The Bioscan 10(4): 1469-1473, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

IN VITRO PLANT REGENERATION STUDIES USING HYPOCOTYL EXPLANT OF BRINJAL (*SOLANUM MELONGENA*)

PUJA RATTAN*[1], SANJEEV KUMAR[2] AND R. K. SALGOTRA[3]
[1]Department of Agriculture, DAV University, Jalandhar - 144 001, Punjab, INDIA
[2]Division of Vegetable Science and Floriculture SKUAST-J, Chatha, Jammu - 180 009, J&K. INDIA
[3]School of Biotechnology, SKUAST-J, Chatha, Jammu - 180 009, J&K. INDIA
e-mail: rattanpooja16@gmail.Com

KEYWORDS
Embryo Rescue
Hypocotyle
Brinjal
Solanum Melongena
Shoot Regeneration
Root Regeneration

Received on :
07.06.2015

Accepted on :
24.10.2015

***Corresponding author**

ABSTRACT
The present study was done to standardize the protocol of embryo rescue technique in which the seeds of indigenous collection of *Solanum melongena* were used as explants. The explants hypocotyle was cultured on MS media supplemented with different combinations of auxins and cytokinins such as BAP (1 to 2.5 mg/l) + IAA (0.2 to 0.4 mg/l) and Kinnetin (1 to 2.5 mg/l) + IAA (0.2 to 0.4 mg/l) for shoot regeneration. Callus initiation and shoot initiation was observed after 15 - 20 days and 25days of inoculation, respectively on media supplemented with BAP-IAA. While, it took 25 and 30-35 days for callus initiation and shoot initiation, respectively on media supplemented with Kinnetin-IAA. The highest shoot regeneration (65.12%) was observed on MS medium supplemented with 2mg/l BAP + 0.3mg/l IAA after 25 days of inoculation. For root regeneration, regenerated shoots were transferred to root regeneration medium having MS medium supplemented with different concentration of auxins IAA (0.5-1mg/l) and IBA (0.5-1mg/l), so as to obtain the complete plantlets. The highest root regeneration was observed when MS medium was supplemented with IBA (1mg/lt). Hence the plant regeneration from hypocotyle of brinjal was observed to be influenced by different combinations and concentrations of auxins and cytokinins.

INTRODUCTION

Eggplant (*Solanum melongena* L., 2n = 2x = 24) is a widely adaptive and highly productive vegetable crop of tropical and subtropical regions world. The crop finds place in kitchen gardens besides being grown for commercial scale production. It is considered to be important source of nutrition and cash income. It suffers from various abiotic and biotic stresses particularly insect-pests (Sharma and Rajam, 1995; Kaur *et al*., 2004). These exert a deleterious effect on yield, market quality and storability of brinjal. In brinjal the cross incompatibility with wild resistant relatives (*Solanum mammosum*, *Solanum incanum* and *Solanum grandiflorum*) and inadvertent linkage drag of undesirable genes are problems in developing resistance in brinjal through conventional breeding methods (Baksh and Iqbal, 1979). Thus, use of biotechnological techniques can be an alternative approach to tackle such issues. However, transfer of genes depends upon the efficient and rapid regeneration protocol.

Several reports on *in vitro* regeneration in brinjal are available and in majority are of indirect plantlets regeneration through a callus phase (Sharma and Rajam, 1995; Franklin, *et al*., 2004; Singh *et al*., 2000; Mir *et al*., 2011; Bhatt *et al*., 2013; Sidhu *et al*., 2014). Various protocols on *in vitro* regeneration in egg plant have been carried out using various auxins and cytokinins either alone (Mukherjee *et al*., 1991; Magioli *et al*.,1998) or in combinations (Matsuoka and Hinata 1979) using various explants. *In vitro* plant regeneration studies using hypocotyl as explant was also carried out by various researchers (Bardhan *et al*., 2012 and Panwar *et al*., 2013). It is generally accepted that plant *in vitro* culture response is influenced by the donor genotype (Henry *et al*., 1994) and type of explants used (Sharma and Rajam, 1995). Although the present study was a part of standardization of embryo rescue technique, the aim was to get higher number of regenerated plants. Therefore, the present study was undertaken to standardize the rapid and efficient protocol for high frequency shoot regeneration of brinjal using hypocotyl as explants.

MATERIALS AND METHODS

Plant material

The present study was a part of standardization of protocol for embryo rescue technique, the plant material used were the seeds of indigenous collection of *Solanum melongena* L. IC 203585 obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. This genotype was quite responsive in wild hybridization with *Solanum khasianum*. Seeds were thoroughly washed in running tap water and then surface sterilized with 0.1 % mercuric chloride ($HgCl_2$), followed by two to three times rinsing in sterile distilled water and in sterile distilled water overnight. These seeds were inoculated into half-strength MS medium (Murashige and Skoog1962) devoid of any growth regulators. Hypocotyl explant of brinjal was obtained from the 15 days old *in vitro*

grown seedlings. The explant were sterilized with 0.1% $HgCl_2$ for 60 seconds under laminar flow, followed by their 3-4 times washing with distilled water to remove traces of mercury chloride. The explant was cut into 1.0 cm size and then cultured on MS medium containing different concentrations of auxins and cytokinines. Basal MS medium containing all the macro and micro nutrients, vitamins, 100mg/lt mesoinositol with 3% sucrose and 0.8% agar was used as cultural medium (Murashige and Skoog, 1962). After inoculation cultures were kept in the culture room at 26 ± 2°C with 16h photoperiod.

Culture media and shoot regeneration

For shoot regeneration, the explant was kept on MS medium supplemented with different combinations of auxins and cytokinins such as, MS + 1mg/L BAP + 0.2mg/L IAA, MS + 2mg/L BAP + 0.3mg/L IAA, MS + 2.5mg/L BAP + 0.4mg/L IAA, MS + 1mg/L kinnetin + 0.2mg/L IAA, MS + 2mg/L kinnetin + 0.3mg/L IAA, and MS + 2.5mg/l kinnetin + 0.4mg/L IAA. Explants were evaluated for number of shoots per explant and percent shoot regeneration. After culturing the cultures were kept at 26 ± 2°C for 30 days and then process of sub culturing was followed on fresh medium for further shoot multiplication.

Root regeneration

The regenerated shoots developed from hypocotyl explants were transferred to root regeneration medium having MS medium with different concentration of auxins so as to obtain the complete plantlets. Different combinations used for root regeneration were basal MS medium + 0.05mg/L IAA, MS medium + 1.0mg/L IAA, MS medium + 0.05mg /L IBA and MS medium + 1.0mg/L IBA.

Hardening of the rooted shoots

In vitro developed plantlets were taken out and washed gently with distilled water to remove adhering agar medium in the laminar flow. The plants were then transferred in the tissue culture pots containing mixture of cocopeat: vermiculite: perlite (1:1:1) which was presterlized by autoclaving at 15lbs/inch^2 pressure for 1hour at 121°C. The plantlets were then watered with ½ MS medium biweekly kept under varying conditions of humidity and light intensity and observed for growth.

Statistical Analysis

The statistical analysis was performed using sigma stat for windows. The values of number of shoot /explant were represented in terms of means for 5 random replicates

RESULTS

Shoot regeneration

MS medium with 2 mg/L BAP + 0.3 mg/L IAA was found to be optimum in obtaining high frequency of shoot regeneration in brinjal. After regeneration the hypocotyl explant (1.0 cm) were inoculated on various shoot regeneration medium supplemented with different combinations and concentrations of BAP-IAA and Kinetin-IAA. (Figure 1)

Effect of BAP-IAA on shoot regeneration

Three different concentrations (MS + 1mg/l BAP + 0.2mg/L IAA, MS + 2mg/L BAP + 0.3mg/L IAA, MS + 2.5mg/L BAP + 0.4mg/L IAA) of BAP-IAA were used for shoot regeneration. Callus initiation was observed after 10-12 days. Shoot initiation started after 25days. As depicted in the (Figure 2&3) maximum shoot regeneration (65.12%) and average number of shoots per explant (0.5) was observed on MS medium supplemented with 2mg/L BAP + 0.3mg/L IAA.

Effect of kinetin and IAA

Three different concentrations i.e. (MS + 1mg/l kinetin + 0.2mg/l IAA, MS + 2mg/l kinetin + 0.3mg/l IAA, and MS + 2.5mg/l kinetin + 0.4mg/l of kinetin) and IAA were used for shoot regeneration. The callus initiation was observed after 20 days and shoot initiation started after 30-35 days. Out of these three combinations maximum shoot regeneration (58.52%) and average number of shoots per explant (0.2) were observed on MS + 2mg/l kinetin + 0.3mg/l IAA. (Table 2 & Fig. 3)

Multiplication and elongation

The regenerated shoots from both the explants were separated and sub-cultured on same medium containing best shoot regeneration medium + growth regulators (2mg/l BAP + 0.3mg/l IAA). Elongation of shoots was obtained after 45-50 days after sub culturing.

Root regeneration

The regenerated shoots were sub-cultured in rooting media which contained different concentrations of auxins such as MS medium + 0.05mg/l IAA, MS medium + 1.0mg/l IAA, MS medium + 0.05mg /l IBA, and MS medium + 1.0mg/l IBA. Root initiation started after 15 days after inoculation and well developed roots were obtained in 4 weeks. As revealed in the

Table1: Effect of different concentrations of BAP-IAA on shoot regeneration

Medium	Callus Formation	*Mean no. of shoot /explants	% shoot regeneration
MS + 1 mg/l BAP + 0.2 mg/l IAA	+	0.48 ± 0.101	32.08 ± 0.160
MS + 2 mg/l BAP + 0.3 mg/l IAA	+ + +	0.52 ± 0.101	65.12 ± 0.121
MS + 2.5 mg/l BAP + 0.4 mg/l IAA	+ + +	0.81 ± 0.100	48.16 ± 0.106

*values were calculated from 5 replicates (n = 5)

Table 2: Effect of different concentrations of Kinetin and IAA on shoot regeneration

Medium	Callus Formation	*Mean no. of shoot /explants	% shoot regeneration
MS + 1 mg/l Kinnetin + 0.2 mg/l IAA	+	0.52 ± 0.128	30.06 ± 0.104
MS + 2 mg/l Kinnetin + 0.3 mg/l IAA	+ +	0.20 ± 0.100	58.52 ± 0.143
MS + 2.5 mg/l Kinnetin + 0.4 mg/l IAA	+ +	0.15 ± 0.106	42.06 ± 0.138

*values were calculated from 5 replicates (n = 5)

Table 3: Effect of different concentrations of auxins on root regeneration

Medium	Mean no. of roots	% root regeneration
MS + 0.05mg/l IAA	4.6	62.08
MS + 1mg/l IAA	10.06	72.16
MS + 0.05mg/l IBA	9.81	75.08
MS + 1mg/l IBA	11.08	91.06
CD	1.06	1.13

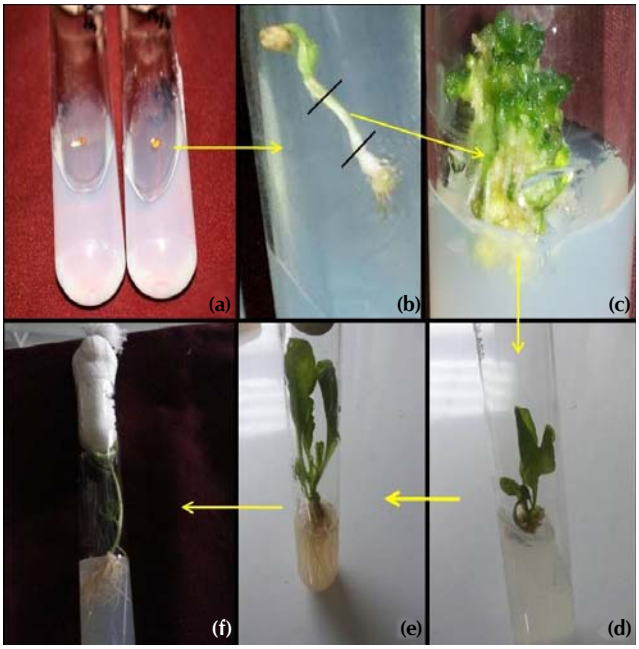

Figure 1:(a-f): Regeneration studes in brinjal from hypocoty a) Emeregence of embryo from the seed, b) Fully eveloped seedling, c) multiple shoot regeneration from hypocoty explantyl explant on shoot regeneration medium, d) Fully developed shoot explant on shoot regeneration medium, e) Emergence of roots on the root regeneration medium and f) Fully developed plant ready for the hardening

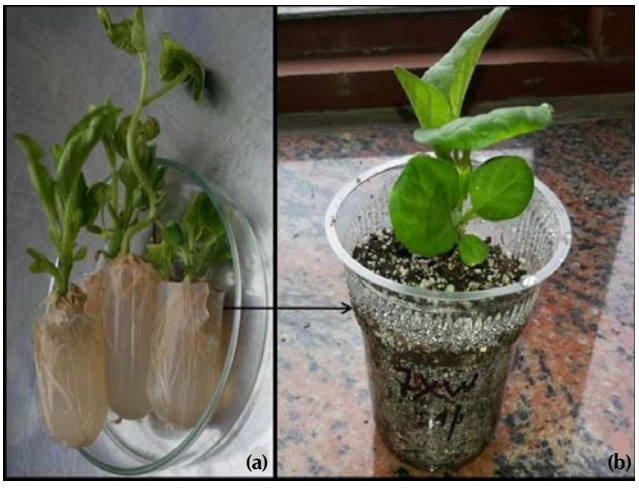

Figure 2(a-b) Hardening of *in vitro* devloped plantes of brinjal. a) regenerated plantlets of brinjal showing healthy root system and b) *in vitro* regenerated plantlets kept for hardening in presterelized mixture of perlite: cocopeat: vermiculite (1:1:1) in pots at 5 day

(Table & Fig. 3) maximum percentage of root regeneration (91.06%) was observed \on MS media containing IBA 1mg/L. After root regeneration the completely formed plantlets were transferred to pre sterilized mixture of cocopeat: vermiculite: perlite (1:1:1) in pots. (PLATE-2).

DISCUSSION

Plant regeneration is a crucial aspect of plant tissue culture methodology that is boon for the production of genetically engineered plants, somaclonal variants and the rapid propagation of difficult to propagate species. Effective *in vitro* regeneration is required for genetic improvement of brinjal through tissue culture and genetic engineering approaches. The aim of present investigation was to determine high frequency shoot regeneration in the brinjal. Callus mediated regeneration with the use of different concentration of BAP (cytokinnins) and auxins (IAA or IBA) have been reported in hypocotyls in brinjal by (Sagare and Mohanty 2012). However some of the earlier workers obtained direct or indirect organogenesis from hypocotyl explants by manipulating the concentrations of growth regulators (Alicchio, 1982; Magioli and Mansur 2005). The present investigation was a part of study which aimed at the regeneration of brinjal plantlets from immature ovules. In these hypocotyls of brinjal seedlings were used as explants for the regeneration, it was observed that regeneration was mediated through callus formation. In several other experiments a range of kinetins and TDZ either alone or in combination with NAA or IAA had been used which resulted in callus mediated response in brinjal as reported by (Alicchio *et al*., 1982; Magioli *et al*., 1998; Khatun *et al*., 2006; Bardhan *et al*., 2012). Combination and balance of auxins and cytokinnins is the main criteria in growing medium which determine the morphogenesis of callus. Furthermore, intrinsic plant growth regulator levels in explants make it respond better on a particular ratio and concentration of plant growth regulators supplemented in culture medium depends upon genotype and crop (Franklin *et al*., 2004). Hence there is wide response of different combinations of plant growth regulators on MS medium supplemented with different auxins and cytokinines.

Gradual increase in the regeneration frequency and number of shoots per explant was observed with the increasing concentration of BAP and highest shoot regeneration (65.12%) was observed on MS medium supplemented with 2mg/L BAP+ 0.3mg/L IAA after 25 days of inoculation, however it was observed that with increase in concentration of BAP, there was 26 % decrease in shoot regeneration in the present study. Zayova *et al*. (2000) and Bhat *et al*. (2013) had also observed higher regeneration 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) had also observed that increase in BAP concentration above 2.5mg/L resulted in decreased regeneration capacity and number of buds on all the explants. Pawar *et al*. (2012) and Sagare and Manty (2012) also observed the shoot regeneration mediated through callus on medium containing BAP@ 1.0 -2.0 mg/L with highest shoot regeneration at 2.0 mg/L BAP.

When the hypocotyl was cultured on MS medium

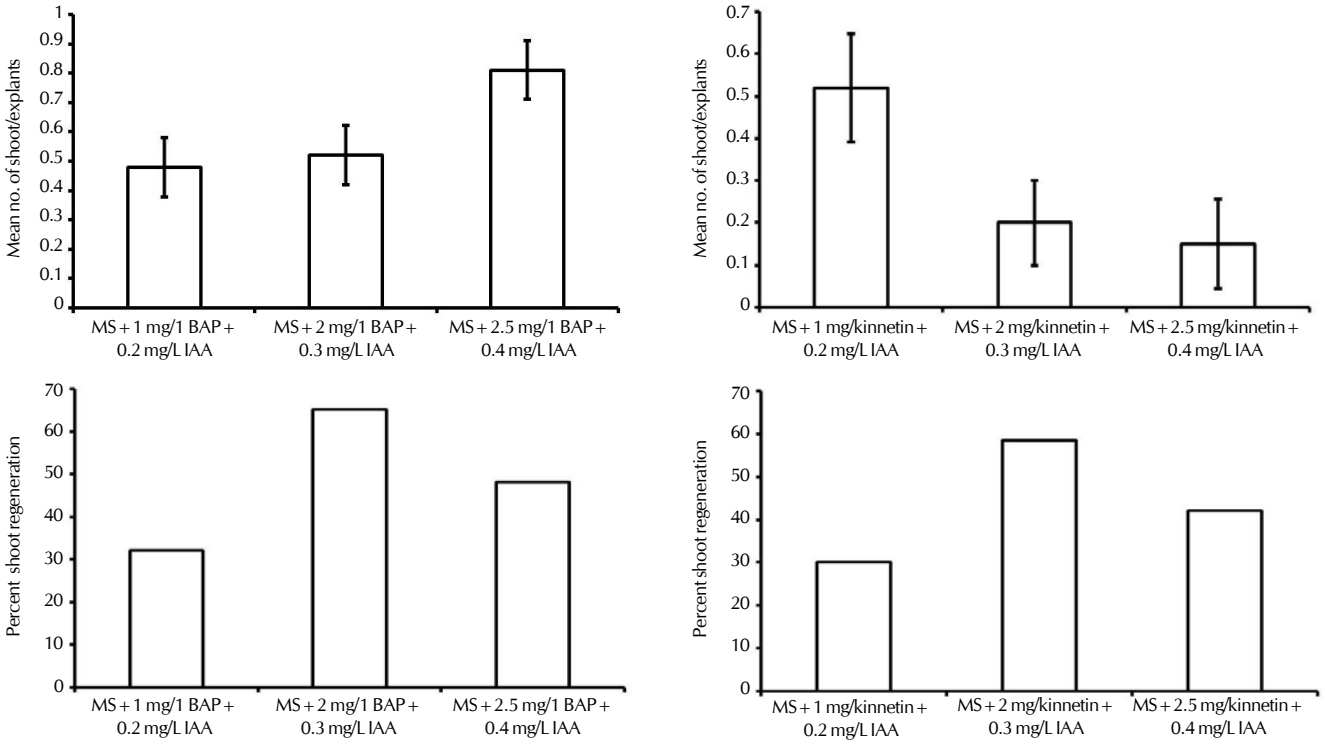

Figure 1: Impact of different combination on regeneration

supplemented with kinetin, an increase in regeneration efficiency of shoot was observed with maximum (58.52%) at MS media supplemented with 2 mg/L Kinetin + 0.3 mg/L IAA. These findings are in line with (Sagare and Manty, 2012; Bhat *et al.,* 2013) who also observed the increase in shoot regeneration with increase in kinetin level using cotyledonary leaves as 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 explant.

Different auxins *i.e.* IAA, IBA of various concentrations was used for root regeneration from *in vitro* developed shoots of brinjal. Root regeneration was observed after 20 days in culture room in media containing IAA and IBA. IBA is widely used for efficient root regeneration in brinjal (Sharma and Rajam, 1995; Singh *et al.,* 2000) although several suggested IAA as efficient root regeneration hormone (Bardhan *et al.,* 2012) In the present study, highest root regeneration (91.06%) was observed when MS medium was supplemented with IBA 1mg/L. These findings are similar to findings of (Sharma and Rajam, 1995; Hossain *et al.,* 2007; Chakravarthi *et al.,* 2009; Panwar *et al.,* 2013), who also observed higher root regeneration with addition of IBA.

In the present study it has been observed that an efficient plant regeneration protocols would make a platform for exploitation of useful somaclonal variations, mutation breeding, induction of di-haploids and genetic transformation with economically important genes for the improvement of eggplant by transfer of economically important traits.

ACKNOWLEDGMENT

The authors are thankful to National Bureau of Plant Genetic Resources, New Delhi, India for providing the germplasm and Department of Biotechnology, Ministry of Science and Technology, New Delhi, India, for sanctioning the project entitled, "Androgenesis mediated introgression of fruit and shoot borer (*Leucinodes orbonalis*) resistant genes into cultivated eggplant (*Solanum melongena*)" under the Bio-CARe scheme for women under which the present work was undertaken.

REFERENCES

Alicchio, R. Del Gross, E. and Boshueri, E. 1982. Tissue culture and plant regeneration from different explants in six cultivars of (Solanum melongena). *Experientia.* **38:** 449-450.

Baksh, S. Iqbal, M. 1979. Compatibility relationship in some of tuberous species of Solanum. *J. Hortic. Sci.* **54:** 163.

Bardhan, S. K. Sharma, C. and Srivatava, D. K. 2012. In vitro plant regeneration studies in brinjal. *J. Cell and Tissue Research.* **12(2):** 3213-3218.

Bhat, S. V., Jadhav, A. S, Pawar, B. D., Kale, A. A., Chimote, V. P. and Pawar, S. V. 2013. In vitro shoot organogenesis and plantley regeneration in brinjal (Solanum melongena). *The Bioscan.* **8(3):** 821-823.

Chakravarthi Havala, V. N. and Prabavathi, K. 2009. Effect of explant age, hormone on somatic embryogenesis and production of multiple shoot from cotyledonary leaf explants of S. trilobatum. *Af. J. Biotechnol.* **8:** 630-634.

Christianson, M. A. and Warnick, D. A. 1985. Temporal requirement for phytohormone balance in the control of organogenesis in vitro. *Dev. Biol.* **112(2):** 494-497.

Franklin, G., Sheeba, C. J. and Lakshmi Sita, G. 2004. Regeneration of eggplant (Solanum melongena L.) from root explants. *In Vitro Cell Dev. Biol. Plant.* **40:** 188-191.

Hossain, M. J., Raman, M. and Bari, A. 2007. Establishment of cell suspension culture and plantlet regeneration of brinjal (Solanum melongena). *J. Plant Sci.* **2:** 407-415.

Jahan, M. A. A. and Hadiuzzama, S. 1996. Callus induction and plant regeneration from different explants of Solanum nigrum L seedlings. *Plant Tiss. Cult.* **6(1):** 57-62.

Henry, Y., Vain, P. and De Buyser, J. 1994. Genetic analysis of in vitro plant tissue culture responses and regeneration capacities. *Euphytica.* **79(1-2):** 45-48

Kaur, S., Bal, S. S., Singh, G., Sidhu, A. S. and Dhillon, T. S. 2004. Management of brinjal shoot and fruit borer, Leucinodes orbonalis Guenee through net house cultivation. *Acta Hort.* **659:** 345-350.

Kaur, M., Dhatt, A. S., Sandhu, J. S. and Gosal, S. S. 2011. In vitro plant regeneration in brinjal from cultured seedling explants. *Indian J. Hortic.* **68(1):** 61-65.

Khatun, F., Meah, M. B. and Nasirudin, K. M. 2006. Regeneration of eggplant through anther culture. *Pak. J. Biol. Sci.* **9:** 48-53.

Magioli, C. and Mansur, E. 2005. Eggplant: tissue culture, genetic transformation and use as an alternative model plant. *Acta Bot. Bras.* **19:** 139-148.

Magioli, C., Rocha, A. P. M,, De., Oliveria, D. E. and Mansur, E. 1998. Efficient shoot organogenesis of eggplant induced by thiodiazuron. *Plant Cell Rep.* **17:** 661-663.

Matsuoka, H. and Hinata, K. 1979. NAA-induced organogenesis and embryogenesis in hypocotyl callus of Solanum melongena L. *J. Exp. Bot.* **30:** 363-370.

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

Muhammad, Danish Jamil, Muhammad Parvaiz, Muhammad Tufail, Junaid Arshad, Sajid Hussain and Sahar Imtiaz. 2013. Callogenesis, regeneration of shoot and root of brinjal (Solanum melongena). *World Applied Science J.* **26(8):**1039-1045.

Mukherjee, S. K., Rathinasabapathi, B. and Gupta, N. 1991. Low sugar and osmotic requirements for shoot regeneration from leaf pieces of Solanum melongena L. *Plant Cell Tissue Organ Cultur.* **25:** 13-16.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobbaco tissue cultures. *Physiol. Plant.* **15:** 473-497.

Pawar, B. D., Jadhav, A. S., Kale, A. A., Chimote, V. P. and Pawar, S. V. 2012. Zeatin induced direct in vitro shoot regeneration in tomato ((Solanum lycopersicon L.). *The Bioscan.* **7(2):** 247-250.

Panwar, B. D., Jadhav, A. S., Kale, A. A., Chimote, V. P. and Pawar, S. V. 2013. In vitro Plant Regeneration in Brinjal (Solanum melongena L.) Using Cotyledon, Hypocotyl and Root Explants. *Vegetos.* **26(2):** 312-317.

Rahman M., Asaduzzaman M., Nahar N. and Bari M. A. 2006. Efficient plant regeneration from cotyledon and midrib derived callus in eggplant (Solanum melongena). *J. Biol. Sci.* **14:** 31-38.

Sagare, D. B. and Mohanty, I. C. 2012. Development of moisture stress tolerant brinjal cv. utkal anushree (Solanum melongena L.) using Agrobacterium mediated gene transformation. *J. Agric. Sci.* **4(8):** 141-148.

Sarkar, R. H., Yesmin, S. and Hoque, M. I. 2006. Multiple shoot formation in eggplant (Solanum melongena), Plant Tiss Cult and Biotechnol. **16:** 53-61.

Sharma, P. and Rajam, M. V. 1995. Genotype, explants and position effects on organogenesis and embryogenesis in eggplant (Solanum melongena). *J. Exp. Bot.* **16:** 135-141.

Shivraj, G. and Rao, S. 2011. Rapid and efficient regeneration of eggplant (Solanum melongena L.). *Indian J. Biotechnol.* **10:** 125-129.

Sidhu, M. K., Dhatt, A. S. and Sidhu, J. S. 2014. Plant regeneration in eggplant (Solanum melongena L): A review. *Afr. J. Biotechnol.* **13(6):** 714-722.

Singh, S. V., Singh, K. S. and Malik, Y. P. 2000. Seasonal abundance and economic losses of shoot and fruit borer (Leucinodes orbonalis) on brinjal. *Indian J. Entomol.* **52:** 247-252.

Taha, R. M. and Tijan, M. 2002. An in vitro production and field transfer protocol for Solanum melongena plants. *South Afr. J. Bot.* **68:** 447-450

Xing Y., Yu Y., Luo, X., Zhang, J . N., Zhao, B. and Guo, Y. D. 2010. High efficiency organogenesis and analysis of genetic stability of the regenerants in Solanum melongena. *Biol. Plant.* **54(2):** 231-236

Zayova, E., Vassi levska Ivanova, R., Kraptcher, B., and Stoeva, D. 2012. Indirect shoot organogenesis of eggplant (Solanum melongena). *J. Cent. Eur .Agr.* **13(3):** 446-457.

APPLICATION FORM

NATIONAL ENVIRONMENTALISTS ASSOCIATION (N.E.A.)

To,
The Secretary,
National Environmentalists Association,
D-13, H.H.Colony,
Ranchi - 834 002, Jharkhand, India

Sir,
I wish to become an Annual / Life member and Fellow* of the association and will abide by the rules and regulations of the association

Name _____

Mailing Address _____

_____

Official Address _____

_____

E-mail _____Ph. No._____(R)_____(O)

Date of Birth _____ Mobile No. _____

Qualification _____

Field of specialization & research _____

Extension work (if done) _____

_____

Please find enclosed a D/D of Rs..... No. ..... Dated ..... as an *Annual / Life membership fee*.

*Attach **Bio-data and some recent publications along with the application form when applying for the Fellowship of the association.**

Correspondance for membership and/ or Fellowship should be done on the following address :

SECRETARY,
National Environmentalists Association,
D-13, H.H.Colony,
Ranchi - 834002
Jharkhand, India
E-mails : m_psinha@yahoo.com Cell : 9431360645
dr.mp.sinha@gmail.com Ph. : 0651-2244071