Solanum melongena L.). In: G. Kalloo, (Ed.), Genetic Improvement of Vegetable 573 Crops. Pergamon Press, Oxford; pp: 587-604.

Krishnaiah K. (1980). Assessment of crop losses due to pests and diseases. In: Govindu HC (ed), University of Agricultural Sciences Technology Series 33, Bangalore pp: 259-267.

Kumar V, Gururaj H B, Prasad B C N, Giridhar P, Ravishankar G A. (2005). Direct shoot organogenesis on shoot apex from seedling explants of [Capsicum annuum (L.) Sci Hortic]. 106:237-246.

Magioli C, Rocha A P M and Oliveria D E. (1998). Efficient shoot organogenesis of eggplant (S. Melongena L.) induced by thidiazuron. Plant Cell Reports. 17: 661-663.

Matsuoka H and Hinata K. (1979). NAA induced organogenesis and embryogenesis in hypocotyl callus of Solanum melongena L. Journal of Experimental Botany. 30:363-370.

Mir K A, Dhatt A S, Sandhu J S and Sidhu A S. (2011). Effect of genotype, explant and culture medium on organogenesis in brinjal. Indian Journal of Horticulture. 68(3): 332-335.

Murashige T and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiolgia Plantarum. 15:473-497.

Padma Mallaya N and Ravishankar G A. (2013). In vitro propagation and genetic fidelity study of plant regenerated from inverted hypocotyl explants of eggplant (Solanum melongena L.) cv. Arka Shristi. Biotech. 3:45-52.

Pei Ching Foo, Ze Hong Lee, Chee Keong Chin, Sreeramanam Subramaniam and Bee Lynn Chew. (2018). Shoot induction
in white eggplant (Solanum melongena (L.) cv. bulat putih) using 6-benzyl amino purine and kinetin. Tropical Life Sciences Research. 29(2): 119-129.

Pratap D, Kumar S, Raj S K and Sharma A K. (2011). Agrobacterium-mediated transformation of eggplant (Solanum melongena L.) using cotyledon explants and coat protein gene of cucumber mosaic virus. Indian Journal of Biotechnology. 10(1): 19-24.

Rao P V L and Singh B. (1991). Plantlet regeneration from encapsulated somatic embryos of hybrid Solanum melongena L. Plant Cell Reports. 10:7-11.

Samuels J. (2009). The Solanaceae: novel crops with high potential. Organic Grower. 9: 32-34.

Sarker R, Yesmin S and Hoque M. (2006). Multiple shoot formation in eggplant (Solanum melongena L.). Plant Tissue Culture and Biotechnology. 16(1): 53-61.

Sparjanbabu D S, Naveen Kumar P, Krishna M S R, Ramajayam D, Kalyana Babu B and Susanthi B. (2019). Effect of culture media, plant growth regulators and genotypes on growth and developmental stages of oil palm (Elaeis guineensis Jacq.) zygotic embryos. Indian J. Agric. Res. 53(2): 143-150.

Tajo Abraham and Smrithi P K. (2016). In vitro propagation of sweet herb; Stevia rebaudiana Bert. Indian Journal of Plant Sciences. 5(4): 1-6

Zayova E, Nikova V, Ilieva K and Philipov P. (2008). Callusogenesis of eggplant (Solanum melongena L.). Comptes Rendus de l’Academie Bulgare Des Sciences. 63(12): 1749-1756.