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In vitro Plant Regeneration Studies in Brinjal (*Solanum melongena* L)

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ABSTRACT

The present investigation was undertaken to standardize the protocol for high frequency *invitro* regeneration of brinjal variety Navkiran which is the essential pre-requisite step of application of biotechnological interventions for crop improvement. Multiple shoot regeneration via callus induction was achieved using epicotyl and hypocotyl as explant at various concentrations and combinations NAA (Naphthalene acetic acid) (0.5mg/L and 1mg/L) and BAP (6-Benzyl Amino Purine) (1.0mg/L, 1.5mg/L and 2.0mg/L) in MS media. Earliest callus induction (6.33±0.33d), was observed when epicotyls was inoculated in MS media fortified with 0.5mg/L NAA and 2.0mg/L BAP. However, maximum callus weight (1.35±0.10g) was obtained in MS media fortified with 0.5mg/L NAA and 2.0mg/L BAP when hypocotyls was used as explant. Ninety percent callus induction was obtained in MS media supplemented with 0.5mg/L NAA and 2.0mg/L BAP in both the explants. Regarding the quality characters of callus, both explants showed variable response in different treatments used under the study. Colour of the callus was dark green, light green and cream, texture of callus was loose, friable and compact and abundance was plenty, moderate and poor. Among the explants used, epicotyl gave the best response to shoot regeneration, it was 52.77±2.61 and took minimum days of shoot regeneration (29.33±0.88), maximum shoot length (3.96 ± 0.06) in MS media fortified with 0.5mg/L NAA and 2.0mg/L BAP. In vitro rooting (68.82±2.70) was obtained within 14.33±1.20 days with 19.10±0.86 number of roots in MS media supplemented with 1.0 mg/L IBA. The rooted plantlets were successfully hardened in a mixture of cocopeat, vermiculite and perlite in ratio of 1:1:1 for 15 days and then transplanted in the main field with survival rate of 95 per cent.

Key Words: BAP, Callogenesis, Eggplant, Hardening, IBA, *Invitro*, NAA, Root regeneration, Shoot regeneration.

INTRODUCTION

Eggplant (*Solanum melongena* L., 2n=2x=24) is also known as brinjal belongs to family Solanaceae. It is widely adaptive and highly productive vegetable crop of tropical and subtropical regions of the world. It is an important non tuberous solanaceous crop grown primarily for its large oval fruit. It is normally warm season crop and can be grown three times in a year *i.e.,* spring summer, rainy season and autumn winter. Its production is suffered due to its infestation by different pathogens such as viruses, fungi, bacteria, mycoplasma, nematode and insect-pest which cause huge yield losses. Controlling the pests and diseases by the use of pesticides and insecticides is costly and simultaneously causes pollution. Development of resistant varieties is one of the most effective methods for the control of losses caused by diseases and pests. Conventional breeding approaches for developing insect resistance for brinjal have not been very successful due to sexual incompatibilities and difficulties in obtaining fertile progenies (Pratap *et al,* 2011). Thus, use of biotechnological techniques can be an alternative approach to tackle such issues. The regeneration of plants from cell and tissue culture is a pre-requisite for the exploitation of various

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biotechnological applications. It can serve as a platform for the transfer of economically important traits through genetic engineering, inducing somaclonal variations, *in vitro* mutations, double-haploid induction, development and utilization of somatic hybrids, determining herbicide or pesticide tolerance limits in eggplant. The application of *in vitro* methodologies to brinjal improvement has resulted in considerable success. Its tissues have 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). Therefore, a remarkable progress can be achieved in eggplant through the combination of conventional and biotechnological approaches. The main factor affecting explant's regeneration capacity is the choice of segment used in tissue culture as explant. Different explants had differential response to regeneration (Kanna and Jayabalan, 2010; Kaur *et al*, 2013) on different media combinations containing cytokinins and auxins. Nature and concentrations of a given growth regulator in association with specific genotype and explants can cause significant result in morphogenetic response of brinjal (Magioli *et al*, 1998). Hence, keeping in view the importance of the crop and need to standardize the explants and concentrations of growth regulators for morphogenesis in the desired cultivar the present investigation was executed.

MATERIALS AND METHODS

The F1 hybrid seeds of brinjal (*Solanummelongena*L. cv. Navkiran) were procured from local seed market. The seeds were washed gently with Tween-20 under running tap water for 20 min. The surface sterilization of seeds was carried out under a laminar air flow chamber. The seeds were treated with 0.1 percent $HgCl_2$ (mercuric chloride) for 30 second afterwards seeds were washed with sterilized distilled water for 3 times to remove the forces of $HgCl_2$. These surface sterilized seeds were then ready for inoculation on the MS medium without any growth hormone. All the aseptic manipulations were carried out under vertical laminar flow chamber. The inoculations were carried out under laminar air flow. After inoculation, the cultures were kept in culture room at 26±2°C with 16/8 hr photo-period for normal seedling growth. Aseptically grown 14-15 d old seedlings of brinjal were used as a source of explants *viz.,* epicotyl and hypocotyl for plant regeneration studies. The seedlings were cut into two parts and sub cultured in the test tubes on MS medium supplemented with two different concentrations and combinations of Naphthalene acetic acid (NAA) (0.5mg/l and 1mg/l) and three different concentrations of 6-Benzyl Amino Purine (BAP) (1mg/l, 1.5mg/l & 2mg/l) to study the various callogenesis parameters and shoot regeneration. The root regeneration studies were carried out on MS medium containing 1 mg/l IBA and MS medium without hormone to get complete plantlet by the formation of roots. The hardening of regenerated plantlets was done on mixture of cocopeat: vermiculite: perlite (1:1:1). The effect of different concentrations and combinations on callus induction, plant regeneration and root regeneration data were recorded for days to seed germination, days to callus initiation, number of callus initiated, percent callus induced, weight of callus, colour of callus, nature of callus, abundance of callus, number of days taken to shoot regeneration, shoot regeneration percentage, number of shoots regenerated per callus, shoot length of regenerated shoots, number of days taken for root regeneration, number of roots regenerated, root regeneration percentage, root length of regenerated roots. The experiment was laid in complete randomized design (CRD) with three replications and data were analyzed by using OP STAT statistical software package for agricultural research workers.

RESULTS AND DISCUSSION

Effect of different treatments on callogenesis

Invitro regeneration was achieved in MS media supplemented with two different concentrations and combinations of NAA (0.5mg/l and 1mg/l) and three

different concentrations of BAP (1mg/l, 1.5mg/l & 2mg/l) (Table 1). The number of callus/explant and percentage of callus initiation was studied to find the best combination and concentration of hormones in MS media for the hypocotyl and epicotyl. It was observed that MS media supplemented with 0.5mg/l NAA+2.0mg/l BAP resulted in highest callus initiation percentage (90.00±0.00) from epicotyl and hypocotyl. These findings were in line with the findings of Ray *et al* (2011) and Naseer and Mahmood (2014) who also observed higher callus induction/explant in brinjal when higher concentration of BAP was supplemented in the MS media. Mir *et al* (2011) also reported 100 per cent callusing in both cotyledon and hypocotyl explants on medium supplemented with auxin and cytokinin.

Weight of the callus was maximum (1.04±0.13g) in the MS media supplemented with 0.5mg/l NAA+2.0mg/l BAP when epicotyl was used as explant and (1.35±0.10) when hypocotyl was used as explant in the same media. The findings of the present investigation were in accordance with the findings of Ray *et al* (2011) who also observed highest callus weight when MS media was supplemented with 0.5 mg/l of NAA + 2.0 mg/l BAP.

Size, colour, nature of the callus affects the regeneration potential of the callus (Taha and Tijan, 2002). Green calli have greater organogenic potential Zayova *et al* (2010). Dark green, loose and plenty of callus was observed in most responsive concentration of NAA and BAP *i.e.,* MS media supplemented with 0.5mg/l NAA+2.0mg/l BAP in both the explant of brinjal. However, variable response of different explants in different concentrations and combinations of hormone in brinjal with respect to the colour, size, and nature of the callus has been observed, which is in agreement with Alim *et al* (2014).

Effect of different treatments on shoot regeneration

The type and concentration of given growth hormone in association with specific genotype and explant used can cause significant difference in shoot regeneration response of brinjal (Kanna and Jayabalan, 2010; Shivraj and Srinath, 2011; Kaur *et al*, 2013; Muktadir *et al*, 2016). Rapid and efficient shoot regeneration from callus is essential for complete plantlet development. In the present investigation epicotyl explant was more responsive than hypocotyl for shoot regeneration from the callus (Table 2). These findings were in line with the findings of earlier researcher (Zhang, 1999) who reported different morphological potential of hypocotyl and cotyledon explant for organogenesis and (Sarker *et al*, 2006) also reported that cotyledon explants are more suitable explant as compared to hypocotyl.

Most rapid shoot regeneration (29.33±0.88) was observed when MS media was supplemented with 0.5mg/l NAA+2mg/l BAP. These findings are in agreement with the findings of Ray *et al* (2011) who also observed rapid shoot regeneration from callus obtained from MS media supplemented with 0.5mg/l NAA+2mg/l BAP and Pawar *et al* (2013) reported that cotyledon explant showed less days to shoot initiation as compare to the hypocotyl explant. Highest number of shoots/callus (4.66±0.33) and highest shoot regeneration percentage (52.77±2.61) was observed from epicotyl explant in MS media supplemented with 0.5mg/l NAA+2mg/l BAP. These findings were in agreement (Pawar *et al*, 2012; Sagare and Mohanty, 2012; Zayova *et al*, 2012 and Naseer and Mahmood, 2014) who also reported highest frequency of shoot regeneration and maximum number of shoot/explant when MS media was supplemented with 2.0mg/l BAP. Longest shoot length (3.96±0.06) were observed in MS media supplemented with 0.5mg/l NAA+2mg/l BAP. These findings were in line with Naseer and Mahmood (2014) who also reported longest shoots when MS media was supplemented with 2.0mg/l BAP.

Effect of different treatments on root regenration

Rooting is the vital part for getting complete

Table 1. Effect of different combinations and concentrations of NAA and BAP in MS media on *invitro* regeneration of callus from epicotyl and hypocotyl explants of brinjal*(Solanum melongena)* variety Navkiran.

Treatments MS media + Hormone (mg/l))	Days to callus initiation in epicotyl	No. of callus initiated in epicoty	Percent callus initiated in epicotyl	Weight of callus in epicotyls (g)	Days to callus initiation in hypocotyl	No. of Callus initiated in hypocotyl	Percentage (%) of callus initiated in hypocotyl	Weight of Callus in hypocotyl
0.5 NAA + 1 BAP	8.66±0.66	8.83±0.16	70.08±1.45	0.96±0.10	10.66±0.33	8.66±0.72	72.38±9.04	1.02±0.06
0.5 NAA +1.5 BAP	8.33±0.33	9.83±0.16	85.68±4.31	0.99±0.12	9.83±0.16	9.33±0.33	77.69±6.15	0.99±0.02
0.5 NAA + 2 BAP	6.33±0.33	10.00±0.00	90.00±0.00	1.04±0.13	8.33±0.33	10.00±00	90.00±0.00	1.35±0.10
1 NAA + 1 BAP	8.16±0.16	10.00±0.00	90.00±0.00	0.97±0.25	9.33±0.33	9.66±0.33	83.84±6.15	1.26±0.37
1 NAA + 1.5 BAP	9.33±1.45	8.66±0.33	68.82±2.70	0.83±0.01	10.33±0.88	8.16±0.16	64.68±1.25	1.08±0.08
1 NAA + 2 BAP	10.33±0.33	7.667±0.66	61.69±4.92	0.80±0.03	12.66±0.33	6.33±0.33	52.75±2.00	0.93±0.03
CD (5%)	2.17	0.99	9.20	N.S.	1.42	1.19	16.25	N.S.

NAA (Naphthalene acetic acid) and BAP (6-Benzyl Amino Purine)

Table 2. Effect of different combinations and concentrations of NAA and BAP in MS media on colour, nature and abundance of callus from epicotyl and hypocotyl of brinjal*(Solanum melongena)* variety Navkiran.

Treatments MS media + Hormone (mg/l))	Colour of Callus in epicotyl	Nature of Callus in epicotyl	Abundance of callus in epicotyl	Colour of Callus in hypocotyl	Nature of Callus in hypocotyl	Abundance of callus in hypocotyl
0.5 NAA + 1 BAP	Light green	Friable	Moderate	Light green	Friable	Plenty
0.5 NAA +1.5 BAP	Light green	Loose	Plenty	Light green	Friable	Plenty
0.5 NAA + 2 BAP	Dark green	Loose	Plenty	Dark green	Loose	Plenty
1 NAA + 1 BAP	Light green	Loose	Plenty	Light green	Loose	Plenty
1 NAA + 1.5 BAP	Cream	Friable	Moderate	Cream	Friable	Moderate
1 NAA + 2 BAP	Cream	Compact	Poor	Cream	Compact	Poor

NAA (Naphthalene acetic acid) and BAP (6-Benzyl Amino Purine)

Table-3 Effect of different concentration and combinations of NAA and BAP in MS media on shoot regeneration of brinjal*(Solanummelongena)* variety Navkiran.

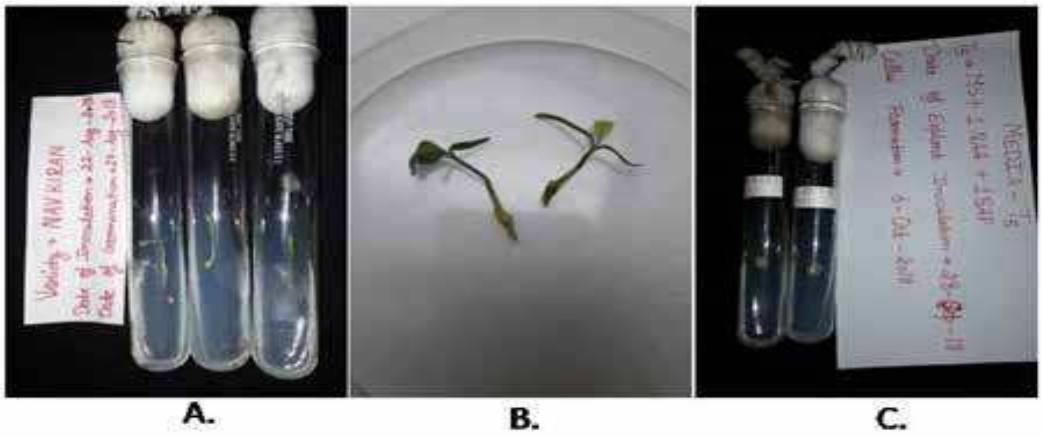
Treatments	No. of days for shoot regeneration from epicotyl callus	No. of days for shoot regeneration hypocotyl callus	Number of shoots/ epicotyl callus	Number of shoots/ hypocotyl callus	Percent shoot regeneration in epicotyls callus	Percent shoot regeneration in hypocotyl callus	Shoot length in epicotyls callus	Shoot length in hypocotyl callus
0.5 NAA + 1 BAP	No response	No response	No response	No response	No response	No response	No response	No response
0.5 NAA +1.5 BAP	42.33±0.33	No response	2.33±0.33	No response	36.22±1.73	No response	1.93±0.21	No response
0.5 NAA + 2 BAP	29.33±0.88	No response	4.66±0.33	No response	52.77±2.61	No response	3.96±0.06	No response
1 NAA + 1 BAP	33.33±0.66	No response	3.83±0.16	No response	44.02±3.46	No response	2.73±0.37	No response
1 NAA + 1.5 BAP	35.33±0.88	No response	2.83±0.16	No response	38.22±0.98	No response	2.33±0.17	No response
1 NAA + 2 BAP	No response	35.33±0.88	No response	2.56±0.21	No response	33.14±1.81	No response	2.10±0.10
CD (5%)	1.84	1.12	0.67	0.27	6.07	2.30	0.59	0.12

NAA (Naphthalene acetic acid) and BAP (6-Benzyl Amino Purine)

Table 4. Effect of IBA and No hormone in MS media on *in vitro* root regenration of brinjal*(Solanum melongena)* variety Navkiran.

Treatments MS media + Hormone (mg/l))	No. of days required for root regeneration	No. of roots regenerated	Percentage root regeneration	Root length at the time of hardening
MS media + 1mg/l IBA	14.33±1.20	19.10±0.86	68.82±2.70	11.13±0.95
MS media without hormone	27.33±0.88	12.77±1.39	54.76±2.00	7.56±0.61
CD 5%	4.25	4.67	9.61	3.22

NAA (Naphthalene acetic acid) and BAP (6-Benzyl Amino Purine)



A. B. C.

D. E. F.

G. H. I.

A. Seedlings germinated B. Seedlings at the time of inoculation C Callus initiation D. Shoot regeneration E. Root regeneration F. Plantlet G.Washing of plantlet H. Hardening of regenerated plantlet. I. Potting of regenerated plants after 15 days of hardening

plantlets from in vitro shoots. Healthy root development helps in better establishment of plant in soil. Therefore, adequate root generation is necessary (Anisuzzaman *et al*, 2008). For the initiation of roots the shoots were excised and transferred to MS medium supplemented with 1mg/l IBA and MS medium without hormone (Table 3). Rapid root regeneration was observed in (14.33±1.20) days, maximum number of roots regenerated (19.10±0.86), highest root regeneration percentage (68.82±2.70) and longest roots (11.13±0.95) was observed in MS medium supplemented with 1mg/l IBA. These findings are in line with (Mallaya *et al*, 2013 and Rattan *et al*, 2015) who also reported 1 mg/l IBA was best for root induction in eggplant. (Hossain *et al*, 2007; Chakravarthi *et al*, 2009; Shivraj and Rao, 2010; Zayova *et al*, 2012; Panwar *et al*, 2013) they also observed higher root regeneration with addition of IBA.

Hardening of plants

After proper *invitro* development, the plantlets were taken out of the test tubes without damaging the delicate root system. The roots were washed gently under running tap water to remove adhering agar completely. The plantlets were treated with 0.5 % bavistin to avoid the fungal contamination and then transferred to potting mixture. For hardening of regenerated plantlets mixture of cocopeat: vermiculite: perlite (1:1:1) was sterilized in an autoclave at 15 lbs/inch² for 15-20 min at 121.5°C. After sterilization the plastic cups were filled with mixture of (1:1:1) cocopeat: vermiculite: perlite and plantlets with washed roots were transferred to the pots. After 15 days of hardening the regenerated plants were shifted to soil for further development and growth.The flowering in tissue culture raised and acclimatized plants was observed after 55-60d. A protocol for plant regeneration from epicotyl and hypocotyl explants has been standardized inbrinjal (*Solanum melongena* L.).

CONCLUSION

The present research work was undertaken with an objective to standardize a protocol for efficient plant regeneration in brinjal (*Solanum melongena* L. cv. Navkiran) and to find out the most effective explant for efficient plant regeneration in brinjal.

It was concluded that the epicotyl explant was more responsive than hypocotyl explant. The earliest callusing highest number of callus induced, highest callus initiation percentage, heaviest callus and dark green, loose and plenty of callus was observed when MS media was supplemented with 0.5mg/l NAA+2mg/l BAP from epicotyl explants. Earliest shooting ,highest number of shoots per explant, best shoot regeneration and longest shoot length from callus obtained from epicotyl explant were observed when MS media was fortified with 0.5mg/l NAA+2mg/l BAP. The earliest rooting, highest number of regenerated roots, highest root regeneration percentage and longest root length were observed when MS media was supplemented with 1mg/l IBA.

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