International Journal of Multidisciplinary and Current Research

Research Article

ISSN: 2321-3124
Available at: http://ijmcr.com

Micropragation of axillary shoot buds in *Sapindus emarginatus* Vahl.

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Accepted 05 April 2014, Available online 15 April 2014, **Vol.2 (March/April 2014 issue)**

Abstract

In the recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under in vitro conditions. In vitro propagation also called micropropagation is in fact the miniature version of conventional propagation, which is carried out under aseptic conditions. The advent of in vitro tissue culture technique has offered a new approach to the morphogenetic investigations. In the present study an attempt has been made to formulate a suitable Protocol for efficient micropropagation from seedling of axillary shoot buds explants of Sapindus emarginatus Vahl.

Keywords: *Sapindus emarginatus, axillary Shoot buds, Thioduzuron (TDZ) ,6-Benzyl amino purine (BAP) .*

Introduction

The goal of 'Micropropagation' is to obtain a large number of genetically identical, physiologically uniform and developmentally normal plantlets, preferably with a high photoautotrophic potential to survive the harsh *ex-vitro* conditions, in a reduced time period and at a lowered cost. Although true-to-type plantlets are the desired goals of micropropagation, many times, somaclonal variants are obtained due to the involvement of tissue culture components.. Micropropagation aims at producing exact replicas of the original plant selected for its desirable characters and considerable progress has been made in the recent years in micrpropagation of many plant species [1-2]. These can be used for selection of new useful variants, which can then be propagated on a large scale by micropropagation eg., stress and disease tolerant variants. Micropropagation provides a means of germplasm storage for maintenance of disease-free stock [3]. Micropropagated plants can be exploited commercially [4-6].

Materials and Methodology

Sapindus emarginatus Vahl. seeds were collected from Aromatic and Medicinal Garden, Department of Botany, Kakatiya University, Warangal. The shoot tips were collected from one year old healthy plants shoots comprising the apical dome and 2 or 3 leaf primordials were excised. The shoot tip explants were washed in a mild non phytotoxic liquid detergent (2% labolene) stirred for about 3 min and then washed in tap water. It was followed by a dip in 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 2 minutes. Finally the axillary bud explants were washed thoroughly with sterile water before the inoculation on to sterilized nutrient agar media prepared in culture tubes. All the above operations were performed under aseptic conditions in a laminar air flow cabinet.

Culture media and culture conditions

MS medium containing 3.0% sucrose and supplemented with different concentrations and combinations of cytokinins viz., BAP/Kn/TDZ (0.5-3.0mg/L) and the initial pH in the culture media was adjusted to 5.8 before addition of 0.8% (w/v) agar-agar. The medium was dispensed into culture tubes (25 x 150mm) each containing 15ml of the culture medium, capped with non-absorbent cotton and was autoclaved at 121^{o}C for 15 minutes. In each culture tube one shoot-tip explant was implanted. The cultures were maintained under than 16 h light provided with white fluorescent tubes (40 µ mol $m^{-2}s^{-1}$) at 25 ± 2^{o}C.

Shoot apices (1.0-1.5 cm) were trimmed from four week-old seedlings and inoculated on a shoot bud induction medium consisting of MS basal medium supplemented with different concentrations of cytokines (0.5–3.0 mg/L) like 6-benzyl amino purine (BAP) or kinetin (Kn) or thioduzuron (TDZ). The percentage of shoot buds formed were counted after six weeks. The elongated shoots (about 2 cm long) obtained from shoot-tip

Table – 1 Direct multiple shoots proliferation from Shoot tip explants of *Sapindus emarginatus* Vahl on MS medium supplemented with various concentrations of BAP, Kn and TDZ

Hormone concentration (mg/L)	% of cultures response	Mean number of shoots /explants ±(S.E.)*
BAP		
0.5	60	4.0 ± 0.32
1.0	70	5.0 ± 0.35
1.5	80	5.6 ± 0.32
2.0	90	6.0 ± 0.32
2.5	85	5.8 ± 0.36
3.0	60	5.5 ± 0.23
Kn		
0.5	62	4.3 ± 0.25
1.0	73	5.4 ± 0.32
1.5	85	5.8 ± 0.32
2.0	92	7.0 ± 0.35
2.5	88	6.6 ± 0.32
3.0	65	6.2 ± 0.23
TDZ		
0.5	65	5.0 ± 0.32
1.0	75	5.6 ± 0.32
1.5	86	6.0 ± 0.32
2.0	95	7.8 ± 0.42
2.5	80	7.0 ± 0.32
3.0	68	6.8 ± 0.32

*SE Standard Error

Table -2 Rooting ability of regenerated shoots from Leaf explants/cotyledonary explants /Shoot tip explants culture of *Sapindus emarginatus* Vahl cultured on MS medium supplemented with IBA.

Growth Hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IBA			
	0.5	54	4.3 ± 0.36
	1.0	73	8.3 ± 0.87
	2.0	70	6.3 ± 0.36

* Mean ± Standard Error

explants were excised and cultured in 250 ml flasks containing 70 ml of rooting media consisting of MS medium supplemented with different concentrations of auxin, 0.5-2.0 mg/L of indole-3-butyric acid (IBA) for the rooting of shoot buds. (Table -2) (Plate-IIa).

The number of roots (including the main roots and their branches) and axillary shoots were induced on four week-old rooted plantlets. These plantlets having 5-9 leaves were decapitated for inducing axillary shoot development by cutting the tips with a sterile blade. Axillary shoots developing in the axils of leaves of the decapitated plantlets were used for further multiple shoot bud induction by culturing on a medium containing 0.5-3.0 mg/L of BAP/Kn/TDZ and the number of shoot buds were counted after six weeks.

The shoot buds proliferated from axillary shoot-tip explants were excised and cultured on a rooting medium consisting of MS medium supplemented with 0.5-2.0 mg/L of IBA. The rooted plantlets were gently removed from the flasks and the roots were washed in tap water to remove traces of agar. The plantlets were then transplanted in perforated paper cups containing sand: soil (1:1) and kept covered with clear polythene bags having a few holes on it for the initial 10 days. The plantlets were kept in a 50% shaded net-house and watered daily with tap water to maintain high humidity. After 10 days, humidity was gradually decreased by increasing the size of holes in the polythene bags and the polythene bags were finally removed. Four week-old hardened plants were then transplanted to bigger earthen pots or to the field.

All cultures were maintained in a growth chamber at a temperature of 25±2°C and 16-h photoperiod provided by white fluorescent tubes (30 µmol m-2S-1). All the experiments were repeated thrice and each treatment for shoot bud induction from the shoot-tip explants and rooting of the shoot buds consisted of ten replicates.

Results

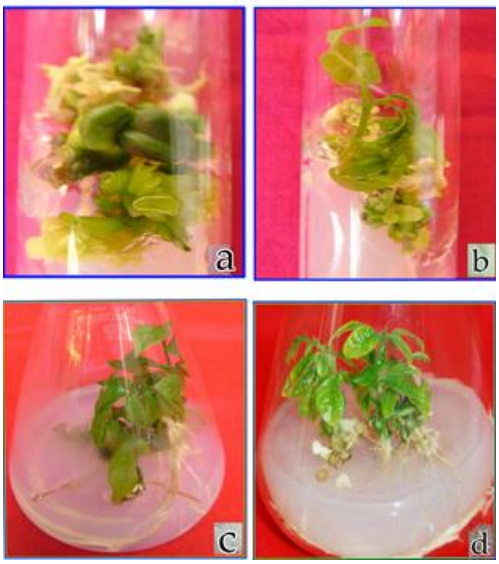
Data on multiple shoot induction from shoot tip explants cultured on MS medium fortified with different concentrations of BAP/Kn/TDZ is presented in (Table -1). The root induction from regenerated shoot tip explants

cultured on MS medium supplemented with different concentrations of IBA is presented in (Table-2) and shown in figures (Plate I and Plate II).

Effect of BAP

Direct multiple shoot proliferation was observed in shoot tip explants cultures and callus formation was not observed in the above treatment. After 6 weeks, shoot tip explant culture developed multiple shoots (Plate I, Fig-a). The various treatments tested on MS medium with BAP at 2.0 mg/L resulted in maximum number of shoots (6.0 ± 0.32). But at high concentration of BAP (3.0 mg/L), shoot induction was found to be considerably reduced. As the concentration of BAP was increased up to 0.5mg/L, the multiple shoots number was increased. But as the concentration of BAP reduced from 2.0mg/L to 3.0 mg/L, the number of shoots were reduced (Table-1).

PLATE - I



Effect of Kn

Shoot tip explants were capable of directly developing multiple shoots on MS basal medium containing different concentrations of Kn (0.5-3.0 mg/L). Multiple shoot initiation from shoot tip explants was observed within six weeks of inoculation. Highest number of shoots (7.0 ± 0.35) were observed in 2.0 mg/L Kn wherein 92% cultures responded. When the Kn was increased up to (0.5-2.0mg/L), the multiple number of shoots and frequency were also increased (Table-1). (Plate I, Fig-b).

Effect of TDZ

The highest result on shoot tip/axillary bud of *Sapindus emarginatus* Vahl on MS medium supplemented with TDZ (0.5–3.0 mg/L) was observed in the study. High percentage (95%) of responding cultures were found at 2.0 mg/L of TDZ compared to all other concentrations tested. Most number of shoots were regenerated from shoot tip explants at 2.0 mg/L of TDZ (7.8± 042 shoots/explant) followed by 2.5 mg/L TDZ. At 0.5, 1.0 and 1.5 mg/L of TDZ, 5.0 ± 0.32, 5.6 ± 0.32 and 6.0 ± 0.32 of shoots were observed. With 65, 75 and 86 percentage of cultures response was recorded. (Table- 1) (Plate I, Fig-d).

***In vitro* rooting**

Fully elongated healthy shoots were transferred on to half strength MS root induction medium of Murashige and skoog [1] fortified with different concentration of IBA (0.5-2.0 mg/L).

Profuse rhizogenesis was observed in IBA with MS medium containing 1.5 mg/L IBA whereas 96% of plants produced roots with 14.3 ± 0.27 roots. (Table -2) (Plate I Fig-c).

Discussion

The result of present investigation showed that the shoot tip explants from mature plants of *Sapindus emarginatus* Vahl could be induced to produce multiple shoots *in vitro.* Maximum number of shoots was induced on MS medium fortified with various concentrations of BAP, Kn and TDZ. These results are in agreement with those on *Tectona grandis* [7] and *Abizzia lebbeck* [8]. Multiple shoot induction was also observed in *Ziziphus manritiana* [9] and *Vanilla plantifolia* [10] shoot tips cultured on MS + cytokinin which is in tune with the results of the present study.

Zamen *et. al*., [11] have studied the effect of different cytokinins viz., BAP, Kn, 2-IP and zeatin on multiple shoot induction from shoot tip culture in mulberry. According to their observation, BAP and Kn were superior to 2-IP and zeatin. The superiority of BAP over other cytokinins for multiple shoot formation has been also reported in Mulberry [12].

Das and Mitra [13] and Roy *et. al*., [14] have also reported the requirement of both auxin and cytokinin for induction of multiple shoos in *Eucalyptus tereticorvis* and jack fruit respectively. Thus, a combination of both auxin and cytokinin improved the efficiency of multiple shoots development although it depended on the individual combinations and concentrations employed.

Khanan *et. al*., [15] have recorded maximum number of shoots / culture (22.5) on MS medium containing 5.0 mg/L BAP in strawberry shoot tip culture. Gupta *et. al*., [16] reported multiple shoot bud induction from shoot apex cultured on MS medium containing BAP in cotton. Nasir *et. al*., [17] have studied the shoot meristem culture in 16 cultures of cotton using several media formation. They observed the best shoot development in MS media containing Kn alone compared to other media with NAA / IAA in combination with Kn. These results are according to the present observation in *Sapindus emarginatus* Vahl which also exhibit that cytokinins increased the number of shoots/ explant. Sharma and Dhiman [18] have also

observed similar results when they cultured the shoot tips of F1 hybrids of *Paulownia*.

The capacity of shoot bud differentiation and shoot proliferation from shoot tip explants of *Sapindus emarginatus* Vahl depended on hormonal variation. There was good shoot bud induction and proliferation response only in the presence of cytokinin and no response in the basal medium. Similar results were also documented in several medicinal plants viz., *ocimum sps.* [19], *Bixa ovellana* L. [20], *Emblica officinale* [21] and *Withania somnifera* [22]. The present study clearly indicates that 2.0 mg/L BAP, KN and TDZ were significantly more effective for inducing shoot organogenesis. Well-developed shoot lets transferred to rooting medium containing 1.5mg/L of IBA induced higher frequency of roots. Similar effect of IBA was also reported in *Ocimum americanum*, *O.canum* and *O.sanctum* by Patnaik and Chand [19]. Further, IBA at 1.5mg/L was found to be the best rooting hormone than other auxins. Auxin - support *in vitro* multiplication was observed in Sunflower [23], Mulberry [24] and Coriander [25]. From our experimental data, it is evident that BAP and KN are best suited for inducing multiple shoots where as IBA is best suited for rooting. Thus, this communication describes an efficient rapid propagation system of *Sapindus emarginatus* Vahl.

Acclimatization

Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar.

PLATE - II

Then the plantlets were transferred to polypots containing pre- soaked vermiculite and maintained inside a growth chamber set at 28 ^{o} C and 70 - 80 % relative humidity. After three weeks, they were transplanted to poly bags containing 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 solution every 3 days for a period of 3 weeks. (Plate II Fig-a and b).

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