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Direct Multiple Shoot Induction and Plantlet Regeneration from Cotyledonary Explants of *Sapindus emarginatus* Vahl (Soapnut)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors VM and SD managed the analyses of the study. Author UT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Multiple shoot induction in *S. emarginatus* has been achieved by two methods: (1) Direct germination of *S. emarginatus in vitro* cotyledon explants in BAP/Kn/TDZ (1.0-3.0 mg/L) supplemented MS medium and (2) in plant treatment with BAP/Kn/TDZ (3.0 mg/ L) in combination of 1AA (0.5 mg/L) of the cotyledon explants of plants and maintained under sterile conditions. While the former method resulted in as many as (7.5±8.6 shoot buds) from the cotyledonary explants within four weeks, the latter yielded on average approximately 8 shoot buds from each treated node in eight weeks. The cytokinin treatment in plant consisted of placing sterile filter paper moistened with sterile distilled water over the node and adding different concentrations of 6-benzylaminopurine. The best results for shoot bud regeneration were obtained with cotyledons, when cultured in the presence of (0.5 mg/L) IAA in combination with (3.0 mg/L). The shoots elongated and rooted directly in vermiculite after a pulse treatment with IBA (2.5 mg/L) for 15 min.

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Fungus growth, a serious problem in *S. emarginatus* tissue culture, was controlled using a fungicide, Bavistin, along with elimination of organic nutrients from the growth medium.

Keywords: Sapindus emarginatus; 6-benzylaminopurine; kinetin; thioduzuron; indole acetic acid and cotyledonary explant.

1. INTRODUCTION

Sapindus emarginatus Vahl. (Family: Sapindaceae) popularly known as 'Ritha' and 'Soapnut', is a important deciduous tree of tropical and sub- tropical regions of Asia. The fruit of this tree contains saponins, the most active secondary metabolites extracted from this plant. It is a good substitute for washing soap and is as such used in preparation of quality shampoos, detergents etc .The fruit is of considerable importance for its medicinal value for treating a number of diseases like common cold, pimples, epilepsy, constipation, nausea etc. It is also used as expectorant and anthelmintic in small doses. It is utilized by Indian jewelers for restoring the brightness of tarnished ornaments made of gold, silver and other precious metals. It is also used for washing and bleaching cardamoms [1]. Central Drug Research Institute, Lucknow India has developed a contraceptive cream from this fruit which has anti-*Trichomonas* activity. The Micropropagation of this tree do not yield satisfactory results and propagation through seeds is also unreliable because the per cent survival of the seedlings is low to heavy incidence of mortality at seedlings stage in the natural habitat. Moreover, the seeds have hard seed coat due to which they became physically dormant. Due to these limitations, the conventional methods of Micropropagation of this species are not easy. Conventional propagation rate through stem cuttings is very slow and percent survival of plant progeny raised from seed also proved to be meager due to the incidence and mortality at seedlings stages in the natural habitat, through *In vitro* propagation of soap nut via meristematic culture and seed culture has been reported earlier there are no reports on its direct regeneration through cotyledonary explants culture [2,3]. Present study was conducted for rapid multiplied of soap nut through direct organogenesis cotyledon explants.

2. METHODOLOGY

2.1Plant Material

Seeds of *S. emarginatus* were collected from Botanical garden Department, of Botany Kakatiya University, Warangal India. Dried mature seeds were soaked in Dilute Sulphuric acid (H_2SO_4) for 24 hrs and sterilized with 0.1% (w/v) aqueous Mercuric chloride ($HgCl_2$) for 3-5 minutes followed by washing 3 times with sterile distilled water later these were dried on sterile tissue paper and 20 seeds per culture bottle were germinated aseptically on MS basal medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. These culture bottles were incubated at 25±1ºC under 16 hours photoperiod. Light was provided by cool white fluorescent tubes with an intensity of 50-60 μ mol m^{-2} $s^{-1.}$

2.2 Acclimatization

After *In vitro* rooting the regenerated plantlets were taken out and were washed carefully to remove agar and then transferred to pots containing sterile vermiculite. Each pot was enclosed in a polyethylene bag after watering and maintained in a plant growth chamber at 25+1ºC under 16-h illumination with fluorescent lamps. Bags were progressively opened weekly. After 3 weeks of field. The percentage of survival was found to be 70% and the plants were morphologically identical to the acclimatization, plantlets were transferred to large pots filled with garden soil and farmyard manure (1:1) in the open parental plants. In all experiments a minimum of three plates were cultured. Each single treatment consisted of five to ten explants per plate. Data recorded at Data recorded at three weeks included the number of shoots per explants, length of shoots and rooting were statically analyzed using one way analysis of variance.

2.3 Data Analysis

Each treatment contained 20 replicates. Each treatment was repeated at least once with similar results. Data were recorded after 8 weeks of culture. The following formulas were used for statistical analysis:

$$\text{Mean} = \frac{\Sigma x}{n}$$

$$\text{Variance } S^{2} = \sum x^{2} = \frac{\sum x^{2} - \frac{(\sum x)^{2}}{n}}{n-1}$$

$$\text{Standard Deviation (S.D)} = \sqrt{\text{variance}}$$

$$\text{Standard Error (S.E)} = \frac{\text{S.D.}}{\sqrt{n}}$$

3. RESULTS

The role of cytokinin and Auxin- cytokinin combinations on direct plant regeneration and adventitious bud induction from cotyledon explants was studied in order to find out the efficient protocol and potential on MS medium fortified with different concentrations of cytokinins alone and auxin's (0.5 mg/L) in combination with various concentrations of cytokinins such as BAP/Kn/TDZ (1.0-5.0 mg/L). These explants were enlarged 3-4 fold within one week of culture initiation. Morphogenic change as were apparent after 6 weeks of culture. The Cotyledon explants developed shoot primordial in large numbers directly from all cut surfaces in contact with medium in all the concentrations and combinations of phytohormones used even in combination with (0.5 mg/L) IAA. The results are presented in (Tables 1 and 2).

3.1 Cotyledonary Explants

Cotyledonary explants cultured on MS medium containing various concentrations of cytokinins BAP/Kn/TDZ alone the direct shoot regeneration (Table 1) and (0.5 mg/L) IAA in combination with (1.0-5.0mg/L) BAP/Kn/TDZ showed variable response in cotyledonary explants culture of *S. emarginatus.* These explants were elongated 3-4 folds within one week of culture initiation. Morphogenic changes were apparent after 6 weeks of culture. The results are presented in (Tables 1 and 2) and shown in (Fig. 1).

3.2 Effect of BAP

Cotyledonary explants were cultured on MS medium amended with various concentrations of BAP (1.0-5.0 mg/L) as role growth regulators showed the direct organogenesis (Table 1) Maximum number of shoot bud proliferation (4.5±0.35 shoots /explant) was found at 3.0 mg/L BAP. At 1.0, and 2.0 mg/L BAP induced 2.6±0.32 and 3.0±0.35 shoots/explant with 50 and 53% of cultures responded. When the concentration was increased and above 3.0 mg/L BAP gradually induction of multiple shoots were reduced. At 4.0 and 5.0 mg/L BAP induced 4.2±0.45 and 4.0±0.35 shoots/explants with 55 and 48 percentage of cultures were responded. The number of shoot bud induction was found to be decreased as the concentration of BAP increased. At high concentration of BAP showed less number of shoots per explants.

3.3 Effect of Kn

Cotyledonary explants were cultured on MS medium containing different concentrations of cytokinin Kn (1.0- 5.0 mg/L) as role growth regulators showed the direct organogenesis/ shoot formation (Table 1) to find out the difference between BAP and Kn in inducing the direct plant regeneration from cotyledonary explants in *S. emarginatus.* Maximum number of shoot bud proliferation (6.0±0.35 shoots/ explant) was found at 3.0 mg/L compared to all other concentrations of Kn at 1.0 and 2.0 mg/L Induced 3.8±0.35 and 4.8±0.35 mg/L shoots / explant with 56 and 60 percentage of cultures responded. Percentage of response was gradually increased up to 3.0 mg/L Kn. At above 3.0 mg/L concentration less number of shoots were recorded. At 4.0 and 5.0 mg/L Kn induced 5.8±0.22 and 5.2±0.34 shoots/explants with 58 and 50 percentage of cultures were also recorded on MS + Kn. However high induction of ability was found in all the concentrations of Kn compared to BAP.

3.4 Effect of TDZ

TDZ was more responsive compared to BAP and Kn in inducing shoot buds from the explants (Table 1) with 1.0 mg/L TDZ the cotyledon explants produced 4.0±0.35 shoots/ explants and 55% cultures responded 3.0 mg/L TDZ was more responsive in inducing maximum number of shoots (7.0±0.32 shoots/ explants) with a greater frequency 65% TDZ at 4.0 and 5.0 mg/L produced 6.2±0.23 and 5.3 ± 0.33 shoots/ explants with 62% and 57% culture responding. As the concentrations of TDZ were increased from 3.0 mg/L to 5.0 mg/L the number of shoots per explants was considerably reduced (Table 1 and Fig. 1b).

Table 1. Effect of BAP, Kn and TDZ on Direct shoot bud proliferation of *S. emarginatus* from cotyledonary explants

Hormone cone (mg/L)	Percentage (%) of shoot regeneration	Average no of shoots/exlants (S.E.)*
BAP		
1.0	50	2.6±0.32
2.0	53	3.0±0.35
3.0	60	4.5±0.35
4.0	55	4.2±0.45
5.0	48	4.0±0.35
Kn		
1.0	56	3.8±0.35
2.0	60	4.8±0.35
3.0	64	6.0±0.35
4.0	58	5.8±0.22
5.0	50	5.2±0.34
TDZ		
1.0	55	4.0±0.35
2.0	63	5.3±0.32
3.0	65	7.0±0.32
4.0	62	6.2±0.23
5.0	57	5.3±0.33

**SE Standard Error*

Table 2. Effect of IAA in combination with BAP, Kn and TDZ on Direct shoot bud proliferation of *S. emarginatus* from Cotyledonary explants

Hormone concentration (mg/L)	Percentage of frequency of plantlet production	Mean number of shoots /explants±SE*
IAA + BAP		
0.5 + 1.0	50	5.6±0.35
0.5 + 2.0	60	6.2±0.34
0.5 + 3.0	64	6.4±0.34
0.5 + 4.0	54	5.2±0.34
0.5 + 5.0	52	4.3±0.34
IAA + Kn		
0.5 + 1.0	56	6.0±0.35
0.5 + 2.0	62	6.5±0.32
0.5 + 3.0	66	6.8±0.32
0.5 + 4.0	56	5.0±0.43
0.5 + 5.0	50	4.6±0.32
IAA + TDZ		
0.5 + 1.0	58	6.7±0.03
0.5 + 2.0	65	7.0±0.34
0.5 + 3.0	68	7.2±0.23
0.5 + 4.0	63	6.0±0.34
0.5 + 5.0	60	5.3±0.45

**SE Standard Error*

3.5 Effect of IAA + BAP

To find out the influence of auxin-cytokinin combination on direct regeneration. The cotyledonary explants were cultured on MS medium fortified with (0.5 mg/L) IAA and different concentrations of cytokinin such as BAP (1.0 -5.0 mg/L). IAA in combination with various concentrations of BAP showed variable results (Table 2). Highest percentage of response was observed at (0.5 mg/L) IAA + (3.0 mg/L) BAP. The percentage of response and number of shoots proliferation was increased up to (1.0 mg/L) BAP and later gradually decreased at

above 3.0 mg/L BAP. At 1.0, 2.0 and 3.0 mg/L BAP with IAA (0.5mg/L) induced 5.6±0.35, 6.2±0.34 and 6.4±0.34 shoots/explants with 50, 60 and 64 percentage of cultures were recorded. At concentrations of BAP was gradually increased above 3.0 mg/L after wards decreased the number of shoots. 4.0 and 5.0 mg/L BAP+ IAA (0.5 mg/L) induced 5.2±0.34 and 4.3±0.34 shoots/explants with 54 and 52 percentage of response was recorded (Table 2).

3.6 Effect of IAA+Kn

Cotyledonary explants cultured on MS medium augmented with (0.5 mg/L) IAA and different concentrations of Kn (1.0 -5.0 mg/L) Showed variable response was recorded (Table 2). Maximum shoot bud induction was recorded at 3.0 mg/L (6.8±0.32 shoots/ explants) with 66 percentage of response was recorded. At 1.0 and 2.0 mg/L Kn + 0.5 mg/L IAA induced. 6.0±0.35 and 6.5±0.32 shoots/ explants with 56 and 62 percentage of cultures were recorded. When the Kn concentration was increased from 3.0 mg/L to 5.0 mg/L it was found that the shoot regeneration and percentage of responses was decreased (Table 2).

3.7 Effect of IAA +TDZ

Cotyledonary explants cultured on MS medium augmented with IAA (0.5 mg/L) and different concentrations of TDZ (1.0 -5.0 mg/L) showed variable response was recorded (Table 10). Maximum shoot bud proliferation was recorded at 3.0 mg/L (7.2±0.23 shoots/ explants) (Fig. 1C) with 68 percentage of response was recorded. At 1.0 and 2.0 mg/L TDZ + 0.5 mg/L IAA induced. 6.7±0.03 and 7.0±0.34 shoots/ explants with 58 and 65 percentage of cultures were recorded. When the concentration of TDZ was increased from 3.0 mg/L to 5.0 mg/L it was found that the shoot regeneration and percentage of responses was decreased (Table 2).

Fig. 1. Direct shoot induction of cotyledon culture of *S. emarginatus* (soapnut)

a) In vitro grown plants after 30 days of seed culture; b) Direct shoots formation on (3.0mg/L) TDZ from cotyledon culture; c) Induction of multiple shoots on MS + IAA(0.5mg/L)+(3.0mg/L) TDZ from Cotyledon culture; d) Induction of multiple roots formation from regenerated shoots from cotyledon explants on IAA(0.5mg/L) after six weeks; e) Hardened Cotyledon explants regenerated plant after 8 weeks of culture

3.8 *In vitro* Rooting

Fully elongated healthy shoots were transferred on to full strength MS root induction Medium (RIM) [4] fortified with different concentration of IAA (0.5 – 2.0 mg/L) and IBA (0.5 – 2.0 mg/L).

Profuse rhizogenesis was observed on (1.5 mg/L) IAA, compared to (0.5 -2.0 mg/L) IAA/ IBA on MS medium containing (1.5 mg/L) IBA whereas 96% of plants produced roots with 14.3±0.27 roots/ explants (Table 3 and Fig 1d). mixture of soil + s and + manure in 1: 1: 1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland's soulation every 3 days for a period of 3 weeks) (Fig 1e).

4. DISCUSSION

We were successful in direct regenerating plants from cotyledonary explants of *S. emarginatus* cultures on MS medium fortified with different concentrations of cytokinins i.e. BAP/Kn/ TDZ (1.0-5.0 mg/L) individually and also in combination with (0.5 mg/L) IAA. Maximum number of shoot buds was induced at 3.0 mg/L TDZ in comparison to Kn/BAP as role growth regulators. When the low level of auxin (0.5 mg/L) IAA were added to the medium containing BAP/Kn/TDZ. It was interesting find out that the shoots induction was enhanced in all the concentrations of cytokinins. However the shoot bud proliferation was found to more on (0.5 mg/L) IAA in combination with TDZ compared to (0.5 mg/L) in combination with Kn/BAP but the combination of IAA+TDZ induced highest number of plantlet regeneration among all hormonal combinations and concentrations were used in *S. emarginatus*.

Similarly [5] have reported the high frequency of plant regeneration on MS medium containing 2.0 mg/L BAP in combination with 0.5 mg/L IAA from cotyledon derived callus in *Momordica dioica,* of the cytokinins used Kn proved as most effective than BAP in inducing shoots, the same findings were recorded in *Capsisum* Spp. [6 and 7].

The explants cotyledon hypocotyls and leaf cultured on MS medium supplemented with IAA+BAP combination was found to be more effective in inducing maximum number of shoots in *Capsicum Spp* [8], similarly it was also reported in *Capsicum annuum* [9] and *Solanum melongena* cv Pusa round [10]. The combination of IAA+BAP showed superiority over IAA+Kn in all the explants. Similar finding were also made in *Petunia* [11] *Lycoperiscon esculentum* [12] and *Solanum incanum* [13] and in two species of Niger [14].

[15] have also observed the highest frequency of direct shoot regeneration on lower level of Auxin and high level of Cytokinin (4.0 mg/L) BAP+(0.5 mg/L) IAA) in leaf explants of *Solanum nigrum*. Similar results were obtained in *Dalbergia Canceolats* [16]. However on lower level of BAP the frequency of shoot regeneration was decreased similar to our present observations. Highest numbers of shoots per explant were developed on MS Medium containing 0.5 mg/L IAA+ 2.5 mg/L BAP in leaf explants of *Solanum sisymbrifolium* [17] compared to all other concentrations of BAP alone and also in combination with 0.5 mg/L IAA.

Direct shoot regeneration occurred on MS medium containing BAP/Kn and in combination with IAA in hypocotyl, stem and leaf cultures of *Solanum viarum*. They have also observed the maximum shoot regeneration on IAA+BAP, 2-iP and superiority of BAP over Kn similar to the present observation [18] reported the induction of large number of shoot buds from leaf disc explants of *Passiflora caerulea* when cultured on

Table 3. Rooting ability of regenerated shoots from cotyledonary explants culture of *S. emarginatus* cultured on MS medium supplemented with IAA and IBA.

Growth hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IAA	IBA		
00	00	23	1.0±0.12
0.5	-	60	2.3±0.37
1.0	-	70	3.2±0.38
2.0	-	73	5.6±0.38
-	0.5	54	4.3±0.36
-	1.0	73	8.3±0.87
-	2.0	70	6.3±0.36

** Mean ± Standard*

MS medium fortified with BAP+IAA [19] have also reported direct shoot regeneration from seedling explants of *Passiflora edulis* on MS medium supplemented with BAP+ Coconut water. Similarly, maximum numbers of shoots were obtained when leaf explants of moth bean and pigeon pea were cultured on MS medium containing BAP+IAA [20].

[21] have reported the direct plantlet regeneration from different explants, i.e. hypocotyl, epicotyls, cotyledon and leaves cultured on IAA+BAP combination, but they found the highest average number of shoot buds per leaf explant in *Azadhiracta indica*. Similarly it was also reported in *Capsicum annuum* [9] and 7 Genotypes of *Capsicum annuum* [7].

As with our result, *S. emarginatus* large numbers of adventitious shoot buds were induced by TDZ, but not by other cytokinins of BAP/Kn [22]. Variation in the activity of different cytokinins can be explained by their differential uptake rate reported in different genomes.

TDZ, when used in combination with auxins, drastically reduced the percentage of response (Table 2) Moreover there was no significant increase in the number of shoots regeneration per explants. In contrast, Yildirim and Turker [22] observed a significant increase in the percentage of explants forming shoot and the mean shoot number per explants when TDZ was used in combination with IAA.

A similar pattern of shoot bud development from Cotyledon explants was reported by [23]. Although it was difficult to count the number of shoot buds, it was clearly visible that the number of shoot buds increases with every increase in TDZ concentration. In lower concentrations of TDZ the shoots elongated with some expanded leaves, whereas in the higher concentration the shoot buds were highly vitrified and fail to elongate.

The efficacy of TDZ for induction of direct shoot organogenesis is well documented in several woody plants [24, 25 and 26]. Moreover some researchers [27] have reported TDZ to be an essential growth regulator for shoot induction from Cotyledon explant of Ericaceae family. It was emphasized that the efficiency of TDZ may be due to its ability to induce cytokinin accumulation [28] or enhance the accumulation and translocation of auxin within the tissue [29] TDZ is also suspected of promoting regulated morphogenesis in plants through the modulation of endogenous cytokinin and auxin [30,31] emphasized the potential use of TDZ in the regulation of adventitious shoot production and hypothesize on the synergism existing between TDZ and both endogenous and exogenous auxin.

From the foregoing discussion, Cytokinins BAP / Kn/TDZ alone or in combination with IAA was effective in inducing shoot regeneration in the cotyledon explants and Leaf explants of *S. emarginatus.* But cotyledon explants proved most effective than leaf explants in inducing shoots. However, 3.0 mg/L Kn / BAP with 0.5 mg/L IAA combination induced highest number of shoots in Cotyledon explants. Thus, the plants regenerated *in vitro* by direct organogenesis may exhibit greater genetic stability than those produced from callus [32]. The regeneration protocols developed from Cotyledon explants in the present investigation can be used for mass propagation of the species and also for genetic manipulation studies to introduce agronomically important traits.

5. CONCLUSION

Successful plant regeneration via direct organogenesis from cotyledon explants was achieved in *S. emarginatus* Vahl the cotyledon explants, cultured on MS medium supplemented with different concentrations of BAP/Kn/TDZ (1.0–5.0 mg/L) alone and also in combination with (0.5 mg/L) IAA showed direct multiple shoot bud proliferation from the cut surfaces of the explants. But at (3.0 mg/L) BAP/Kn/TDZ and in combination with IAA (0.5 mg/L) too, the formation of shoot buds were reduced at above (3.0 mg/L) BAP /Kn/TDZ concentrations. Average number of shoots/explants was observed to be higher at (3.0 mg/L) BAP/Kn/TDZ alone in cotyledon explants studied. When (0.5 mg/L) IAA added to the MS medium augmented with BAP/Kn/TDZ induced the enhanced shoot bud proliferation cotyledon explants. High frequency of shoots per explants was recorded on (0.5 mg/L) IAA + (3.0 mg/L) BAP/Kn/TDZ hormonal combination from these results it can be concluded that TDZ showed as efficient growth regulator in comparison to Kn/BAP either alone or in combination with IAA. Thus the regeneration protocols developed in the present investigation can be used for different type of experiments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anonymous. The useful Plants of India. Publications & Information Directorate. CSIR. New Delhi; 1992.
2. Philomia NS, Rao JVS. Multiple shoot production from seed culture of soap nut (*S. mukorossi*). Phytomorphology. 1999;49:419-423.
3. Philomia NS, Rao JVS. Micropropagation of soap nut (*S. mukorossi*). Indian J. Exp Biology. 2000;38:621-624.
4. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 1962;15:473-497.
5. Hoque A, Islam R, Arima S. High frequency plant regeneration from cotyledon derived callus of *Mimordica dioica* (Roxb) Wild. Phytomorphology. 2000;50:267-272.
6. Phillips GC, Hubsten Berger JF. Organogenesis in pepper tissue culture plant cell Tiss. Org. Cult. 1985;4:262-269.
7. Venkataiah B, Subhash K. Genotype explant and medium effects of on adventitious shoot bud formation and plant regeneration *Capsicum annuum L.* J. Genet & Breed. 2001;55:143-149.
8. Gunay A, Rao PS. *In vitro* plant regeneration from hypocotyls and cotyledon explants of red pepper (*Capsicum*). Plant Sci. Lett. 1978;11:365-372.
9. Christophar T, Rajam MV. Effect of genotype explant & medium on *In vitro* regeneration of red pepper. Plant Cell Tissue Org. Cult. 1996;46:245-250.
10. Sharma P, Rajam MV. Genotype, explant & position effects on organogenesis & Somatic embryogenesis in egg plant (*Solanum melongena*L.). J. Exptl. Bot. 1995;46:135-141.
11. Rao PS, Hard H, Reddy GP. Hormonal regulation of morphogenesis in organ culture of Petunia inflates *Autirhinum majas* and Pharbitis vil. In Plant Growth substances, Hirokwa Tokyo. 1973;22:1113-1120.
12. Kartha KK, Gamborg OL, Shyluk JP, Constabel F, Morphogenic investigation on *In vitro* leaf culture of Tomato and high frequency plant regeneration. Z. Pflanzenphysiol. 1976;77:292-301.
13. Sadanandam A, Fauoqui MA. Induction and selection of Lincomycin-Resistant Plants in *Solanum melongena* L. Plant Sci. 1997;79:237-239.
14. Jadimathi VG, Murthy HN, Ashok Kumar HG, Ravi Shankar B. Plant regeneration from leaf cultures of *Guizotia abyssinica (*Niger) and *Guizotia scabra*. Phytomorphology. 1998;48:131-135.
15. Shahzad A, Hasan H, Siddiqui AS Callus induction and regeneration in *Solanum nigrum L*. *In vitro* Phytomorphology. 1999;49:215-220.
16. Dwari M, Chand PK. Evaluation of explant growth regulators and callus assays for enhanced callus induction proliferation & plant regeneration in three Legumes *Dalbergia lanceoloaria.* Phytomorphology. 1996;46:123-131.
17. Rao CS, Eganathan P, Anand A, Balacrishna P, Reddy TP Protocol for *In vitro* propagation of *Excoecaria agallocha* L, a medicinally important mangrove species. Plant Cell Reports. 1998;17:861-865.
18. Jas Rai YT, Mudgil Y, Remakanthan A, Kannan VR. Direct shoot regeneration from cultivated Leaves of *Passiflora caerules* L. and field per formance of regenerated Plants. Phytomorphology. 1999;49:289-293.
19. Desai HV, Mehta AR. Organogenesis in cultured leaf discs of *Passiflora* Spp. In: Hand book of Plant Tissue & Cell Culture. (eds). AR Mehta & P.N. Bhatta, Acad Book centre, Ahmedabad. 1990;28-30.
20. Kunjumon A, Kannan VR, Jasrai VT. Plant regeneration from leaf explants of three grain legumes on same medium. J. Plant Biochem. Biotech. 1996;5:27-29.
21. Neeta D.S., Sing H., Tivarekar S, Eapen S. Plant Regeneration from different explants of Neem Plant cell Tiss org. Cult. 2001;65:159-162.
22. Blakesey D Uptake and metabolism of 6-benzyladenine in shoot proliferation of *Musa* and *Rhododendron*. Plant Cell Tissue Organ Cult. 1990;25:69–74.
23. Banerjee S, Tripathi J, Verma PC, Dwivedi PD, Khanuja SPS, Bagchi GD; 2004.
24. Graham J, Iasi L, Millam S. Genotype-specific regeneration from a number of *Rubus* cultivars. Plant Cell Tissue Organ Cult. 1997;48:167–73.

25. Leblay C, Chevreau E, Raboin LM. Adventitious shoot regeneration from *In vitro* leaves of several Pear cultivars (*Pyrus communis* L.). Plant Cell Tissue Organ Cult. 1991;25:99–105.
26. Preece JE, Imel MR. Plant regeneration form leaf explants of Rhododendron P.J.M. hybrids. Sci. Hort. 1991;48:159-170.
27. Hsia CH, Korban SS. The influence of cytokinins and ionic strength of Anderson's medium on shoot establishment and proliferation of evergreen azalea. Euphytica. 1997;93:1–7.
28. Victor JMR, Murthy BNS, Murch SJ, Krishnaraj S, Saxena P. Studies of Endogenous Purine metabolism in Thidiazuron-induced somatic embryogenesis of peanut (*Arachis hypogea* L.). Plant Growth Regul. 1999;28,41–7.
29. Murthy BNS, Murch SJ, Saxena PK. Thidiazuron: A potent *In vitro* plant morphogenesis. *In vitro* dev Biol Plant. 1998;34:267–7.
30. Gill R, Saxena PK. Direct somatic embryogenesis and regeneration of plants from seedling explants of Peanut (*Arachis hypogeae*) promotive role of Thidiazuron. Can J Plant Sci. 1992;70:1186–92.
31. Huetteman Carl A, John E, Preece Thidiazuron. A potent cytokinin for woody plant tissue culture. Plant Cell Tissue Organ Cult. 1993;33:105–19.
32. Lee M, Phillips RI. The chromosomal basis of soma clonal variation. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1988;39:413-437.

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