ISSN-1991-3036 (Online) & ISSN-2075-1621 (Optical)
Int. J. Sustain. Crop Prod. 5(4):5-10(November 2010)

IN VITRO DIRECT AND INDIRECT PLANTLETS REGENERATION FROM NODAL SEGMENT OF *Boerhaavia repens* L.

A. ROWNAQ AND M.S. HOSSAIN[1]

Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.

[1] Corresponding author & address: M.S. Hossain, Email: shahadat94@yahoo.com
Accepted for publication on 15 October 2010

ABSTRACT

Rownaq A, Hossain MS (2010) *In vitro* Direct and Indirect Plantlets Regneration from Nodal Segment of *Boerhaavia repens* L. *Int. J. Sustain. Crop Prod.* 5(4), 5-10.

An efficient protocol for *in vitro* propagation of *Boerhaavia repens* L. was established using nodal segment. The highest direct shoot regeneration from nodal segment was noted (94%) in the MS medium having 2.0 mg/l BAP+0.2 mg/l NAA. The mean number of shoots per culture (12.51) and mean length of shoot per culture (5.10 cm) were also highest in the same medium. Callus induction and plantlet regeneration were observed using different concentrations and combinations of growth regulators. The highest frequency (100%) of organogenic callus was observed in the MS medium containing 1.0 mg/l NAA+1.0 mg/l BAP. Again, the highest (91%) shoot was produced in the MS medium in the combination with 2.0 mg/l BAP+1.5 mg/l Kn+0.5 mg/l NAA was proved to be most suitable in shoot formation from callus. The elongated shoots developed maximum (100%) roots on a half strength MS medium containing 1mg/l IBA. The well rooted plantlets were successfully established in natural condition, where the survival rate of plantlets was 80%.

***Key words**: In vitro regeneration, nodal segment, Boerhaavia repens* L.

INTRODUCTION

Boerhavia repens L. is an important medicinal plant. It has a pan tropical distribution, and possibly originates from the old World. It occurs throughout Africa including the Mediterranean countries and South Africa and is especially common in regions with a distinct dry season. It is found everywhere in Bangladesh. In bengali, it is commonly known as Punornova. This plant is herbaceous**,** annual or perennial, sometimes suffrutescent at base, cylinder, often glandular, glabrous, pubescent from slender and soft or stout, woody and ropelike or has fusiform tap root. Stems are procumbent, decumbent, ascending or erect, unarmed, with or without glutinous bands on internodes and leaves are petiolate, blade thin or thick and slightly fleshy. Inflorescences are terminal and auxiliary, cymose, paniculate or thyrsiform, recemose and umbellate. Various parts of this plant has an excellent medicinal uses. It is useful in blood impurities, anaemia, inflammation, heart disease, asthma, tumour, spleen enlargement, abdominal pains and joint pain (Mishra 1980). For a general liver tonic, one cup of a whole herb or root decoction or 2 ml of a 4:1 tincture is taken once daily. This same dosage is taken two to three times daily for various liver and kidney disorders. For a natural diuretic, 500 mg of the root in capsules or tablets can be taken twice daily. As a menstrual aid (to reduce menstrual pain, cramping, and excessive bleeding) one cup of a whole herb or root decoction or 1–2 g in tablets or capsules can be taken two to three times daily as needed (Gaitonde 1974). The root of this plant has an important role in herbal medicine in Brazil, India and Bangladesh. Novel plant chemicals have been found Punornova, including flavoids, steroids and alkaloids, many of which drive its documented biological activities (Pandey *et al*. 2005). In spite of its many medicinal values, there are no reports available on *in vitro* propagation of this plant. Therefore, the present investigation was undertaken to examine the *in vitro* regeneration technique that can be used as a potential tool for large scale production of this valuable plant.

MATERIALS AND METHODS

The experiment was carried out at the Professor Ali Md. Eunus laboratory, Department of Genetic Engineering and Biotechnology, Rajshahi University, Bangladesh. Nodal segments of Punornova (Boerhavia repens L.) were collected from field grown six months old plants from Rajshahi University campus and washed thoroughly under running tap water and treated with 1% Tween-80 for eight minutes. This was followed by three successive washing with distilled water to make the material free from Tween-80. Further sterilization was done under aseptic condition in a Laminar Airflow Hood. Surface sterilization was carried out with 0.1% HgCl2 for 3.5 minutes followed by gentle shaking. The material then washed sterilized doubled distilled water for 4-5 times to remove HgCl2. Explants with single nodal segment of 1 cm in length was cut from the sterilized shoot. Then explants were transferred to 25×150 mm culture tube with 15ml MS (Murashige and Skoog, 1962) medium supplemented with different hormonal concentrations for direct shoot regeneration. The pH was adjusted to 5.7 prior to autoclaving. The cultures were maintained at 25 ± 2°C under the cool white fluorescent tubes at 3000 lux with a photoperiod of 16 hours. Direct shoot regeneration from nodal segment was cultured in medium with different combinations of BAP (Benzylamino purine), Kn (Kinetin), NAA (1- Naphthalene acetic acid) and IAA (Indole-3-acetic acid). Callus induction from nodal segment was suitable in combinations of BAP and NAA. Shoot formation from callus was cultured in medium with combinations of BAP, Kn and NAA. Well developed shoots were excised and sub cultured

on MS medium with different concentrations of NAA, IBA and IAA for root induction. After development of sufficient root system the regenerated plantlets were considered ready to transfer to soil. The roots of the plantlets are gently washed under running tap water to remove agar attached to the root zone. Immediately after that they were transferred to small pots containing a mixture of sand, soil and humus in the ratio of 1:2:2. After sufficient acclimatization, the plantlets were transferred into the larger pot containing soil, where 80% plantlets were survived.

RESULT AND DISCUSSION

Different growth regulators including BAP, Kn and NAA were used in different concentrations and different combinations for induction of direct shoot buds from nodal explants of *B. repens*. The highest (94%) shoot proliferation was noted in the medium having 2.0 mg/l BAP+0.2 mg/l NAA. The mean number of shoots per culture (12.51) and mean length of shoot per culture (5.10cm) were also highest in the same combination (Table 1 & Fig. A).

Callus induction was observed on MS media supplemented with NAA (0.5-1.5mg/l) in combination with BAP (0.25-1.0mg/l) (Table 2). The results of these experiments were taken after 6-10 days depending upon the concentration and combination of hormonal treatment of culture. Maximum (100%) callus was induced in the medium containing 1.0 mg/l NAA+1.0 mg/l BAP and the induced callus was light green and friable (Table 2 & Fig. B) followed by 96% in the medium combinations of both 1.0 mg/l NAA + 0.5%mg/l BAP and 0.5mg/l NAA + 1.0mg/l BAP respectively. Many reports are observed on callus initiation from herbaceous and vegetable crop species (Misra *et al*. 1983; Jain *et al*. 1985 and Asao *et al.* 1989) in egg plants and Lin *et al*. in *Polygonum multiflorum.*

The highest percentage of shoot formation was 91% in the MS medium containing of 2.0mg/l BAP + 1.5mg/l Kn + 0.5 mg/l NAA and highest number of shoots per callus was 10.24 in the same media (Table 3 and Fig. C) followed by the media in combinations of both 2.0mg/l BAP + 1.5 mg/l Kn + 1.0 mg/l NAA and 2.0 mg/l BAP + 1.0 mg/l Kn + 0.5 mg/l NAA. On the other, the lowest percentage 51% of shoots was found in the MS medium having 2.0 mg/l BAP + 2.0 mg/l Kn + 1.0 mg/l NAA. Thus, 2.0 mg/l BAP + 1.5 mg/l Kn + 0.5 mg/l NAA was appeared to be a suitable treatment for shoot regeneration as well as elongation. Shoot regeneration was reported in plant species including *Withania somnifera* (Rani and Grover, 1999) and *Polygonum multiflorum* (Lin *et al*. 2003).

5-7cm long *in vitro* grown shoots were separated and transferred to rooting media. The highest (100%) root induction was taken place along with highest mean number 10.48 and mean length of root 5.33cm per culture were obtained from half strength MS medium with 1.0 mg/l IBA (Table 4 & Fig. D) followed by the media in combination with 0.5 mg/l IBA. On the contrary, medium having 0.1 mg/l NAA exhibited the lowest (33%) root induction as well as minimum number of roots and minimum root length per culture. Thus, 1.0mg/l IBA was found to be an ideal treatment for root induction as well as root elongation. Similar result of *in vitro* root induction was reported in other plants, such as, Chaplot *et al*. 2006 in *Plambaga zeylamica* Linn. and Biswas *et al.* 2007 in *Abrus precatorius*. Rooted healthy plantlets were transferred to plastic pots with garden soil to maintain the high humidity. Subsequently the plantlets were planted in small pots containing potting mixture of sterile sand, soil and humus in the ratio of 1:2:2 for hardening (Fig. E). Finally, acclimatized plantlets were transferred to the larger pot (Fig. F) in the natural environment, where 80% plantlets were survived and grown successfully.

ACKNOWLEDGEMENT

The authors wish to thank Professor Dr. Biswanath Sikdar, Chairman, Department of Genetic Engineering and Biotechnology, Rajshahi University, Bangladesh for giving financial support and laboratory facilities to conduct this research work.

Table 1. Effect of different concentrations and combinations of auxin and cytokinin on direct shoot regeneration from nodal explants of *Boerhavia repens* L. (Data collected after 30 days of culture)

Growth regulators (mg/l)	% of culture response (M ± SE) *	Mean no. of shoot per culture (M ± SE) *	Mean length of shoot (cm) per culture (M ± SE) *
BAP + NAA			
1.0+0.2	60±0.52	3.26 ±0.18	1.78 ±0.16
1.0+0.3	51±0.88	2.82 ±0.14	1.40 ±0.09
1.0+0.4	31±0.88	1.67 ±0.16	0.76 ±0.08
2.0+0.2	94±1.45	12.51 ±0.43	5.10 ±0.17
2.0+0.3	65±0.88	6.35±0.34	3.16 ±o.13
2.0+0.4	51±0.67	5.04 ±0.22	2.42 ±0.26
3.0+0.2	69±0.65	7.15 ±0.25	3.16 ±0.14
3.0+0.3	51±0.57	3.77 ±0.06	1.92 ±0.21
3.0+0.4	40±0.81	3.11 ±0.25	1.75 ±0.16
Kn + NAA			
1.0+0.1	31±0.33	1.04±0.10	0.63±0.08
1.0+0.2	29±0.88	0.93±0.11	0.59±0.08
1.0+0.3	20±1.45	0.73±0.14	0.47±0.09
2.0+0.1	40±0.57	1.64±0.18	0.86±0.14
2.0+0.2	49±0.33	2.06±0.14	1.54±0.09
2.0+0.3	20±0.57	0.87±0.13	0.58±0.15
3.0+0.1	60±1.15	4.06±0.19	1.81±0.12
3.0+0.2	49±0.33	3.53±0.14	1.54±0.13
3.0+0.3	31±0.33	2.36±0.23	1.03±0.09

Note: The experiments were repeated thrice and each experiment consisting of 10 replicates following RCBD trial.
*M = Mean and SE = Standard error

Table 2. Effect of different concentrations and combinations of auxin and cytokinin in MS medium on callus induction from nodal explants

Growth regulators (mg/l)	Days to callus initiation	% of callus induction (M ± SE)*	Nature of callus
NAA+BAP			
0.5 + 0.25	7-10	80±0.57	Wf*
0.5+0.5	8-10	91±0.33	Wf
0.5+1.0	8-10	96±0.57	Wf
1.0 + 0.25	7-8	87±0.88	Lgf*
1.0+0.5	6-7	96±1.15	Lgf
1.0+1.0	6-7	100±0.57	Lgf
1.5 + 0.25	6-10	78±0.66	Lgf
1.5+0.5	8-10	80±0.33	Lgf
1.5+1.0	8-10	91±0.57	Lgf

Note: The experiments were repeated thrice and each experiment consisting of 10 replicates following RCBD trial.
*M = Mean and SE = Standard error, *****Wf = White friable and *Lgf = Light green friable

Table 3. Effect of BAP + Kn + NAA on adventitious shoot regeneration from callus of *B. repens* L. (Data collected after 28 days of culture)

Plantgrowth regulators (mg/l)	% response of culture (M ± SE)*	Mean no. of shoot per culture (M ± SE)*	Mean length (cm) of shoot per culture (M ± SE)*
BAP + Kn + NAA			
2.0+1.0+0.5	78±0.33	8.14±0.33	4.53±0.18
2.0+1.0+1.0	60±0.88	5.22±0.33	2.20±0.19
2.0+1.5+0.5	91±0.57	10.24±0.20	5.53±0.23
2.0+1.5+1.0	78±0.57	8.93±0.21	4.23±0.13
2.0+2.0+0.5	71±0.67	5.88±0.23	2.91±0.18
2.0+2.0+1.0	51±0.33	4.01±0.26	2.03±0.28

Note: The experiments were repeated thrice and each experiment consisting of 10 replicates following RCBD trial.
*M = Mean and SE = Standard error

Table 4. Effect of different concentration of Auxins (IBA, NAA and IAA) for rooting (Data collected after 25 days of culture)

Growth regulators mg/l	% of culture response (M ± SE) *	Mean no. of roots per explant (M ± SE) *	Length of root (cm) (M ± SE) *
IBA			
0.1	60±0.33	5.37±0.55	2.43±0.20
0.5	93±0.57	8.64±0.26	4.34±0.32
1.0	100±0.33	10.48±0.06	5.33±0.11
NAA			
0.1	33±0.57	1.64±0.29	1.02±0.10
0.5	71±0.88	5.82±0.12	2.83±0.14
1.0	60±0.67	4.46±0.13	2.21±0.19
IAA			
0.1	40±0.33	2.03±0.05	1.60±0.23
0.5	48±0.67	2.46±0.21	1.83±0.16
1.0	54±o.57	2.77±0.15	2.11±0.15

Note: The experiments were repeated thrice and each experiment consisting of 10 replicates following RCBD trial.
* M = Mean and SE = Standard error.

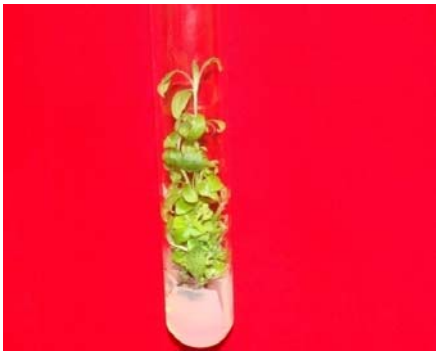

Fig A: Direct shoot regeneration

Fig B: Callus induction from nodal explants

Fig C: Shoot regeneration from callus

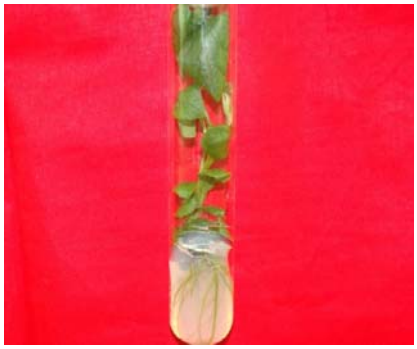

Fig D: Root induction and elongation

Fig E: Hardening and acclimatization of *In vitro* grown plantlet

Fig F: Transplantation of acclimatized plantlet in the larger pot

CONCLUSION

In the investigation it was observed that the callus induction and regeneration ability of the nodal explants has the potential for future biotechnological studies for the improvement of *Boerhaavia repens* L.

REFERENCES

Asao H, Tanigawa M, Arai S, Kobatake H (1989) Plant regeneration from mesophyll protoplasts of the egg plant and its wild species, *Bull. Nara. Agric. Expt. Station.* Japan. 20, 73-78.

Biswas A, Roy M, Bari Miah MA, Bhadra SK (2007) *In vitro* propagation of *Abrus precatorius* L. A Rare Medicinal Plant of Chittagong Hill Tracts. *Plant Tiss. Cult. Biotechnol.* 17(1), 59-64.

Chaplot BB, Dave AM, Jasrai YT (2006) A valued medicinal plant-Chitrak (*Plumbaga zeylanica* Linn.): Successful plant regeneration through various explants and field performance. *Plant Tiss. Cult. Biotechnol.* 16(2), 77-84.

Gaitonde BB (1974) Diuretic activity of punarnava (*Boerhaavia diffusa*). *Haffkine Inst. Bull*. 24, 3-7.

Jain RK, Chowdhury JB, Sharma DR (1985) Selection and Characterization of methionine and sulfoimine reistant with cultures of brinjal (*Solanum melongena* L.) Current Sci. 37, 389-396.

Lin LC, Nalawade SM, Mulabagal V, Yeh MS, Tsay HS (2003) Micropropagation of *Polygonum multiflorum* THUNB and Quantitative Analysis of the Anthraquinones. Emodin And Physician Formed in *in vitro* Propagated Shoots and Plants. *Biol Pharm. Bull.* 26, 1467-1471.

Mishra JP (1980) Studies on the effect of indigenous drug *Boerhaavia diffusa* Rom. on kidney regeneration. *Indian J. Pharm* 12, 59.

Misra NRTM, Verghese N, Maherchandani, Jain RK (1983) Studies on Induction and differectiation of androgenetic callus of *Solanum melongena* L. In: Plant cell culture in crop improvement. *Basic life sci.* 22, 465-486.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant. *Physiol*. 15, 473-497.

Pandey R, Maurya R, Singh G, Sathiamoorthy B, Naik S (2005) Immunosuppressive properties of flavonoids isolated from *Boerhaavia diffusa* Linn. *Int. Immunopharmacol* 5(3), 541-53.

Rani G, Grover IS (1999) *In vitro* callus induction and regeneration studies in *Withania somnifera*. *Plant Cell Tiss. Org Cult.*, 57, 23-27.