academicJournals

Vol. 7(37), pp. 2761-2768, 3 October, 2013
DOI 10.5897/JMPR12.1318
ISSN 1996-0875 © 2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Adventitious plantlet regeneration from different explants of *Aegle marmelos* (L.) Corr.

Priya R. Behera[1], Manikkannan Thirunavoukkarasu[1]* and Pradeep K. Chand[2]

[1]Bioresources Engineering Department, CSIR- Institute of Minerals & Materials Technology, Bhubaneswar-751 013, India.
[2]Post Graduate Department of Botany, Utkal University, Bhubaneswar-751 004, India.

Accepted 17 September, 2013

Plant regeneration was achieved from the diverse explants such as epicotyl, cotyledon, hypocotyl and root explants of important medicinal plant *Aegle marmelos*. Explants were obtained from 4 week old axenic seedlings of *A. marmelos* and cultured on Murashige and Skoog (MS) medium supplemented with plant growth regulators to ascertain the suitable explants and media composition for mass production. Cytokinins benzylaminopurine (BAP) and kinetin (Kin) were used for multiple shoot induction. Between two cytokinin tested, BAP was more efficient than Kin with respect to the initiation and subsequent proliferation of shoot buds. Addition of an auxin along with cytokinin improved the shoot production capacity. Shoot buds could be initiated from all the explants tested, with epicotyl explants producing the highest average number of shoots/explant. MS medium supplemented with 2.2 µM BAP + 1.425 µM indole-3-acetic acid (IAA) produced maximum number of shoots. The regenerated shoots were further multiplied by sub culturing on fresh medium. The elongated shoots were shifted to half-strength MS medium supplemented with different concentrations of IAA, α-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) for root induction. Rooting was better in the medium augmented with 2.85 µM IAA. About 90% of the rooted plantlets survived acclimatization and transfer to the field.

Key words: *Aegle marmelos*, medicinal plant, micropropagation, seedling explants, cytokinin.

INTRODUCTION

Aegle marmelos (L.) Corr. (Family: Rutaceae) is a spiny tree sparsely distributed throughout India on the plains and in hilly tracts up to 1300 m elevations. It is commonly known as the 'Bael fruit' or 'Bengal Quince' with religious and medicinal values. Though more prized for its medicinal purposes than its edible quality, this interesting tree is, nevertheless, of sufficient importance as an edible fruit. Fruits, leaves and roots are used for treating various ailments. In pharmacological trials, both fruits and roots have shown antiamoebic and hypoglycemic activities (Poonachan et al., 1993). The alkaloid aegeline present in the leaf is a potent antiasthamatic agent (Haravey, 1968). The ripe fruit is used for curing dyspepsia (Jauhari et al., 1969; Satyavati et al., 1976) and the infusion of dried unripe fruits has been used as antidiarrhea and antidysentery agents, the juice from crushed leaves has been used for the treatment of bronchitis, and the decoction of root barks has also been used as anti-malarial drug (Mishra et al., 2010). In addition, young leaves are used as vegetable. Green leaves have anti-inflamatory, antipyretic and analgesic properties (Shoba and Thomas, 2001; Arul et al., 2005). The chemical investigation of the leaves of this plant has revealed the presence of a number of alkaloids (Phuwapraisirisan et al., 2008). Roots of *A. marmelos* have antidote to snake venom, anti-imflammatory and wound healing properties

*Corresponding author. E-mail: mtarasu@yahoo.com

(Shoba and Thomas, 2001). Due its multiple utilities in pharmaceutical industries, the species is being exploited ruthlessly.

A. marmelos shows a wide genetic variability in terms of quality, form and size of the fruits (Bhati et al., 1992). Apart from the heterozygosity problems, the seeds have short span of viability and are prone to insect attack. Hence, propagation through seeds is not a viable route to produce the large number of plants. Also, propagation through root suckers is very slow, difficult and cumbersome. Alternatively, *in vitro* mass propagation techniques offer opportunities for multiplying desired planting materials in a larger quantity within a short span of time. In recent years, a number of workers have attempted to multiply *A. marmelos* using different explants such as cotyledon (Hossain et al., 1994; Hazeena and Sulekha, 2008; Puhan and Thirunavoukkarasu, 2011), hypocotyls (Hossain et al., 1995), root segments (Bhati et al., 1992), nucellar tissue (Hossain et al., 1993; Islam et al., 1995), single node segments (Ajithkumar and Seeni, 1998) and cotyledonary node (Nayak et al., 2007). In order to ascertain the suitable explants for optimal response, the comparative response of various explants such as hypocotyl, cotyledon, epicotyl and root segments from axenic culture of *A. marmelos* was studied.

MATERIALS AND METHODS

Study site and source materials

The study was under taken at the CSIR-Institute of Minerals and Materials Technology (IMMT), Bhubaneswar (20° 17′ 45″ N latitude and 85° 49′ 15″ E longitudes at the altitude of 200 ft). Ripe fruits of *A. marmelos* were collected from an elite tree (20 to 22 years old) growing in the adjoining forest area. Authentication of the specimen was done in the herbaria of the institute by comparing the specimen material maintained in the herbarium. The seeds were extracted from the fruits, dried under shade for 2 to 3 days and used as source material for the present studies.

Nutrient media preparation and culture conditions

The nutrient medium consisted of Murashige and Skoog (MS, 1962) basal medium supplemented with sucrose (3% w/v). Depending on the requirement, the media was further augmented with or without growth regulators. For *in vitro* seed germination MS medium without any growth regulators was used. Shoot induction medium consisted of different plant growth regulators such as benzylaminopurine (BAP, 2.2 to 8.8 µM), kinetin (Kin, 2.35 to 9.4 µM), indole-3-acetic acid (IAA, 1.425 µM), and α-naphthaleneacetic acid (NAA, 1.35 µM) were added either alone or in combination to MS basal medium. For root induction, half-strength MS basal medium was supplemented with IAA, NAA or indole-3-butyric acid (IBA), and the pH was adjusted to 5.8. The agar-gelled (0.8% w/v) MS basal medium with or without growth hormones was dispensed into either 150 × 25 mm test tubes or in conical flasks of 250 ml capacity. The medium was sterilized at 121°C and 1.1 kg cm^{-2} pressure for 16 min. All the cultures were incubated in a culture room maintained at 25 ± 2°C under 16/8 h light/dark cycle, 45 µM m^{-2} s^{-1} irradiance level provided by cool white fluorescent tubes with 55 to 60% relative humidity (RH). Each treatment consisted of 10 explants and was repeated thrice.

In vitro seed germination and explant preparation

The shade-dried seeds were washed thoroughly in a liquid detergent solution followed by thorough washing in tap water. Then, they were surface sterilized in 0.1% $HgCl_2$ solution for 3 to 4 min followed by washing in sterile, double distilled water three to four times under aseptic conditions. The sterilized seeds were aseptically transferred to conical flasks of 250 ml capacity containing sterile half-strength MS basal medium without any additives. Five to eight seeds were explanted in each flask, and for each experiment, 200 seeds were inoculated. When the seedlings were 4 weeks old, epicotyl, hypocotyl, cotyledon and roots were aseptically excised and cultured on different nutrient media for shoot bud induction.

Data scoring

Data were recorded on percentage of response of different seedling explants (epicotyls, hypocotyls, cotyledons, roots) on shoot proliferation; shoot number and shoot length after 7 weeks of culture on MS basal medium with or without addition of plant growth regulators (PGRs). Similarly, data were recorded on percentage of rooting, root number and root length after 4 weeks of culture in the root induction medium. Data was analyzed statistically using analysis of variance (ANOVA) for a completely randomized design. Duncan's new multiple range test (DMRT) was used to separate the mean for significant effect (Gomez and Gomez, 1984). Ten replicates were used for every treatment and repeated thrice.

RESULTS

In vitro seed germination and explant preparation

In vitro cultured seeds showed 70% germination after 1 to 2 weeks of inoculation and attain a height of 6 to 7 cm in 4 to 5 weeks time. Seedlings of 4 weeks old were used to excise the epicotyl, hypocotyls, cotyledon and roots explants.

Adventitious shoot bud initiation and development

Explants prepared from aseptic seedlings were cultured on to MS basal medium augmented with or without plant growth regulators and showed varied response (Table 1). No significant response was noted in the absence of plant growth regulators on any of the cultured explants. Addition of plant growth hormones to the medium had a positive effect on shoot bud formation. Shoot bud formation and further development varied with the explants and type and combination of plant growth regulators used. Epicotyls produced the highest mean number of shoot followed by cotyledon and hypocotyl explants. Root explants produced comparatively least number of shoots. Initiation of multiple shoots in all the four explants in most of the treatments ranged from 7

Table 1. Effect of different PGRs on shoot multiplication from the various explants on MS medium in *A. marmelos* after 7 wks of incubation

MS + PGRs (µM)	Epicotyl explants			Cotyledon explants			Hypocotyl explants			Root explants		
	Response (%)	Mean shoot number	Mean shoot length (cm)	Response (%)	Mean shoot number	Mean shoot length (cm)	Response (%)	Mean shoot number	Mean shoot length (cm)	Response (%)	Mean shoot number	Mean shoot length (cm)
Control	0	0	0	0	0	0	0	0	0	0	0	0
BAP (2.2)	100	211^{b}	1.62^{b}	70	16.17^{h}	1.25^{bcde}	83.3	14.17^{j}	1.24^{bcde}	80	9.33^{n}	1.41^{bc}
BAP (4.4)	96.6	70.3^{c}	1.1^{bcdef}	80	24.83^{f}	1.39^{bc}	70	10.43^{m}	0.85^{cdef}	70	5.37^{p}	0.82^{cdef}
BAP (8.8)	96.6	56.9^{d}	0.84^{cdef}	66.6	11.30^{l}	0.87^{cdef}	63.3	6.67^{o}	0.71^{ef}	53.3	3.17^{rs}	0.6^{f}
Kin (2.35)	80	6.3^{o}	0.96^{cdef}	70	2.53^{tv}	0.83^{cdef}	70	2.83^{st}	0.91^{cdef}	70	2.23^{tv}	0.82^{cdef}
Kin (4.7)	90	16.4^{h}	$1.26bc^{de}$	76.6	5.07^{pq}	0.99^{bcdef}	80	4.57^{q}	1.24^{bcde}	76.6	3.67^{r}	1.1^{bcdef}
Kin (9.4)	86.6	13.1^{k}	1.05^{bcdef}	53.3	2.0^{v}	0.74^{def}	66.6	2.5^{tv}	0.82^{cdef}	66.6	1.97^{v}	0.92^{cdef}
BAP (2.2) + IAA (1.425)	100	341.73^{a}	2.34^{a}	86.6	56.3^{d}	1.42^{bc}	83.3	21.3^{g}	1.35^{bcd}	80	15.3^{i}	1.4^{bc}
BAP (2.2) + NAA (1.35)	70	35.4^{f}	0.95^{cdef}	66.6	13.2^{k}	0.7^{ef}	63.3	9.3^{n}	0.74^{def}	60	6.6^{o}	0.6^{f}

Data pooled from three independent experiments each with 10 replicates. Data collected after 7 weeks of culture.*Mean values within column followed by the same letter are not significantly different ($p \leq 0.05$; Duncan's New Multiple Range Test).

to10 days of inoculation. Rapid and early shoots initiation was observed in epicotyl explants (Figure 1a) where shoot initiation started in seven days after culture, and in three weeks time most of the epicotyls produced shoots (Figure 1b). Further shoot elongation was achieved on the same medium after 4 weeks of transfer where the shoots attained a length of 3 to 4 cm (Figure 1c). Cotyledon explants produced shoot buds after 8 to 10 days of culture (Figure 1d) and the shoot buds emerged from the cut surface of the explant (Figure 1e). After 4 weeks of culture period, the regenerating shoot clumps were transferred to fresh medium of same composition where shoots showed elongation (Figure 1f). Hypocotyl explants (Figure 1g) showed the first visible shoot proliferation on 10th day of inoculation and the shoots emerged from the cut ends and further shoot elongation achieved after 2 sub culture at 4 weeks interval (Figure 1h and i).

Effect of cytokinins

Between two cytokinin tested, BAP was more efficient than Kin with respect to the initiation and subsequent proliferation of shoot buds. Invariably all the explants showed highest regenerative response in the BAP supplemented medium (Table 1). When BAP was used as sole PGR supplement, the best shoot proliferation was observed in MS + BAP, 2.2 µM. In this treatment all the epicotyl segments produced shoots averaging of 211 shoots per explant (Significant at $p \leq 0.05$). Cotyledon explants responded better in MS + BAP, 4.4 µM wherein 80% culture produced shoots with an average of 24.83 shoots per explant. Root and hypocotyl explants responded better in MS + BAP, 2.2 µM resulting in 83.3 and 80% response, respectively. In such concentrations, root explants produced a mean shoot number of 14.17 and 9.33 shoots per explant was produced from hypocotyl explants. There was invariably delayed shoot initiation in explants cultured in Kin supplemented medium. In such cases, time taken to initiate shoot bud formation varied from 14 to 25 days depending on the explant type. Epicotyl explants was quicker (14 to 16 days) in initiating shoot buds followed by cotyledon and root explants (18 to 20 days) and hypocotyls took the maximum time (23 to 25 days) to form shoot bud initiation. An average of 16.4 shoot numbers were recorded as the highest response from epicotyl explants which were cultured in MS + Kin, 4.7 µM. In the rest of the explants, there was no significant shoot formation observed in any of the concentration level of Kin (Table 1). Optimal concentration requirement of cytokinins varied to different explants studied. For instance, 2.2 µM concentration of BA or 4.7 µM Kin is the optimal strength for epicotyl, hypocotyl and root cultures, whereas cotyledon explants

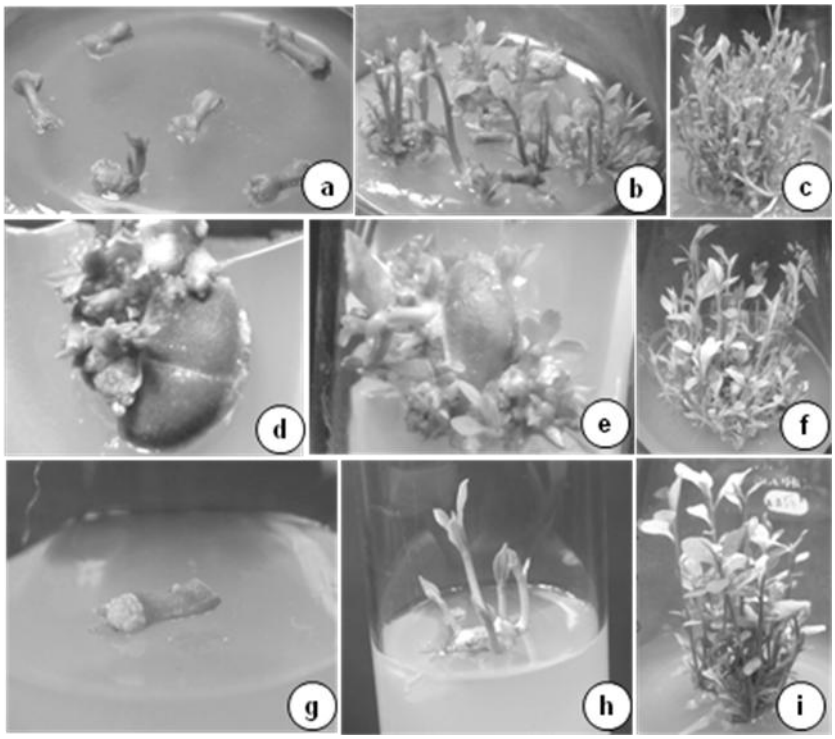

Figure 1. Adventitious shoot regeneration from different explants of *A. marmelos*. (a) Epicotyls cultured on MS + BAP (2.2 µM) + IAA (1.425 µM) medium (1 week old). (b) Development of shoots on the cultured epicotyls on the same medium (3 weeks old). (c) Individual epicotyl sub-cultured on the same medium showing shoot elongation (7 weeks old). (d) Cotyledon segment cultured on MS + BAP (4.4 µM) + IAA (1.425 µM) medium showing shoot bud formation (2 week old). (e) Shoot development all along the cultured cotyledon on the same medium (4 weeks old). (f) Shoot elongation from cotyledon cultured after sub culturing on the same medium composition (7 weeks old). (g) Hypocotyl cultured on MS + BAP (2.2 µM) + IAA (1.425 µM) medium showing bulging of explant (1 week old). (h) Shoot development on the cultured hypocotyl (3 week old). (i) Sub-cultured hypocotyl showing the shoot elongation on the same medium composition (7 week old).

responded maximal in 4.4 µM of BAP or 4.7 µM of Kin. The regeneration frequency decreased with increase beyond the optimal concentration of cytokinins.

Effect of cytokinins and auxins

In order to test the beneficial effect of auxin and cytokinin, only BAP at 2.2 µM concentration was used (based on the results obtained in all cultures) along with 1.425 µM of IAA or 1.35 µM of NAA. Addition of IAA in BAP containing medium was beneficial while, addition of NAA had inhibitory effect and resulted in low regeneration from all the explants. Enhanced shoot formation was achieved in BAP and IAA combinations. Cultures maintained in MS basal medium containing 2.2 µM BAP + IAA 1.425 µM, shoot formation was increased to around 50 to 100% (Table 1). It is clearly evident from the tabulated data (Table 1) that an average of 341.73 shoots per explant was recorded in epicotyl cultures against 211 shoots per explant when the epicotyls were cultured in BAP supplemented medium alone. Such enhanced shoot production also observed in cotyledon, hypocoty and root cultures (Table 1). After 4 weeks of culture initiation medium, the cultures were sub-cultured on to the fresh medium of same strength for further shoot elongation. After 4 weeks of transfer to fresh medium, the cultures formed compact clumps of shoots especially from epicotyl explants.

Adventitious root induction

Well developed elongated shoots (3 to 4 cm) regenerated from different explant source were excised and transferred to half-strength MS medium supplemented with a single auxin, namely, IAA, IBA, or NAA for root induction. Shoots cultured on an auxin-free medium (control) failed to form roots. Depending on the auxin type and concentration, root numbers ranging from 1 to 6 were

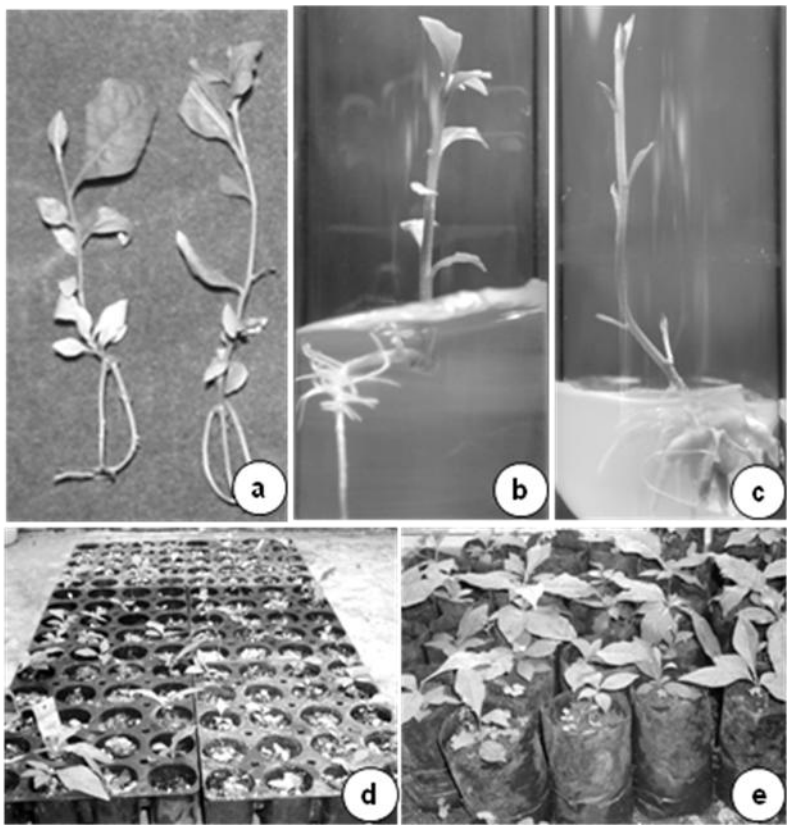

Figure 2. *In vitro* rooting of micro shoots and transplantation of *A. marmelos* plantlets. (a) Microshoot rooted on ½ strength MS + 2.85 µM of IAA (after 5 weeks of culture). (b) Microshoot rooted on ½ strength MS + 9.8 µM of IBA (after 5 weeks of culture). (c) Microshoot cultured on ½ strength MS + 5.4 µM of NAA showing callused base with spongy roots (after 5 weeks of culture). (d) Rooted plantlets planted in plastic root trainer containing pre-soaked vermiculite (3 week after transfer). (e) Plants transferred to the polybags containing soil medium (soil + Farm Yard Manure) showing normal growth.

observed in the auxin supplemented medium. Among the three auxins (IAA, IBA and NAA) tested for rooting, IAA responded better than the other two auxins. Roots were initiated between 10 and 20 days after culture and in majority of cases, one or two roots were produced. Concentration of auxin played a significant role in root formation. Shoots cultured on 2.85 µM IAA, 5.4 µM NAA and 14.7 µM IBA supplemented medium showed a maximum of 93.3, 66.6 and 70% rooting response with an average root of 2.07, 1.8 and 2.6 per shoot, respectively. As the concentration increased, there was decline in percent response and root number. The medium fortified with 2.85 µM IAA showed the highest rooting response, where about 93.3% cultures responded for rooting with a mean root numbers of 2.7 and an average length of 2.2 cm (Figure 2a). IBA was not effective at lower concentrations (2.45 to 9.8 µM), but at a higher concentration, that is, 14.7 µM concentration was effective in producing healthy roots (Figure 2b). Shoots incubated in NAA augmented MS basal medium developed callus at the base and swelled a little and latter roots emerged from the swelled region (Figure 2c). NAA induced roots were shorter and thick as compared to IAA and IBA induced roots. Root length was significantly affected by the type of auxin and concentration. Shoots cultured in NAA showed comparatively poor root growth. Although the number of roots produced per shoot was higher in the NAA-supplemented medium, the rooting was accompanied by callus; furthermore, roots in such cultures were short and no further elongation was observed. Shoots cultured in IAA or IBA enriched medium showed elongated roots without any intervening callus (Table 2).

Rooted plantlets were taken out from the culture tubes, washed thoroughly to remove any remains of medium, and planted in plastic root trainer containing pre-soaked vermiculite (Figure 2d). The plantlets were maintained inside a mist chamber for a period of 3 weeks and were subsequently transferred to the polybags containing soil medium (soil + farm yard manure) and the plants showed

Table 2. Effect of different auxins in MS basal medium on root induction on excised in vitro shoots of *A. marmelos* (after 5 weeks of incubation)

MS + PGRs (µM)	Days to root initiation	Response (%)	Mean root numbers	Mean root length (cm)
IAA (1.425)	12-14	40	0.73^{fgh}	0.9^{fg}
IAA (2.85)	10-12	93.3	2.07^{b}	2.2^{a}
IAA (5.7)	10-12	60	1.27^{de}	1.5^{cde}
IAA (11.4)	12-14	40	0.8^{efgh}	0.9^{fg}
IBA (1.225)	-	-	-	-
IBA (2.45)	12-14	33.3	0.53^{gh}	0.62^{gh}
IBA (4.9)	12-14	40	0.9^{defgh}	0.9^{fg}
IBA (9.8)	12-14	46.6	1.2^{def}	1.4^{de}
NAA (1.35)	-	-	-	-
NAA (2.7)	16-20	50	2.17^{ab}	0.92^{fg}
NAA (5.4)	18-20	66.6	2.6^{a}	1.6^{cd}
NAA (10.8)	18-20	63.3	1.93^{b}	1.17^{ef}

Data pooled from three independent experiments each with 10 replicates per treatment. *Mean values within column followed by the same letter are not significantly different ($p \leq 0.05$; Duncan's New Multiple Range Test).

normal growth with 90% survival (Figure 2e).

DISCUSSION

In the present study, plant regeneration has been achieved from diverse explants such as epicotyl, cotyledon, hypocotyl and root explants of *A. marmelos*. In our previous study (Nayak et al., 2007) a protocol was provided for high frequency plant regeneration from cotyledonary nodes of *A. marmelos*. Since only one cotyledonary node explants can be obtained from each axenic seedling, alternative method to obtain more number of explants from single seedling was tried. The adventitious bud formation efficiency of cultured explants showed varied response and seems to be dependent more precisely on the explant type as well as on the plant growth regulator treatments. The epicotyl segment was the best source of explant for multiple shooting in all the media composition tested in comparison to other explants used. Successful plant regeneration from epicotyl explants have been reported in citrus (Costa et al., 2004) and neem (Salvi et al., 2001). It was observed that epicotyl segments that were reared close to cotyledonary node region resulted in the highest number of shoot production than the segment away from the node. Our finding is in close conformity with the result obtained in *Troyer citrange* (Garcia-Luis et al., 1999; Moreira-Dias et al., 2011). The fact may be attributed to increased enzymatic activity at the cotyledon node zone as observed in case of bean (Davis, 1983). Next to epicotyl cultures, cotyledon cultures showed better response over hypocotyl and root cultures. Successful shoot regeneration from cotyledon segments of *A. marmelos* has been reported earlier (Hazeena and Sulekha, 2008; Puhan and Thirunavoukkarasu, 2011). They could obtain an average shoots of 40 as the maximal response which is less than the shoots obtained in the present studies where one could get an average of 56.3 shoots per cotyledon explant.

Between the two cytokinins tested, BAP showed better shoot induction capacity than the Kin. It is evident from the observed data that explants cultured in the BAP enriched medium, has a response around 98 against 85% response in Kin supplemented medium. It may be possible that BAP is more effective than Kin in switching on endogenous cytokinin production (Vankova et al., 1991). Positive effect of BAP over other cytokinins in inducing multiple shoots have been realized in many tree species (Costa et al., 2004; Figueiredo et al., 2001; Walia et al., 2003; Nayak et al., 2010). Concentration of cytokinin also affected the shoot induction potential in *A. marmelose* cultures. Optimal concentration of BAP for effective shoot induction varied with the type of explants used. For instance, 2.2 µM BAP was the best regeneration medium among the singly used cytokinins for epicotyl, root and hypocotyl explants, but cotyledon explants exerted best response at 4.4 µM concentration. Shoot induction efficiency was declined with an increase in concentration of BAP. This is in accordance with the results obtained by Scaria et al. (1993) who have observed that when the stem explants of *A. marmelos* cultured in varied concentration of BAP, there was steady increase in shoot number production from 0.44 to 4.4 µM concentration. Further increase in concentration of BAP resulted in sharp decline in shoot number production. Also, a lower dosages of cytokinins resulted in increased shoot length, while higher strength reduced the shoot length. This study suggest that high concentration of BAP after bud initiation was not essential for shoot development due to the reduction in the number of shoots and high incidences of abnormality. In support of

our findings, there are numerous reports which show the impact of using high level of cytokinin that produces abnormality and affects its genetic variability (Martin et al., 2006; Shirani et al., 2009).

MS basal medium with cytokinin and auxin enhanced the regeneration potential in a number of tree species (Behera et al., 2008; Thirunavoukkarasu et al., 2006) including in *A. marmelos* cultures (Nayak et al., 2007). In the present studies also, synergetic effect of auxin and cytokinin had pronounced effect on shoot bud induction. It is obvious from the tabulated data that when epicotyls cultured on to MS basal medium with BAP (2.2 µM) supplement alone produced an average of 211 shoots, while addition of IAA (1.425 µM) enhanced the shoot production capacity to 343. Addition of NAA to the best regeneration medium (BAP 2.2 µM) resulted in reduction in shoots numbers. This is in corroboration with the results obtained in some tree species, namely, *Psidium guajava* (Singh et al., 2002), *Gmelina arborea* (Behera et al., 2008), including *A. marmelos* (Ajithkumar and Seeni, 1998).

Throughout root induction studies, half strength MS basal medium was used due to the fact that low salt concentrations promote rooting in many woody plants (Nemeth, 1986). An auxin supplement is an essential factor for obtaining rooted shoots in the present investigation. MS basal medium with an auxin did not support rooting on the cultured micro shoots of *A. marmelos*. Concentration and type of auxin in the medium was found to be the critical factor in producing healthy roots. Among the three auxins (IAA, NAA, IBA) used in the present studies, IAA favored the best rooting response (93.3%) at 2.85 µM concentration. Increasing the concentration of IAA, there was decline in rooting response, root number and root length. The highest response of IBA and NAA were 70 and 66.6%, respectively. Delayed rooting and rigorous callus development at the base of the shootlets along with spongy roots was seen in the medium supplemented with NAA. A similar factor also reported in *Ruta graveolens* wherein micros hoots implanted in NAA containing medium resulted in massive callus at the bottom with tiny spongy roots (Bohidar et al., 2008).

In the present study, a protocol has been worked out for adventitious shoot regeneration from epicotyl, hypocotyl, cotyledon and root segments from axenic seedling explant of *A. marmelos* which can be utilized for enhanced production of this highly important medicinal plant and also for genetic transformation studies.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. B. K. Mishra, Director, CSIR-Institute of Minerals and Materials Technology, Bhubaneswar for facilities and support. The Department of ISM, Ministry of Health and Family Welfare, Government of India is duly acknowledged for sponsoring the project.

REFERENCES

Ajithkumar D, Seeni S (1998). Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr. a medicinal tree. Plant Cell Rep. 17:422-426.

Arul V, Miyazaki S, Dhananjayan R (2005). Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* Corr. J. Ethnopharmacol. 96(1-2):159-163.

Behera PR, Nayak P, Thirunavoukkarasu M, Sahoo S (2008). Plant regeneration of *Gmelina arborea* Roxb. from cotyledonary node explants. Indian J. Plant Physiol. 13(3):258-265.

Bhati R, Shekhawat NS, Arya HC (1992). In vitro regeneration of plantlets from root segments of *Aegle marmelos*. Indian J. Exp. Biol. 30:844-845.

Bohidar S, Thirunavoukkarasu M, Rao TV (2008). Propagation of *Ruta graveolens* L. by *in vitro* culture of nodal explants. Indian J. Plant Physiol. 13(2):125-129.

Costa MGC, Alves VS, Lani ERG, Mosquima PR, Carvalho CR, Otoni WC (2004). Morphogenic gradients of adventitious bud and shoot regeneration in epicotyl explants of *Citrus*. Sci. Hort. 100:63-74.

Davis BD (1983). Effects of sugars on α-amylase activity in Pea embryonic axes. Am. J. Bot. 70(6):821-826.

Figueiredo SF, Albarello N, Viana VR (2001). Micropropagation of *Rollina mucosa* (Jacq.) Baill. In Vitro Cell. Dev. Biol. Plant 37(4):471-475.

Garcia-Luis A, Bordon Y, Moreira-Dias JM, Molina RV, Guardiola JL (1999). Explant orientation and polarity determine the morphogenic response of epicotyl segments of *Troyer citrange*. Ann. Bot. 84:715-723.

Gomez KA, Gomez AA (1984). Statistical Procedure for Agricultural Research, John Wiley & Sons, New York. p.680

Haravey SK (1968). A preliminary communication of the action of *Aegle marmelos* (Bael) on heart. Indian J. Med. Res. 56:327.

Hazeena MS, Sulekha GR (2008). Callus induction and regeneration in *Aegle marmelos* (L.) Corr. using cotyledon explant. J. Trop. Agric. 46(1-2):79-84.

Hossain M, Islam R, Islam A, Joarder OI (1995). Direct organogenesis in cultured hypocotyl explants of *Aegle marmelos* Corr. Plant Tiss. Cult. 5(1):21-25.

Hossain M, Islam R, Karim MR, Joarder OI, Biswas BK (1994). Regeneration of plantlets from *in vitro* cultured cotyledons of *Aegle marmelos* Corr. (Rutaceae). Sci. Hort. 57(4):315-321.

Hossain M, Karim MR, Islam R, Joarder OI (1993). Plant regeneration from nucellar tissues of *Aegle marmelos* through organogenesis. Plant Cell Tiss. Org. Cult. 34:199-203.

Islam R, Hossain M, Karim M.R, Joarder OI (1995). Regeneration of *Aegle marmelos* (L.) Corr. Plants in vitro from callus cultures of embryonic tissues. Curr. Sci. 69(6):494-495.

Jauhari OS, Singh RD, Awasthi RK (1969). Survey of some important varieties of Bael (*Aegle marmelos* Correa). Punjab Hort. J. 9:48.

Martin KP, Pachathundikandi S, Zhang CL, Slater A, Madassery J (2006). RAPD analysis of a variant of banana (*Musa* sp.) cv. grande naine and its propagation via shoot tip culture. *In vitro* Cell. Dev. Biol. Plant 42:188–192.

Mishra BB, Singh DD, Kishore N, Tiwari VK, Tripathi V (2010). Antifungal constituents isolated from the seeds of *Aegle marmelos.* Phytochemistry 71:230-234.

Moreira-Dias JM, Molina RV, Guardiola JL, Garcia-Luis A (2001). Day length and photon flux density influence the growth regulator effects on morphogenesis in epicotyl segments of *Troyer citrange*. Sci. Hort. 87:275-290.

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

Nayak P, Behera PR, Thirunavoukkarasu M (2007). High frequency plantlet regeneration from cotyledonary node cultures of *Aegle marmelos* (L.) Corr. In Vitro Cell. Dev. Biol. Plant 43:31-236.

Nayak P, Behera PR, Thirunavoukkarasu M. Chand PK (2010). High frequency plant regeneration through adventitious multiple shoot organogenesis in epicotyl explants of Indian goose berry (*Emblica*

officinalis Gaertn). Sci. Hort. 123: 473-478.
Nemeth G (1986). Induction of rooting. In: Bajaj YPS (ed.) Biotechnology in Agriculture and Forestry 1, Tree 1, Springer, Berlin. pp. 49-86.
Phuwapraisirisan P, Puksasook T, Jong-aramruang J, Kokpol U (2008). Phenylethyl cinnamides: A new series of α-glucosidase inhibitors from the leaves of *Aegle marmelos.* Bioorg. Med. Chem. Lett. 18:4956-4958.
Ponnachan PTC, Paulose CS, Panikkar KR (1993). Hypoglycemic effect of alkaloid preparation from leaves of *Aegle marmelos*. Amala Res. Bull. 13:37-41.
Puhan P, Thirunavoukkarasu M (2011). Direct organogenesis of *Aegle marmelos* (L.) Corr. from cotyledon segments. Af. J. Biotechnol. 10(82):18986-18990.
Salvi NX, Hemraj S, Suchita T, Eapen S, Singh H, Tivarekar S (2001). Plant regeneration from different explants of neem. Plant Cell Tiss. Org. Cult. 65:159-162.
Satyavati GV, Raina MK, Sharma M (1976). Medicinal Plants of India. Vol. 1, Indian Council of Medical Research, New Delhi, India.
Scaria KV, Inamdar JA, Kalia K, Subramanian RB, Nataraj M (1993). Micropropagation of *Aegle marmelos* (L) Corr. Phytomorphology 43(1-2):87-92.
Shirani S, M Fatemeh, Maziah M (2009). Morphological abnormally among regenerated shoots of banana and plantain (*Musa* sps.) after in vitro multiplication with TDZ and BAP from excised shoot tips. Afr. J. Biotechnol. 8:5755-5761.
Shoba FG, Thomas M (2001). Study of anti-diarrhoeal activity of four medicinal plants in castor oil induced diarrhoea. J. Ethnopharmacol. 76:73-76.
Singh SK, Meghwal PR, Sharma HC, Singh SP (2002). Direct shoot regeneration on hypocotyls explants *in vitro* germinated seedlings of *Psidium guajava* L. cv. Allahabad Safeda. Sci. Hort. 95:213-221.
Thirunavoukkarasu M, Parida S, Rath SP, Behera A (2006). Micropropogation of *Enterolobium cyclocarpum* (Jacq.) Griseb. J. Sustain. For. 23(4):1-12.
Vankova R, Hsiao KC, Bornman CH, Gaudinova A (1991). Effects of synthetic cytokinins on levels of endogenous cytokinins and respiration patterns of *Beta vulgaris* cells in suspension. J. Plant Growth Regul. 10:197-200.
Walia N, Sinha S, Babbar SB (2003). Micropropagation of *Cratevia nurvala.* Biol. Plant 46(2):181-185.