IN VITRO PROPAGATION AND BIOCHEMICAL ANALYSIS OF FIELD ESTABLISHED WOOD APPLE (*Aegle marmelos* L.)

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Abstract. This paper describes an improved and rapid protocol for multiple shoot regeneration from nodal segments of wood apple (*Aegle marmelos* L.), a medicinal tree, cultured on Murashige and Skoog (1962) (MS) medium supplemented with various concentrations of auxins and cytokinins individually and in various combinations. BAP was found to be more effective than kinetin for shoot multiplication. Nodal explants responded most favorably at low BAP (2.0 mg/l) producing maximum number of shoots (8.0) and uniform shoots facilitating their simultaneous harvest for rooting. The medium supplemented with 2.0 mg/l BAP + 1.0 mg/l IAA was found to be most prolific combination of the treatments with regard to number and length of shoots. Creamish friable compact callus accompanying multiple shoots (8.0) was achieved from nodal segment on MS medium fortified with 2.0 mg/l BAP + 0.5 mg/l 2,4-D within 8 days of culture. The *in vitro* regenerated shoots were rooted best in half strength MS medium enriched with 1.0 mg/l IAA. The rooted plantlets were successfully established with 60% survival. Besides that the biochemical parameters, like chlorophyll, total sugars, reducing sugars and proteins were estimated in leaf tissue from both *in vivo* and *in vitro* raised plants in order to establish the sustainability of plants.

Keywords: Biochemical analysis, Multiple shoots, Nodal segments, *Aegle marmelos*, Auxins, Cytokinins.

Abbreviations: BAP - Benzyl amino purine; **2,4-D -** 2,4-Dichlorophenoxyacetic acid; **MS -** Murashige and Skoog (1962) basal medium; **NAA -** *a*-naphthalene acetic acid; **IBA -** Indole-3-butyric acid; **IAA -** Indole acetic acid.

INTRODUCTION

Micropropagation of mature trees has been a difficult task due to various factors like exogenous and endogenous infection, presence of phenolic compounds, long complex life cycles, great genetic variations, etc. [4, 43]. It is well established that *in vitro* propagation of plant species is influenced by various factors, like genotype, age and source of initial tissue [10].

Aegle marmelos (L.) Corr., (Rutaceae) is a popular medicinal plant in the Ayurvedic and Siddha systems of medicines used to treat a wide variety of ailments. In India, this plant is known as "Bael Tree". It is mostly found in tropical and subtropical region. The tree grows wild in dry forests on hills and plains of central and southern India, Burma, Pakistan, Bangladesh, Sri Lanka, Northern Malaya, Java and Philippine Islands [19]. It is medium sized tree having profuse dimorphic branched, alternate, trifoliate, deep green leaves, membranous leaflets, large sweet scented, greenish white flowers, large, globose fruits [33]. It flowers from May to July and yields an average of 350-400 fruits (200-250 kg) per tree [27].

Almost all parts of the tree are used in preparing herbal medicine [21].The roots are useful for treating diarrhea, dysentery, and dyspepsia [27]. The aqueous extracts of the stem and root bark are used to treat malaria, fever, jaundice, and skin diseases such as ulcers, urticaria, and eczema [30]. In pharmacological trails, both the fruit and root showed showed antiamoebic and hypoglycaemic activities [32]. The plant is rich in alkaloids, among which aegline, marmesin, marmin, and marmelosin are the major ones. The compounds luvangetin and pyranocoumarin, isolated from seeds showed significant antiulcer activity [12]. Essential oil isolated from the leaf has antifungal activity [35].

It is an out breeder, and is routinely propagated by seeds for cultivation in northern India [38]. As seed progeny are not uniform, no standard varieties are available. Seeds have short viability and are prone to insect attack. Vegetative propagation through root suckers is slow, difficult and cumbersome [1].

Explants collection season is one of the most important factor in establishment and growth of *in vitro* cultures [5, 31]. The position of node of the explant also plays an important role in determining the growth and differentiation of cultures [41].

MATERIALS AND METHODS

Explants of different types- shoot tips (0.5 cm), internodal and nodal segments (0.5-1.0 cm) excised from the 10 year old tree growing in Herbal Garden of Botany Department, Kurukshetra University, Kurukshetra. All the explants were washed with liquid detergent for 10 min and under running tap water for 15 min to remove dust particles. They were then immersed for 2 min in a solution containing 50 mg/l each of ascorbate and citrate to check phenolic exudation, and surface sterilized by passage through 15% (vol/vol) Steriliq for 10 min and 0.1% (vol/vol) $HgCl_2$ for 8 min under aseptic conditions. After this these explants were then thoroughly washed 4-5 times with sterilized double distilled water to remove the traces of mercuric chloride. The nodal segments (1.0-1.5 cm), after trimming the ends, were finally inoculated on MS medium [30] supplemented with various concentrations (0.5-2.0 mg/l) of auxins (IAA, NAA, 2,4-D and IBA) and cytokinins (BAP and Kn) alone and in various combinations for shoot regeneration and callus induction.

The cultures were incubated at a temperature of 25±2ºC and a photoperiod of 16hrs light (intensity of 2000 lux) and 8hrs of dark.

Visual observations like callus induction, growth of callus, number of days taken for bud break, percentage of bud break and number of shoots regenerated per explants were recorded regularly. A mean of 20 replicates was taken per treatments.

The nodal segments containing mature calli were further subcultured on MS medium supplemented with different concentrations (0.5-2.0 mg/l) of BAP and Kn individually and in various combination with IAA and NAA for more callus induction and shoot differentiation.

The *in vitro* raised shoots (2.5-4.0 cm long) were excised and transferred to cultural tubes containing full and half strength MS medium fortified with IBA, IAA and NAA under aseptic conditions for rooting.

The shoots with well developed roots were gradually pulled out from the medium and immersed in water to remove the remains of agar-agar particles sticking to the root system by using a fine brush. These plantlets were transferred to pots containing mixture of sterilized soil, sand and compost (1:1:1). The potted plantlets were covered with a transparent polythene bag to ensure high humidity around the plants. The pots were supplied with MS (half strength) salt solution on alternate days. After about two weeks the polythene bags were removed for 3-4 hours daily to expose the plants to the conditions of natural humidity for acclimatization. These plants were shifted to bigger pots after one month of its transfer and were maintained under green house conditions.

Different biochemical parameters viz. chlorophyll, total sugars, reducing sugars and proteins were estimated in Leaf tissue from *in vivo* and *in vitro* grown plants (21days after *ex vitro* transfer) were taken for biochemical analysis. The analyses were conducted at Plant Physiology Laboratory, Department of Botany, Kurukshetra University, Kurukshetra, India. The content of chlorophyll (a, b and total) was measured according to (Arnon, 1949) [2], reducing and total sugars according to (Hart and Fisher, 1971) [15] and proteins by method of Bradford (1976) [6].

Statistical analysis was done by using the formula:

$$SE = \pm \sqrt{\frac{(X^2)}{n(n-1)}}$$

where:

SE = Standard error;

X = Deviation of mean;

n = Number of replicates [39].

RESULTS

In the present study 4-8 nodal stem segment proved to be the best explants where most of explants showed bud burst in minimum 8 days (Fig 1a). Bud burst frequency reduced and time taken for bud induction enhanced with hard and brown explants from 10- 16 nodal segment.

Direct organogenesis

The apex portion of stem i.e. shoot tip did not respond in culture due to very soft and succumbs toxic effects on sterilents. Nodal stem segment were found to be best explants.

MS basal medium devoid of growth regulators served as control. No bud break was achieved on MS medium devoid of growth regulators. Nodal explants responded to all the concentrations of BAP (0.5-4.0 mg/l) tried. MS medium fortified with 2.0 mg/l BAP supported better results as compared to other concentrations of BAP in terms of period required for bud break, percent bud break, number of shoots regenerated and shoot length (Table 1) (Fig 1a). In case of Kn, maximum numbers of shoots (7.2) per explant was recorded in the medium fortified with 2.0 mg/1 (Fig 1b).

A combined effect of different cytokinins (BAP and Kinetin) and auxins (IAA and NAA) in various combination on the nodal explants was also studied (Table 1). The percent bud break decreases with increase in the concentration of IAA and NAA from 0.5 mg/l to 2.0 mg/l. Among the various media fortified with different concentrations of BAP and IAA, 2.0 mg/l BAP + 1.0 mg/l IAA was proved better in terms of percent bud break and number of shoots differentiated per explant (Fig 1c).

Supplementation of Kn in place of BAP with IAA did not make much difference. In case of Kn + NAA or IAA supplemented media, the medium with Kn (2.0 mg/l) + IAA (0.5 mg/l) showed fifty percent bud break after 10 days of inoculation with (6.3) numbers of shoots per explant (Table 1). It has been found that increase in concentration of IAA resulted in reduction in the number of shoots per explant. Among the three concentrations of NAA applied with BAP and Kn, most favorable results in terms of bud break were obtained on the MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA.

Indirect organogenesis

For callus induction, nodal and internodal stem segments were cultured on MS medium supplemented with different concentrations (0.5-4.0 mg/l) of BAP and 2,4-D. Callus induction was observed within eight to nineteen days of culture from the cut surface of the explants (Fig 1d). BAP was more effective than 2,4-D for callus formation. MS medium fortified with 2.0mg/l BAP resulted in eighty percent callus formation after 10 days of inoculation (Table 2). In case of 2,4-D, medium fortified with 2.0 mg/l 2,4-D resulted in seventy percent callus formation after 12 days of inoculation. In these treatments the induced calli were Creamish in colour and structurally nodular. Shoot regeneration with callus was also noticed at higher concentration of BAP (2.0 and 4.0 mg/l) (Fig 1e).

The nodal explants favoured shoot induction along with callus formation on MS medium supplemented in

combinations of BAP with 2,4-D (Table 3; Fig 1f). The highest number of shoots per callus was 8.0 in MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l 2,4-D followed by 7.0 in MS medium having 2.0 mg/l BAP + 1.0 mg/l 2,4-D.

Table 1. Effect of cytokinins and auxins supplemented individually and in various combinations on nodal segments of *Aegle armelos*.

Auxins/ cytokinins (mg/l)	Concentration of plant growth regulators (mg/l)	bud break (%)	Number of days required for bud break	Number of shoots (Mean±SE)	Shoot Length(cm) (Mean ± SE)
Control	-	-	-	-	-
BAP	0.5	40	12	3.5 ± 0.75	2.7 ± 0.64
	1.0	50	10	7.0 ± 0.81	3.8 ± 0.20
	2.0	80	08	8.0 ± 0.81	4.2 ± 0.25
	4.0	40	10	4.1 ± 0.99	2.0 ± 0.25
Kn	0.5	40	15	3.0 ± 0.74	2.4 ± 0.15
	1.0	50	11	6.8 ± 0.93	2.7 ± 0.29
	2.0	70	09	7.2 ± 0.97	3.3 ± 0.28
	4.0	50	10	4.4 ± 0.96	2.0 ± 0.14
MS+Kn+ IAA	2.0 + 0.5	50	10	6.3 ± 0.74	3.1 ± 0.22
	2.0 + 1.0	50	12	5.8 ± 0.78	2.4 ± 0.21
	2.0 + 2.0	20	15	2.2 ± 0.50	2.1 ± 0.08
MS+Kn+ NAA	2.0 + 0.5	50	14	2.7 ± 0.46	2.3 ± 0.15
	2.0 + 1.0	40	15	2.3 ± 0.82	2.2 ± 0.28
	2.0 + 2.0	20	16	2.3 ± 0.74	2.1 ± 0.05
MS+BAP+ IAA	2.0 + 0.5	50	11	6.0 ± 0.70	3.2 ± 0.29
	2.0 + 1.0	60	09	7.1 ± 0.87	2.3 ± 0.41
	2.0 + 2.0	30	12	4.3 ± 0.81	2.2 ± 0.08
MS+BAP+ NAA	2.0 + 0.5	50	10	3.2 ± 0.88	2.5 ± 0.46
	2.0 + 1.0	40	12	2.1 ± 0.64	2.4 ± 0.27
	2.0 + 2.0	40	15	2.2 ± 0.63	2.2 ± 0.27

(–) No Response
*Data based on 20 explants per treatment and taken after 28 days of culture

Figure 1. *In vitro* propagation of *Aegle marmelos*. **(a)** Shoot bud initiation on MS medium + BAP (2.0 mg/l); **(b)** Shoot formation from nodal explant on MS medium + Kn (2.0 mg/l); **(c)** Shoot formation from nodal explant on MS medium with 2.0 mg/l BAP + 1.0 mg/l IAA; **(d)** Callus initation from internodal segment on MS medium with 2,4-D (2.0 mg/l); **(e)** Callus initation from nodal explant on MS medium with BAP (2.0 mg/l); **(f)** Callus induction along with shoot formation from nodal explant on MS medium with 2.0 mg/l BAP+ 1.0 mg/l 2,4-D; **(g)** Shoot regeneration and callus induction from old nodal segments containing mature calli, when subcultured on MS medium with BAP (2.0 mg/l) + IAA (0.5 mg/l); **(h)** *In vitro* rooting on MS medium half strength + IAA (1.0 mg/l).

Table 2. Effect of cytokinins and auxins supplemented individually on callus formation on internodal explants of *Aegle marmelos*.

Media Composition	Concentration of plant growth regulators (mg/l)	No. of days required for callus induction	Callus induction (%)	Nature of callus	Callus growth
MS control	–	–	–	–	–
MS + BAP	0.5	19	20	Creamish friable, Compact	C+
	1.0	16	40	Creamish friable, Compact	C+
	2.0	10	80	Creamish friable, Compact	C+++
	4.0	12	60	Creamish friable, Compact	C++
MS + 2,4-D	0.5	18	20	Creamish friable, Compact	C+
	1.0	17	30	Creamish friable, Compact	C+
	2.0	12	70	Creamish friable, Compact	C++
	4.0	14	50	Creamish friable, Compact	C++

(–) No Response, (C+) Poor growth, (C++) Moderate growth, (C+++) Good growth.
*Data based on 20 explants per treatment and taken after 28 days of culture

Table 3. Effect of various combinations of BAP with 2,4-D on callus formation and shoot regeneration on nodal explants of *Aegle marmelos.*

Media Composition	Concentration of plant growth regulators (mg/l)	No. of days required for callus induction	Derived callus shoots (%)	Mean no. of shoots	Length of shoots (cm)
MS + BAP + 2,4-D	1.0 + 0.5	8	70	4.2 ± 0.63	4.0 ± 0.29
	1.0 + 1.0	8	50	4.1 ± 0.60	3.4 ± 0.37
MS + BAP + 2,4-D	2.0 + 0.5	8	80	8.0 ± 0.81	5.4 ± 0.39
	2.0 + 1.0	9	50	7.0 ± 0.87	5.0 ± 0.73

(–) No Response, (C+) Poor growth, (C++) Moderate growth, (C+++) Good growth.
*Data based on 20 explants per treatment and taken after 28 days of culture

The 3-4 weeks old nodal segments containing mature calli were further subcultured on MS medium supplemented with different concentrations (0.5-2.0 mg/l) of BAP and Kn individually and in various combination with IAA and NAA for more callus induction and shoot differentiation. Cultures maintained on Kn supplemented medium showed no response. While cultures maintained on higher concentration of BAP supplemented medium showed poor response. The medium supplemented with BAP (2.0 mg/l) + IAA (0.5 mg/l) showed new shoots formation and callus induction after 10-15 days (Fig. 1g).

Rooting of in vitro regenerated shoots

The medium without growth regulators failed to initiate roots. Better rooting was supported by the MS medium fortified with 1.0 mg/l IAA (Table 4; Fig 1h).

Acclimatization and Transfer of plantlets to the soil

Complete regenerated plantlets with sufficient roots were taken out from the culture tubes and transferred to small earthen pots containing mixture of sterilized soil, sand and compost (1:1:1). Sixty percent of the regenerants survived well.

Table 4. Root formation on different media composition in *Aegle marmelos*.

Media composition (mg/l)	Days required for root induction	% age of Root formation	Remarks
MS full strength without growth regulators	–	–	–
MS half strength without growth regulators	–	–	–
MS half strength + 0.5 mg/l IBA	–	–	–
MS half strength + 1.0 mg/l IBA	–	–	–
MS half strength + 2.0 mg/l IBA	22-25	05	Short, Healthy
MS half strength + 0.5 mg/l NAA	20-22	20	Long, Thin
MS half strength + 1.0 mg/l NAA	18-20	30	Long, Thin
MS half strength + 2.0 mg/l NAA	20	20	Long, Thin
MS half strength + 0.5 mg/l IAA	18	50	Long, Thin
MS half strength + 1.0 mg/l IAA	16-18	60	Long, Thin
MS half strength + 2.0 mg/l IAA	17	40	Long, Thin

(-) No Response
*Data based on 20 explants per treatment and taken after 28 days of culture

Table 5. Comparison of various biochemical contents (mg/g) between *in vivo* and *in vitro* regenerated leaves of *Aegle marmelos*.

Biochemical Parameters	Sample 1	Sample 2
Chlorophyll (a+b)	0.104 (0.039 + 0.065)	0.296 (0.110 + 0.86)
Total Sugars	2.10	3.53
Reducing Sugars	1.92	2.24
Proteins	0.63	0.88

Sample 1: Leaves sample collected from *in vitro* raised plants.
Sample 2: Leaves sample collected from *in vivo* plants.

Biochemical analysis of leaf extract

Different biochemical parameters viz. chlorophyll, total sugars, reducing sugars and proteins were higher in the leaves of *in vivo* grown plants than those in *in vitro* regenerated leaves (Table. 5).

DISCUSSION

Explant collected during May- June was found to be ideal because 80% explant shows *in vitro* bud burst and minimum infection where as the bud burst frequency reduced in other months.

Nodal stem segment were found to be best explant due to the presence of protected axillary buds, which during surface sterilization do not get damaged as also suggested by Ajithkumar and Seeni [1].

No bud break was achieved on MS medium devoid of growth regulators. Similarly no shoot buds developed on MS basal medium in *Peganum harmala* [36], *Celastrus paniculatus* [23].

As compared to the BAP treatment, relatively lower responses in terms of period required for bud break and number of shoots differentiated per explant were observed in media supplemented with kinetin. This observation is contradictory to the report by Ajithkumar and Seeni [1] who found both BAP and Kn were equally effective for this plant. The effectiveness of BAP on the induction of bud break and shoot proliferation has been reported in *Vigna radiata* [40]. Regeneration of shoots in response to Kn has been observed in *Kaempferia galangal* [7] and *Phyllanthus niruri* [22]. The present study showed that lower concentration of BAP was sufficient for shoot multiplication in this plant. The use of comparatively lower concentration of growth regulator in present protocol is an important factor worth mentioning, as it minimizes the risk of producing genetically altered individuals [8].

The combination of IAA with cytokinins (BAP or Kn) promoted shoot formation in various plant species like *Vigna radiata, Saussurea involucrata, Vitex negundo* [42, 13, 20].

Presence of 2,4-D has been shown to be essential for callus formation in *Capsicum annum* [14]. Callus formation along with shoot induction was also reported in several medicinal plant species including *Carica papaya* [18], *Amorphophallus albus* [17].

The 3-4 weeks old nodal segments containing mature calli were further subcultured on MS medium supplemented with different concentrations (0.5-2.0 mg/l) of BAP and Kn individually and in various combination with IAA and NAA for more callus induction and shoot differentiation. The medium supplemented with BAP (2.0 mg/l) + IAA (0.5 mg/l) showed new shoots formation and callus induction after 10-15 days. Such variability has also been reported in *Heracleum candicans* [37].

Effectiveness of IAA for root induction was also reported in various types of plants, namely *Rotula aquatica* Lour. [25], *Tylophora indica* [9].

Similar mixture of sterilized soil, sand and compost (1:1:1) composition was used to acclimatize *Celastrus paniculatus* [24], *Dalbergia latifolia* [34], *Dendrocalamus asper* [3]. Further hardening and acclimatization procedures for establishment of micropropagated plantlets were also developed for many species such as *Salvadora persica* [26], *Peganum harmala* [11].

Acknowledgements. The authors are grateful to University Grants Commission, New Delhi for financial assistance and to Kurukshetra University, Kurukshetra for providing laboratory facilities to carry out the work.

REFERENCES

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

[2] Arnon, D.I., (1949): Copper enzymes in isolated chloroplasts polyphenol-oxidase in *Beta vulgari.* Plant Physiology. 24: 1-15.

[3] Arya, I.D., Arya, S., (1997): *In vitro* culture and establishment of exotic bamboo *Dendro calamus asper*. Indian Journal of Experimental Biology, 35: 1252-1255.

[4] Bajaj, Y.P.S., (1997): Medicinal and Miscellaneous Plants. In: *Biotechnology in Agriculture and Forestry.* VI. Springer-Verlag, Berlin. pp. 313-391.

[5] Bhatt, I.D., Dhar, U., (2004): Factors controlling micropropagation of *Myrica esculenta* buch-Ham. ExD.Don: a high value wild edible of Kumaun Himalaya. African Journal of Biotechechnology, 3: 534-540.

[6] Bradford, M.M. (1976): A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Annals of Biochemistry. 72: 248-54.

[7] Chirangini, P., Sinha, S.K., Sharma, G.J., (2005): *In vitro* propagation and microrhizome induction in *Kaempferia galangal* Linn. and *K. rotunda* Linn. Internet Journal of Biotechnology, 4: 404-408.

[8] Edson, J.L., Leege Brusven, A.D., Everett, R.L., Wenny, D.L., (1996): Minimizing growth regulators in shoot cultures of an endangered plant, *Hackelia venusta* (Boraginaceae). *In vitro* Cell Developmental Biology Plant, 32: 267-271.

[9] Faisal, M., Ahmad, N., Anis, M., (2007): An efficient micropropagation system for *Tylophora indica:* an endangered, medicinally important plant. *Plant Biotechnology Reports,* 1: 155-161.

[10] George, E.F., (1993): Plant propagation by Tissue Culture. Part I: The Technology. Exegetics Ltd., England. 520 p.

[11] Goel, N., Singh, N., Saini, R., (2009): Efficient *in vitro* multiplication of Syrian Rue (*Peganum harmala* L.) Using 6-benzylaminopurine pre-conditioned seedling explants. Nature and Science, 7:129-134.

[12] Goel, R.K., Maiti, R.N., Manickam, M., Ray, A.B., (1997): Antiulcer activity of naturally occurring pyrano-coumarin and isocoumarins and their effect on prostanoid synthesis using human colonic mucosa. Indian Journal of Experimental Biology, 35: 1080–1083.

[13] Guo, B., Gao, M., Liu, C.Z., (2007): *In vitro* propagation of an endangered medicinal plant *Saussurea involucrata* Kar. et kir. Plant Cell Reports, 26: 261-265.

[14] Gupta, R.S., Dixit, V.P., Dobhal, M.P., (2002): Hypocholesterolaemic effect of the oleoresin of

Capsicum annum L. in gerbils (*Meriones hurrianae* Jerdon). *Phytotherapy Research,*16: 273-275.
[15] Hart, F.L., Fisher, H.J., (1971): Methods of analysis. pp. 43. In Hart, F.L., Fisher, H.J. (eds.): Modern Food Analysis, Springer, New York.
[16] Hossain, M., Islam, R., Karim, M.R., Rahman, S.M., Joarder, O.I., (1994): Production of plantlets from *Aegle marmelos* nucellar callus. Plant Cell Reports, 13: 570–573.
[17] Hu, J., Li, J., (2008): Morphogenetic pathway in petiole derived callus of *Amorphophallus albus in vitro*. Acta Physiologiae Plantarum, 30: 389-393.
[18] Islam, M., Islam, R., Hossain, M., Alam, M.F., Banu, N.A., (2000): Callus Induction and Plant Regeneration from Immature Zygotic Embryos of *Carica papaya* L.. Bangladesh Journal of Genetics & Biotechnology, 1: 123-128.
[19] Islam, R., Hossain, M., Karim, M.R., Joarder, O.I., (1995): Regeneration of *Aegle marmelos*(L.) Corr., plantlets *in vitro* from callus cultures of embroynic tissues. Current Science, 69: 494-495.
[20] Jawahar, M., Ravipaul, S., Jeyaseelan, M., (2008): *In vitro* regeneration of *Vitex negundo* L.- A Multipurpose woody aromatic medicinal shrub. Plant Tissue Culture & Biotechnology, 18: 37-42.
[21] Kala, C.P. (2006): Ethnobotany and ethnoconservation of *Aegle marmelos* (L) Correa. Indian Journal of Traditional Knowledge. 5: 541-550.
[22] Karthikeyan, K., Chandran, C., Kulothungan, S., (2007): Rapid regeneration of *Phyllanthus niruri* L. from shoot tip and nodal explants. Indian Journal of Applied and Pure Biology. 22: 337-342.
[23] Lal, D., Singh, N., (2010): Mass Multiplication of *Celastrus paniculatus* Willd – An Important Medicinal Plant Under i*n vitro* Conditions using Nodal Segments. Journal of American Science, 6: 55-61.
[24] Martin, G., Geetha, S.P., Balachandran, I., Ravindran, P.N., (2004): Micropropagation of *Celastrus paniculatus* Willd: A highly sought after medicinal plant in Ayurveda. Proceedings of the National Symposium on Biotechnology for a Better Future, Jan. 15-17, St. Aloysius College, Mangalore, pp: 43-43.
[25] Martin, K.P., (2003): Rapid i*n vitro* multiplication and ex vitro rooting of *Rotula aquatica* Lour., a rare rhoeophytic woody medicinal plant. Plant Cell Reports, 21: 415-420.
[26] Mathur, S., Shekhawat, G.S., Batra, A., (2008): Somatic embryogenesis and plantlet regeneration from cotyledon explants of *Salvadora persica* L.. Phytomorphology, 58: 57-63.
[27] Mazumder, R., Bhattacharya, S., Mazumder, A., Pattnaik, A.K., Tiwary, P.M., Chaudhary, S., (2006): Antidiarrhoeal evaluation of *Aegle marmelos* (Correa) Linn. root extract. Phytotherapy Research, 20: 82–84.
[28] Mohapatra, H., Barik, D.P., Rath, S.P., (2008): *In vitro* regeneration of medicinal plant *Centella asiatica.* Biologia Plantarum, 52(2): 339-342.
[29] Murashige, T., Skoog, F., (1962): A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum. 15: 473-495.
[30] Nadkarni, K.M., (1954): Indian material Medica, 3rd edn. Popular Book Depot, Bombay, India, pp. 45-49.
[31] Pati, R., Chandra, R., Chauhan, U.K., Mishra, M., Srivastava, N., (2008): *In vitro* clonal propagation of Bael (*Aegle marmelos* Corr.) CV. CISH-B1 through enhanced axillary branching. Physiology & Molecular Biology of Plants, 14(4): 337-346.
[32] Ponnachan, P.T.C., Paulose, C.S., Panikar, K.R. (1993): Effect of the leaf extract of *Aegle marmelos* (L.) Corr. in diabetic rats. Indian Journal of Experimental Biology, 31:345–7.
[33] Purohit, S.S., Vyas, S. P. (2005): Medicinal Plant Cultivation-A Scientific Approach. (Agrobion. India), pp. 282.
[34] Raghavaswamy, B.V., Himabindu, K., Sita, G.L., (1992): *In vitro* micropropagation of elite rosewood (*Dalbergia latifolia* Roxb.). Plant Cell Reports, 11: 126-131.
[35] Rana, B.K., Sing, U.P., Taneja, V., (1997): Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos* (L.) Corr.. Journal of Ethnopharmacology, 57: 29–34.
[36] Saini, R., Jaiwal, P.K.. (2000): *In vitro* multiplication of *Peganum harmala* – an important medicinal plant. Indian Journal of Experimental Biology, 38: 499-503.
[37] Sharma, R.K., Wakhlu, A.K., (2003): Regeneration of *Heracleum candicans* wall plants from callus cultures through organogenesis. Journal of Plant Biochemistry & Biotechnology, 12: 71-72.
[38] Singh, U.R., Panday, I.C., Prasad, R.S., (1976): Propagation of bael (*Aegle marmelos* Corr.). Punjab Horticulture Journal, 16: 57-59.
[39] Snedecor, G.W., (1956): Statistical method applied to experiments in agriculture and biology. Iowa State College Press, Ames, pp. 534.
[40] Saini, S.R., Singh, R.P., Jaiwal, P.K., (2007): *Agrobacterium tumefaciens* mediated transfer of *Phaseolus vulgaris*-amylase inhibitor-1 gene into mungbean *Vigna radiata* (L.) Wilczek using *bar* as selectable marker. Plant Cell Reports, 26: 187-198.
[41] Vaishnawa, G.R., (1983): Clonal multiplication of elite of *Tecomella undulate* through tissue culture. Ph.D. Thesis, University of Jodhpur, India. In Pati, R., Chandra, R., Chauhan, U.K., Mishra, M., Srivastava, N., (2008): *In vitro* clonal propagation of Bael (*Aegle marmelos* Corr.) CV. CISH-B1 through enhanced axillary branching. Physiology & Molecular Biology of Plants, 14(4): 337-346.
[42] Yasmeen, A., Rao, S., (2005): Regeneration of multiple shoots from cotyledons of *Vigna radiata* (L.) Wilczek. Journal of Indian Botanical Society, 84: 141-143.
[43] Zimmerman, R., (1985): Application of tissue culture propagation to woody plants. pp. 165-177. In: llenke, R.R., Hughes, K.W., Constantin, M.J., Hollaendra, A. (eds.): K.W. Tissue Culture in Forestry and Agriculture, Plenum Press. New York.

Received: 23 November 2010
Accepted: 14 March 2011
Published Online: 18 March 2011
Analele Universităţii din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433