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IN VITRO SHOOT ORGANOGENESIS AND PLANTLET REGENERATION IN BRINJAL (*SOLANUM MELONGENA L*)

S. V. BHAT, A.S. JADHAV, B. D. PAWAR*, A. A. KALE, V.P. CHIMOTE, S. V. PAWAR
State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413722, INDIA
e-mail: bhau.raje@gmail.com

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***Corresponding author**

ABSTRACT
In vitro plant regeneration of brinjal genotype "Manjari Gota" was achieved using cotyledonary leave, nodal segment and shoot tip explants. Best regeneration was observed on MS medium supplemented with 2 mg/l 6-benzylamino purine (BAP) and 1 mg/l kinetin, with regards to regeneration efficiency, days to callus initiation and number of shoots per explant. Among the explants used cotyledonary leaf pair showed highest shoot regeneration efficiency (100 %) followed by shoot tip (96. 66 %) and nodal segment (93.33 %). The highest numbers of shoots per explant (6.66) were obtained while using cotyledonary leaf pair explant. *In vitro* root initiation was obtained within 8 days of culture on MS medium supplemented with 0.5 mg/l indole butyric acid (IBA). *In vitro* rooted plantlets were successfully established in polycarbonated polyhouse with 100 % survival rate. This plant regeneration method can be very useful for genetic transformation of brinjal.

INTRODUCTION

Brinjal (*Solanum melongena* L.; 2n = 24) belong to the Solanaceae family, are native to the South East Asian region and were first domesticated there over 4000 years ago (Luthria 2012). It is one of the most important vegetables worldwide, with a global production 431.74 lakh tones and 17.28 lakh ha area harvested, in 2010-11. India ranked second in brinjal production. In India Brinjal ranked third in vegetable crops in terms of production (118.96 lakh tones) and area harvested (6.8 lakh ha), respectively in the year 2010-11 (anonymous 2011). Brinjal is rich in antioxidant compounds and have hepatoprotective properties (Concellon *et al.,* 2012)

Brinjal is susceptible to several diseases and pests causing massive yield losses. Excessive and indiscriminate use of chemical pesticides not only damages the environment but also affects human health. Conventional breeding approaches for developing insect resistance for brinjal is limited due to sexual incompatibilities, prevalence of sterility in the progeny and lack of natural resistance sources (Shivraj and Rao 2011, Magioli and Mansur 2005). Biotechnology provides tools to develop plants with desired traits which had been difficult to achieve using conventional breeding techniques. To combat yield losses due to insects, introduction of genes and development of transgenic plants through *Agrobacterium* mediated transformation system is very essential.

An efficient regeneration protocol for development of transgenic brinjal is a prerequisite for successful *Agrobacterium* mediated transformation system. Fassuliotis first reported *in vitro* regeneration in wild species of brinjal *(Solanum sisymbriifolium* Lam) (Fassuliotis 1975). Since then there are several reports on *in vitro* regeneration in brinjal. Organogenesis from various explants such as cotyledon (Bardhan *et al.,* 2012, Mir *et al.,* 2011, Shivraj and Rao 2011, Kaur *et al.,* 2011, Sarkar *et al.,* 2006), hypocotyl (Mir *et al.,* 2011; Shivraj and Rao 2011, Bardhan *et al.,* 2012, Ferdausi *et al.,* 2009, Kaur *et al.,* 2011, Sarkar *et al.,* 2006), leaf (Shivraj and Rao 2011, Magioli *et al.* 1998, Kaur *et al.,* 2011), root (Mir *et al.,* 2011; Franklin *et al.,* 2004, Sarkar *et al.,* 2006), shoot tip (Ferdausi *et al.,* 2009, Sarkar *et al.,* 2006), midrib (Ferdausi *et al.,* 2009), epicotyl (Magioli *et al.* 1998), stem nodes (Magioli *et al.* 1998) has been reported previously.

The type and concentration of a growth regulator in association to specific genotypes can cause significant differences in the morphogenetic responses of brinjal (Magioli and Mansur 2005). Keeping in view these factors, aim of our study was to develop efficient and reproducible regeneration protocol in Brinjal using cotyledonary leaf pair, nodal segment and shoot tip explants.

MATERIAL AND METHOD

The seeds of Brinjal (*Solanum melongena* L.) variety "Manjari Gota" were obtained from Vegetable Breeder, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri. Seeds were surface sterilized with 70% (v/v) ethanol for 1 min followed by 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 6 min. The seeds were further rinsed five times with sterile distilled water and then inoculated on a Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. Those were incubated at 25°C temperature with 16/8 h photoperiod. Cotyledonary leaf pairs, nodal segments and shoot tips were aseptically excised from the *in vitro* grown seedling cultured

on MS medium fortified with different levels of 6-benzylamino purine (BAP), zeatin, and kinetin. Those shoots greater than 3 cm in length were excised and cultured on MS medium supplemented with 0.5 mg/l of indole butyric acid (IBA) for rooting. Plantlets with well developed roots were taken out washed with water to remove agar, then rinsed with 0.1 % blitox (fungicide) solution and transferred to portray containing coco peat. Plantlets were initially covered with plastic bags for 5–7 days, and kept at polycarbonated polyhouse. Plants were irrigated with half MS solution for 14 days and finally transferred to pot containing soil: cow dunk (4:1) and irrigated with water at regular intervals. Regeneration efficiency was calculated by calculating number of explants differentiating into complete plantlet.

RESULTS AND DISCUSSION

The present study describes development of a rapid and efficient plantlet regeneration protocol using cotyledonary leaf pair, nodal segment and shoot tip obtained from *in vitro* grown seedling. The type and concentration of a growth regulator was found to have significant impact on callus formation and morphogenetic responses. The results of *in vitro* regeneration from various brinjal explants at different hormone concentrations and combinations are given in Table 1.

All three explants initiated callus and formed shoots on all twelve combinations of plant growth regulators tested. It was observed that callus induction and regeneration was quite permissive over a wide range of plant growth regulators. Cultured explants showed signs of callus induction within 9-12 days. Earliest callus initiation (9.33-9.66 days) was observed on MS medium supplemented with 2 mg/l BAP and 1 mg/l kinetin in all three explants, whereas callus initiation was observed after 10.66-11.66 days on MS medium supplemented with 0.75 mg/l zeatin. Callus initiation was observed at the base of explants (Fig 2). Initially a single preformed shoot got elongated (Fig 3), however as callus proliferated, elongation of preformed shoot was inhibited. During callusing, many new shoot buds emerged (Fig 4) however very few differentiated into shoots (Fig 5).

Shoot regeneration using BAP

In the present study gradual increase in regeneration frequency (60.00-96.66 %) and number of shoots per explant (1-5.66) was observed with increasing concentrations of BAP (1.0 - 2.0 mg/l) in all the explant tested. However at 2.5 mg/l BAP, there is considerable decrease in number of shoots per explant (1.66- 2.33) and regeneration frequency (66.66- 73.33 %). Zayova *et al.,* 2012 had also observed higher regeneration frequency in brinjal with increased BAP concentrations i.e. 0.5, 1, and 2 mg/l. However, they reported that higher BAP level had negative effect on organogenesis leading to shoots vitrification. Kaur *et al.,* 2011 observed that increase in BAP concentration above 2.5 mg/l BAP resulted in decreased in regeneration capability and number of buds on all the explants. Pawar *et al.,* (2012) and Sagare and Mohanty (2012) also observed indirect shoot regeneration on medium comprising MS medium supplemented with BAP (1.0-2.0 mg/l) with highest frequency of shoot regeneration and maximum number of shoots per explant occurring at 2.0 mg/l BAP in combination with 2.0 mg/l BAP.

Shoot regeneration using zeatin

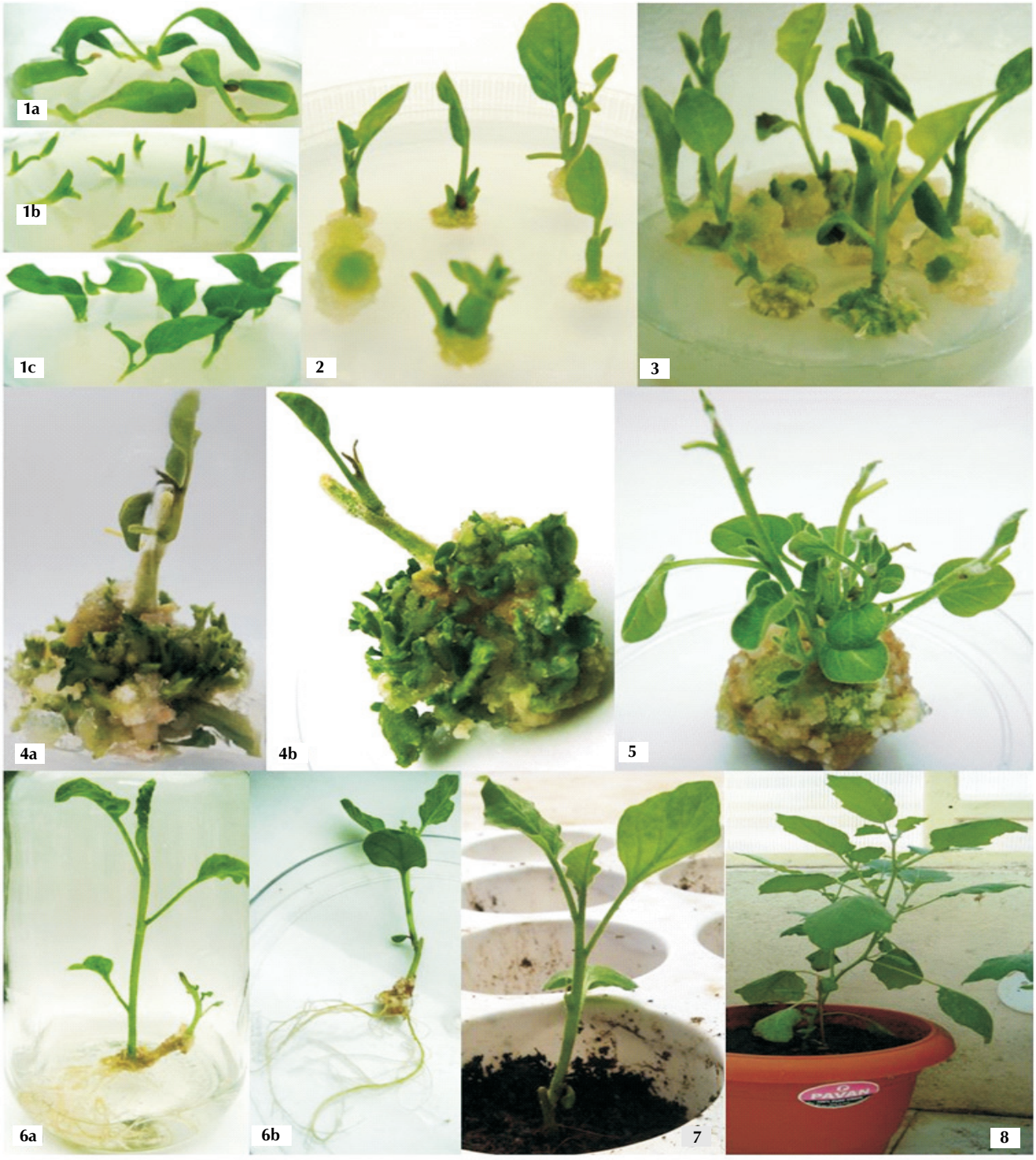
Among four different concentration of zeatin used 1 mg/l zeatin concentration found to be optimum exhibiting 93.33 % regeneration frequency with cotyledonary leaf pair explants followed by shoot tips (90 %) and nodal segments (90 %). Multiple shoots formation was higher in cotyledonary leaf pairs (4 shoots per explant) followed by shoot tip explants (3.66 shoots per explant). Nodal segment responded very poorly to multiple shoot formation (1-1.66 shoots per explant) and didn't formed more than two shoots. Medium supplemented with 1 mg/l zeatin exhibited better results than medium supplemented 1 mg/l BAP. Sarkar *et al.,* 2006 reported that among different concentrations of zeatin, 1.0 and 2.0 mg/l in MS were most effective.

Shoot regeneration using BAP and kinetin

The maximum regeneration efficiency was observed in cotyledonary leaf pair explants (100 %) followed by shoot tip (96.66 %) and nodal segment (93.33 %) when cultured on MS medium supplemented with 2 mg/l BAP and 1 mg/l kinetin. The maximum number of shoots per explant was obtained

Table 1: Effect of medium composition on *in vitro* shoot regeneration

Explant	Cotyledonary leaf pair			Shoot tip			Nodal segment		
Treatment	Days to callus initiation	Regeneration efficiency (%)	No. of shoots per explant	Days to callus initiation	Regeneration efficiency (%)	No. of shoots per explant	Days to callus initiation	Regeneration efficiency (%)	No. of shoots per explant
MS + 1 mg/l BAP	10.33	76.66	1.33	11.66	63.33	1.00	11.00	60.00	1.00
MS + 1.5 mg/l BAP	11.33	90.00	2.33	11.66	70.00	1.66	11.00	83.33	1.33
MS + 2 mg/l BAP	10.00	96.66	5.66	10.00	93.33	4.66	10.00	90.00	400
MS + 2.5 mg/l BAP	10.33	83.33	1.66	11.00	70.00	2.33	11.33	80.00	2.33
MS + 1 mg/l BAP+ 0.5 mg/l Kin	11.33	70.00	1.33	11.33	73.33	1.00	11.33	66.66	1.66
MS + 1.5 mg/l BAP+ 0.75 mg/l Kin	10.66	73.33	2.33	10.66	73.33	1.33	10.66	63.33	2.66
MS + 2 mg/l BAP + 1.0 mg/l Kin	9.33	100	6.66	9.33	96.66	5.33	9.66	93.33	4.33
MS + 2.5 mg/l BAP+ 1.25 mg/l Kin	10.33	70.00	3.00	11.33	80.00	2.33	11.33	73.33	2.33
MS + 0.25 mg/l zeatin	11.33	63.33	1.00	12.00	60.00	1.66	11.66	63.33	1.00
MS + 0.50 mg/l zeatin	11.33	76.66	1.66	12.00	63.33	2.33	11.33	63.33	1.33
MS + 0.75 mg/l zeatin	10.66	80.00	2.00	11.33	70.00	2.33	11.66	80.00	1.33
MS + 1 mg/l zeatin	10.33	93.33	4.00	10.33	90.00	3.66	10.00	90.00	1.66
Standard error	0.31	4.81	0.51	0.43	4.08	0.38	0.39	3.46	0.33
C.D.at 5 percent	0.93	14.04	1.51	1.25	11.91	1.12	1.15	10.12	0.97



Figs. 1-8: 1. a. Cotyledonary leaf pair; b. Nodal segment; c. Shoot tip explants inoculated on MS medium supplemented with 2 mg/l BAP and 1 mg/l kinetin. 2. Callus initiation. 3. Elongation of *in vitro* raised shoot. 4. Shoot bud initiation. 5. Shoot multiplication on MS medium supplemented with 2 mg/l BAP and 1 mg/l kinetin. 6. Rooting on MS medium supplemented with 0.5 mg/l IBA. 7. Primary hardening 8. Well developed brinjal plant in polycarbonated polyhouse

from cotyledonary leaf pair explant (6.6) as compared to shoot tip (5.33) and nodal explant (4.33) when cultured on MS medium supplemented with 2 mg/l BAP and 1 mg/l kinetin. It was observed that addition of kinetin resulted in increase in regeneration efficiency and number of shoots per explant. Sarkar *et al.,* 2006 had observed high frequency direct organogenesis of shoots from cotyledonary leaf in MS supplemented with 1.0 mg/l BAP and 1.0 mg/l Kinetin. Shivraj

and Rao (2011) obtained highest number of shoots on MS medium supplemented with 2 mg/l BAP and 0.5 mg/l kinetin using cotyledonary leaf explant. On the contrary to this Kaur *et al.,* 2011 had reported that addition of kinetin decreased the regeneration capability and number of buds on all the explants.

Root induction

IBA is widely used for efficient root induction in Brinjal (Zayova *et al.,* 2012, Shivraj and Rao 2011), although several authors have demonstrated efficient rooting on medium supplemented with IAA (Bardhan *et al.,* 2012, Magioli *et al.,* 1998), growth regulator free MS medium (Mir *et al.,* 2011, Kaur *et al.,* 2011, Ferdausi *et al.,* 2009, Sarkar *et al.,* 2006), on soilrite (Franklin *et al.,* 2004). In the present study regenerated shoots were successfully rooted when transferred to MS medium supplemented with 0.5 mg/l IBA (Fig 6). The differentiation of roots on the elongated shoots occurred over a period of 8-12 days. Healthy plantlets with well developed roots found to be very sensitive and prone to the fungal attack, when subjected to hardening. 0.1% blitox treatment completely controls fungal infection. Plantlets after primary and secondary hardening resulted in 100 % survival (Fig 7 and 8) and exhibited normal growth. In summary, we report an efficient and reproducible regeneration protocol for *in vitro* plant regeneration in brinjal. Cotyledonary leaf pair explant was found to more responsive for plant regeneration as compared to shoot tip and nodal segment. Combination of BAP and kinetin improved regeneration efficiency as well as number of shoots per explant. This optimized regeneration protocol can be efficiently used for *Agrobacterium* mediated genetic transformation in brinjal.

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