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RESEARCH ARTICLE

Studies on *in vitro* plant regeneration in brinjal (*Solanum melongena* L.)

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SUMMARY

In the present study the rapid regeneration protocol of brinjal was conducted. The shoot tip and hypocotyl explants from the *in vitro* grown sterile seedling were used for regeneration. The MS culture medium containing 2.5 mg l^{-1} BAP + 0.3 mg l^{-1} IAA induced highest callus growth in hypocotyl (1.43 cm) as well as in shoot tip (1.14 cm). The calluses obtained from both explants (shoot tip and hypocotyl) were sub-cultured on responding treatments. After sub-culturing, the highest number of shoots (3.59), shoot length (3.16 cm) and leaves (3.66) were recorded on the medium containing 2.5 mg l^{-1} BAP + 0.3 mg l^{-1} IAA. Root induction frequency was highest in full strength MS medium with 0.5 mg l^{-1} IAA (number of roots = 14.06 and root length = 6.99 cm). In hardening, highest survival percentage (100%) and healthy growth of plantlets were observed in mixture of vermiculite, farmyard manure and cocopeat in 1:1:1 ratio.

Key Words **:** BAP, Brinjal, Hardening, Hypocotyls, IAA, Rapid regeneration, Shoot tip

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Brinjal (*Solanum melongena* L.) is one of the most common, popular and principle vegetable crops grown in India and other parts of the world. The application of *in vitro* methodologies to brinjal improvement has resulted in considerable success. Its tissues present a high morphogenetic potential that is useful for developmental studies as well as for establishing biotechnological approaches to produce improved varieties with resistance to pests and diseases (Collonnier *et al.,* 2001*;* Magioli and Mansur, 2005). The existing reports on organogenesis show that nature and concentrations of a given growth regulator in association with specific genotype and explants can cause significant result in morphogenetic response of brinjal (Matsuoka and Hinata, 1979; Alicchi*o et al.,* 1982; Sharma and Rajam, 1995*;* Magioli *et al.,* 1998). The adventitious shoot regeneration capacity of cells or tissues to be used in transformation studies affects the success of genetic transformation significantly and frequency of explants responding after co-cultivation is severely affected (Chen *et al.,* 1995). Hence, it is advisable to know as well as document the effect of various concentrations of growth regulators on shoot morphogenesis in a crop variety before heading to *in vitro* applications for its genetic improvement. Furthermore, no effort has been made to document the effects of growth regulators on shoot regeneration and *in vitro* root induction in major Indian brinjal cultivars. 'Bhagyamathi' is a predominant, year round, locally well adapted and most preferred brinjal cultivar in India particularly in Andhra Pradesh. The present study was carried out to document effect of growth regulators on morphogenetic response, shoot elongation and *in vitro* root induction in cv. 'BHAGYAMATHI'.

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MATERIALS AND METHODS

The present investigation was carried out at the tissue

culture laboratory, College of Horticulture, Rajendranagar, Hyderabad. The breeder seed material of brinjal cv. 'BHAGYAMATHI' was obtained from the Division of Vegetable Crops, APHU, Rajendranagar, Hyderabad. Aseptic seedling source for explants were raised *in vitro*, on solid MS medium (Murashige and Skoog, 1962) without hormones after surface sterilizing the seeds with 0.1 per cent mercuric chloride for 10 minutes then rinsed 5 times with sterile double distilled water. From 25 days old seedlings, hypocotyl and shoot tips excised and were used as explants. Murashige and Skoog medium (MS) with 3 per cent sucrose and 0.8 per cent agar was used as a basal medium. Growth regulators were added and the pH of the medium was adjusted to 5.8 with NaOH or HCl (0.1 N) before autoclaving. Sterilization of culture medium and instruments was done by autoclaving at 121^{0}C at 15 psi pressure for 15 minutes. Cultures were incubated at light intensity of 30-40 μE^{-2} S^{-1} under 16 h photoperiod and temperature was maintained at 25± 1^{0}C. To study the effect of growth regulators on callus growth and shoot regeneration, hypocotyl and shoot tip explants were cultured on MS medium containing, 2.0 mg l^{-1} and 2.5 mg l^{-1} of BAP (benzylaminopurine) in combination with 0.1, 0.2 and 0.3 mgl^{-1} IAA (Indole-3-acetic acid). The callus obtained from shoot tip and hypocotyl explants after 28 days were removed and sub-cultured on responding treatments *i.e*. 2.5 mg l^{-1} BAP with 0.2 mg l^{-1} IAA and 2.5 mg l^{-1} BAP with 0.3 mg l^{-1} IAA. Experiment was conducted with three replications. After sub-culturing, the experiment was conducted with 10 replications. To study the effect of growth regulator concentrations on rooting, the elongated shoots were cultured on full-strength MS basal medium and half-strength MS basal medium containing 0.1, 0.3 and 0.5 mg l^{-1} IAA along with control was carried out with three replications. The effect of different potting mixtures during hardening of plantlets was also carried out by using vermiculite, farmyard manure, cocopeat, vermiculite + farmyard manure (1:1 ratio), cocopeat + farmyard manure (1:1 ratio) and vermiculite + farmyard manure + cocopeat (1:1:1 ratio). Initially the plantlets were hardened under lab conditions (the light intensity of 30-40 μE^{-2} S^{-1} under 16 h photoperiod and temperature was maintained at 25± 1^{0}C) for 15 days and then transplanted under shade house. Experiments involving hardening studies were conducted with four replications. Observations were recorded after 28 days of culture on callus initiation, regeneration response, *in vitro* rooting and hardening. The experiments were laid out in completely randomized design (CRD) with three replications.

RESULTS AND DISCUSSION

Hormonal balance is a key factor in the regeneration of morphogenesis in cultured explants. In present study, concentrations and ratios of growth regulators were designed based on literature. In tissue culture media, cytokinins are incorporated mainly for cell division and differentiation of adventitious shoots from callus and organs (Bhojwani and Razdan, 2002). Profuse callus were produced in shoot tip (1.14 cm) and hypocotyls (1.43 cm) explants and gave rise to green buds and shoots from the callus (Fig.1 A, B and C). Similar results were noticed when a range of cytokines (BAP, Kinetin, TDZ, Zeatin) either alone or in combination with auxin (IAA, IBA or NAA) were used in callus mediated regeneration response in brinjal (Dobariya and Kachhadiya, 2004; Bhansali and Ramawat, 1993; Sharmina *et al*., 2008; Khatun *et. al.,* 2006, Anwar *et al*., 2002; Solanki *et al*., 2006; Yu Bolan *et al*., 2003). MS culture medium containing 2.5 mg l^{-1} BAP and 0.3 mg l^{-1} IAA induced highest regeneration response in both the explants (Table 1 and 2). The sub-culturing of callus on MS medium containing 2.5 mg l^{-1} BAP in combination with 0.3 mg

Fig. 1 : *In vitro* plant regeneration in brinjal. (A), (B) and (C) Regeneration response of explants on MS culture medium containing 2.5 mg l^{-1} BAP and 0.3 mg l^{-1} IAA; (D) and (E) *In vitro* root induction on different treatments; (F) Initial hardening of rooted plantlets under lab condition; (G) Hardened plantlets in different potting mixture; (H) Hardened plantlets planted under the shade house; (I) Normal growth and fruiting of tissue cultured plants

Table 1 : Effect of MS medium with different treatments on regeneration of explants of brinjal cv. Bhagyamathi

Treatments	Growth regulator combinations	callus size (cm)	number of shoots	shoot length (cm)
T_1	Shoot Tip + Control	-	1.00	0.74
T_2	Shoot tip + 2.0 mg l^{-1} BAP + 0.1 mgl^{-1} IAA	0.46	1.00	0.89
T_3	Shoot tip + 2.0 mgl^{-1} BAP+ 0.2 mgl^{-1} IAA	0.55	1.00	0.90
T_4	Shoot tip + 2.0 mgl^{-1} BAP + 0.3 mgl^{-1} IAA	0.77	1.26	0.95
T_5	Shoot tip + 2.5 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	0.56	1.00	0.95
T_6	Shoot tip + 2.5 mgl^{-1} BAP + 0.2 mgl^{-1} IAA	1.02	2.07	1.61
T_7	Shoot tip + 2.5 mgl^{-1} BAP + 0.3 mgl^{-1} IAA	1.14	2.13	1.67
T_8	Hypocotyls + Control	-	-	-
T_9	Hypocotyls + 2.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	-	-	-
T_{10}	Hypocotyls + 2.0 mgl^{-1} BAP + 0.2 mgl^{-1} IAA	-	-	-
T_{11}	Hypocotyls + 2.0 mgl^{-1} BAP + 0.3 mgl^{-1} IAA	1.09	-	-
T_{12}	Hypocotyls + 2.5 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	0.58	-	-
T_{13}	Hypocotyls + 2.5 mgl^{-1} BAP + 0.2 mgl^{-1} IAA	1.38	1.84	0.55
T_{14}	Hypocotyls + 2.5 mgl^{-1} BAP + 0.3 mgl^{-1} IAA	1.43	1.88	0.60
C.D at 5%		0.013	0.037	0.011

Table 2 : Sub-culturing of callus from the both explants on MS medium with responding treatments

Growth regulator combinations	Number of shoots	Shoot length (cm)	Number of leaves
2.5 mgl^{-1} BAP + 0.2 mgl^{-1} IAA	3.46	3.10	3.21
2.5 mgl^{-1} BAP + 0.3 mgl^{-1} IAA	3.59	3.16	3.66
C.D at 5%	0.012	0.009	0.009

Table 3 : Effect of different treatments on *in vitro* rooting of brinjal cv. Bhagyamathi

Treatments	Growth regulator combinations	Number of roots	Roots length (cm)	Number of leaves
T_1	Full strength MS medium (control)	2.55	3.64	3.29
T_2	Full strength MS medium + 0.1 mgl^{-1} IAA	6.11	5.05	4.10
T_3	Full strength MS medium + 0.3 mgl^{-1} IAA	8.98	6.37	5.37
T_4	Full strength MS medium + 0.5 mgl^{-1} IAA	14.06	6.99	6.27
T_5	Half strength MS medium (control)	2.27	3.07	2.77
T_6	Half strength MS medium + 0.1 mgl^{-1} IAA	5.85	4.89	3.63
T_7	Half strength MS medium + 0.3 mgl^{-1} IAA	8.51	6.30	5.03
T_8	Half strength MS medium + 0.5 mgl^{-1} IAA	13.81	6.44	5.77
C.D at 5%		0.035	0.050	0.107

Table 4 : Effect of potting mixtures for survival and establishment of plantlets of brinjal during hardening

Treatments	Growth regulator combinations	Survival percentage	Shoot length (cm)	Number of leaves
T_1	Vermiculite	100.00	4.72	5.06
T_2	FYM	82.50	2.72	4.51
T_3	Cocopeat	90.00	4.71	5.30
T_4	Vermiculite + FYM (1:1 ratio)	100.00	5.10	6.20
T_5	Cocopeat + FYM (1:1 ratio)	97.50	4.92	5.43
T_6	Cocopeat + FYM + Vermiculite (1:1:1 ratio)	100.00	7.23	6.52
C.D at 5%		4.322	0.029	0.049

l^{-1} IAA produced more number of shoots (3.59) and length of shoots (3.16 cm). Solanki *et al.* (2006) reported that the culture of hypocotyls and cotyledons of brinjal on MS medium supplemented with BAP 2.5 mg l^{-1} with IAA 0.2 mg l^{-1} showed shoot formation. Dobariya and Kanchhadiya (2004) got shoot regeneration from callus derived from young leaf explants on MS medium supplemented with BAP 2.0 mg l^{-1} with 1.0 mg l^{-1} kinetin.

In vitro rooting responses of shoots were significantly influenced by various treatments (Fig. 1. D and E). In addition, IAA at 0.5 mg l^{-1} in full–strength MS culture medium induced more number of roots (14.06) and increased the root length (6.99 cm) compare to other treatments (Table 3). Similarly Sharmina *et al.* (2008) also reported that highest number (15.21) of roots per shoots developed when the shoots of brinjal cv. 'Katabegun' were cultured on 0.5 mg l^{-1} IBA containing MS medium and also Picoli *et al.* (2002) observed highest root number of brinjal shoots on MS medium supplemented with IAA 0.25 mg l^{-1} in combinations with 0.5 mg l^{-1} BAP.

The hardening technique used in the present study was found to be very efficient, which resulted cent per cent plantlet survivability and good growth (Fig. 1. F and G). The survivals of *in vitro* rooted plantlets were significantly affected with different potting mixtures. The plastic cups containing mixture of cocopeat, FYM and vermiculite (1:1:1 ratio) resulted cent per cent plantlets survival during hardening (Table 4) and produced highest shoot length (7.23 cm) and more number of leaves (6.52). Vermiculite has good aeration and water holding properties and also high cation exchange capacity which makes it particularly useful for propagation mixes (Adams *et al.*, 1998). Further those plants exhibited normal growth, flowering and fruiting when they were transferred to field condition (Fig. 1. H and I). Raghuveer *et al.* (1994) reported that all the healthy rooted plantlets of brinjal survived when being transferred to plastic pots containing sterile vermiculite. Kumar and Mehta (1996) also observed that the survival of tissue culture derived plantlets of eggplant was affected by the type of the potting mixture used. Highest percentage of survival was recorded in pots filled with vermiculite with farmyard manure.

The protocol reported here, took less time, *i.e.* 3-4 months from initiation to establishment with very high percentage of establishment of plantlets in shade house.

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