MICROPROPAGATION OF *SYZYGIUM CUMINII* L.USING *IN VITRO* TECHNIQUE

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ABSTRACT

Immature seeds of *Syzygium cuminii* L. produced seedlings after a week when cultured on Murashige and Skoog's medium supplemented with a cytokinin BA (0.05-1.0 mg/l) and NAA (0.01-0.05 mg/l). The seedling explants were induced to form callus on MS medium containing 2,4-D (0.05-1.0 mg/l) with BA (0.5-2.0 mg/l). Such callii subsequently, produced shoot buds when sub cultured on MS medium supplemented with BAP (0.5-2.0 mg/l) singly or in combination with NAA (0.05-0.2 mg/l). Upto 85% of cultures showed shoot formation with about 25 shoots / culture from callus cultured on medium containing 1.0 mg/l of BA along with 0.05 mg/l of NAA. Adventitious shoots showed root initiation when sub cultured on MS medium containing IBA and / or NAA 1.0 mg/l). However further growth and development of rooted shoots occurred only when plantlets were transferred to MS basal medium. About 75% of rooted shoots have successfully been established to soil.

Key Words: in vitro, plantlets, seedlings, explants, Black plum.

INTRODUCTION

The importance of tissue culture approach propagation of woody plants, including conifers, for the tree improvement and reforestation has been discussed as burning issue by several workers (Jones, 1983; Farnum, *et.al.*1983; Bajaj, 1986). Although during last years significant progress has been achieving *in vitro* organogenesis in callus cultures of hardwood trees, only a few studies have led to complete plant regeneration (Mascarenhas and Muralidharan, 1989; Anonymous, 1980; Paranjothy *et.al*).

Syzygium cuminii L. (Black plum), a hard timber tree of tropics is also important for its edible fruit and medicinal value. Its fruit possess considerable nutritive value. It is a good source of iron apart from usual content e.g. Minerals, sugar, protein etc. (Singh, et.al. 1967). The vinegar from fruit is tonic, astringent, carminative and useful in diseases of spleen. The seeds possess remedy for diabetes milieus. Clonal propagation by cuttings and grafting ensures the conservation of this indigenous minor fruit tree but it has been reported in a very few cases. Efforts of plantlet regeneration from explants of mature trees have failed because of recalcitrant nature of this tree. However plantlets could be micro propagated from nodal and shoot tip explants of obtained from seedlings (Yadav, et.al. 1990). Therefore, the present investigation was undertaken to develop a protocol for the large scale propagation of *Syzygium cuminii L*. using *in vitro* technique.

MATERIAL AND METHODS

Immature seeds of *Syzygium cuminii* L. were collected during June and July from a tree situated in the botanical garden of the campus. Then they were brought to the laboratory and thoroughly washed with running tap water for about an hour and then treated with 1.0%(v/v) cetavlon (20% cetrimide w/v, a detergent and antiseptic), for 10 min. After proper rinsing with tap water the seeds were then surface sterilized with 0.1% aqueous mercuric chloride solution for 5 min. followed by thorough washing with sterile distilled water. The seeds were then individually cultured on solidified MS medium (Murashige and Skoog, 1962) containing BAP at various concentrations singly or in combination with NAA. The cultures were stored at 27º C. One or two segments of about 5-8 mm size were excised from the cotyledon part of one week old seedlings and cultured on medium containing different growth regulators singly or in combination. Cultures wee maintained at 26º $\pm$ 1 C with 16 hours illumination at a photon flux density of 50-70u E/m2/s from white fluorescent tubes. For each treatment 12 explants wee used and all the experiments were performed at least thrice. The plantlets thus produced were taken out from the culture tubes fro transfer into soil. Rooted shoots were washed thoroughly under running tap water to remove the traces of agar and were then planted into soil after acclimatization.

Data were subjected to analysis of variance and means were separated by Duncan's multiple range test.

RESULTS

The explants cultured on MS basal medium (medium without growth regulators) and MS medium supplemented with different concentrations of BAP and KN (0.5, 1.0 and 2.0 mg/l) did not showed any callogenic and/or organogenic response.

Explants cultured on medium containing BAP (0.2, 0.5, 1.0 and 2.0 mg/l) and NAA (0.05, 0.2 and 0.5 mg/l) showed smaller green callus. However if these segments were cultured on MS medium containing 2,4-D (0.05-1.0 mg/l) and BAP (0.2-1.0 mg/l) a large amount of light green callus was produced within 7 weeks. Growth of the callus could be maintained by transferring the cultures to fresh medium of the same composition. The 7-week-old calli were sub cultured on medium supplemented on medium supplemented with various concentrations of BAP and NAA. Singly or in combination. After a fortnight light green nodular structures were formed on callus sub cultured on medium supplemented with BAP and NAA (0.5- 2.0 mg/l). These nodular structures subsequently differentiated into shoot buds after 06 weeks of culture and developed into complete plantlet after 7-8 weeks of culture.

Each responsive culture showed the production of about 10-60 shoots on the surface of the callus. The numbers of shoot produced per culture were higher when medium was supplemented with BAP (0.5-1.0mg/l) and NAA (0.02mg/l) as compared to medium the supplemented with BAP alone (Table-1). However BAP (0.5-2.0mg/l) along with higher concentration of NAA (0.5mg/l) reduced the number of shoots /culture. Shoots attained the length of about 4-8 cm. after 6 weeks of sub culture (Fig.- 1C, D). A few shoots showed inhibitory growth however when these shoots were sub cultured to another fresh medium of same composition the non elongated shoots also attained the length of 5-7 cm. within 2 weeks of sub culture. For inducing shoot buds from cotyledon explants of *S. cuminii* a transfer of callus to a medium containing BAP alone or with the combination of NAA was necessary. All these shoots were then either used for rooting or cultured on shoot proliferating medium for multiplication. More pronounced growth of the shoots

were observed when proliferating shoots were sub cultured at 2 weeks interval on medium of same composition i.e. 1.0 mg/l of BAP and 0.02 mg/l of NAA on which callus was producing green nodules.

Shoots 2-6 cm long were isolated from callus and were sub cultured on MS basal medium supplemented with IAA and/or NAA (0.5 and 1.0 mg/l). Rooting of shoots was observed on medium containing auxins singly or in combination. Only one or two roots /shoot were observed when any of the auxin was supplied singly. However in combination of NAA and IBA, each at 1.0 mg/l, root number was increased significantly but length attained was only 0.5 cm or less (Fig. 1E). Initiation of roots was observed within 15 days after but after 3 weeks the leaves of shoots started to defoliate and ultimately caused the death of plantlets via apical dieback.

Therefore the shoots were first placed on auxin enriched rooting medium for about two weeks and then transferred to MS basal medium. This allowed shoots to produce numerous roots (4-8) with laterals which were 3 to 5 cm long. After sub culturing shoots on MS basal medium after root initiation the first leaf of shoot unfolds and then growth of the shoots and roots occurred until complete plantlets were formed (Fig. 1F).

The rooted shoots (plantlets) were then transferred to the pots containing equal amount of garden soil and compost. As the plantlets established into the soil they exhibit good growth. New leaves were produced within 30 to 45 days of transfer. These plantlets showed primary branching after 3 to 4 months of transfer and secondary and tertiary branches were produced with in subsequent months. About 80% of plants survived well in soil and no variation was observed among the plantlets suggesting phenotypic stability.

DISCUSSIONS

In the present study 2, 4-D was found to be essential for the initiation of callus. However, a combination of 2, 4-D and BAP favored vigorous callusing of the explants. Presence of 2, 4-D has also been found to be associated with callus formation in stem explants of *Citrus paradisi (*Bhansali and Arya, 1978), *Azadirachta indica* and *Ficus religiosa* (Narayan, 1984) and hypocotyls segments of *Santalum album* (Rao and Bapat, 1978) The presence of BAP alone can induce the shoot bud from callus of *S.cuminii* and induction of shoots on medium containing BAP alone was found in watermelon also (Srivastav, *et. al*. 1989) using hypocotyls explants. However, Loh and Rao (1989) suggested that 0.1 mg/l of BAP gave best results in case of guava instead of 1.0 mg/l of BAP. A combination of cytokinin (BAP) and an auxin (NAA) showed better response of shoot proliferation as compared to cytokinin (BAP) alone. Working with Euclyptus Laskmi Sita in (1981) has reported that NA in combination with BAP produced more sturdy shoots. Synergistic effect of an auxin and cytokinin on shoot proliferation has been observed by Upadhaya and Chandra (1983) in *Albizia lebbek* as well. However contrary to this a combination of cytokine and auxin reduce the shoot proliferation in case of *Morus nigra L. (*Yadav et al., 1990*)*

In vitro grown shoots of *S.cuminii* showed 100% rooting with 1 or2 roots on MS medium supplemented with 1.0 mg/l produces 5 to 6 roots. However, in combination, growth of the roots was totally checked. Nevertheless transfer of these shoots after root induction to auxin free medium produced large number of roots with sufficient growth.Similiar findings were made by Williams et al.(1984) for Dampiera shoots.This may be due to otherwise supraoptimal levels of applied auxin.It has been suggested that auxin levels which promote root initiation may be unsuitable for root growth and transfer of these cultures to basal medium after root initiation but before root emergence may be beneficial(Welander, 1983).Our results illustrates the potential of cotyledon explants to form rooted shoots,and provides a practical method for multiplication of this valuable tree.

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Figure 1

LEGENDS FOR FIGURE - 1

Figure 1: Plantlet regeneration from Cotyledon explants of Syzygium *cuminii* L.

A. Callus initiation from cut ends on MS+BAP (0.5 mgl^{-1}) + 2, 4-D (0.05 mgl^{-1}) after 3 weeks of culture. X 1.1.

B. Shoot bud initiation after 2 weeks of subculture on MS+BAP (0.2 mgl^{-1}) + NAA (0.02 mgl^{-1}). X 1.8.

C&D. Multiple shoots produced on same medium after 4 weeks of subculture. X 1.4 and after 7 weeks of subculture showing elongated shoots. X 1.2.

E. Root formation on MS+1.0 mgl^{-1} IBA and 1.0 mgl^{-1} NAA after 4 weeks of culture.

(Note: shoot as well as root growth has been checked). X 1..8.

F. Rooting of shoots of MS medium containing 1.0 mgl^{-1} of IBA after 4 weeks of subculture. X 1.2

C = callus, **s** = shoot, **Sb** = shoot bud, r = root.

Table 1: Effect of BAP and/or NAA on frequency of adventitious shoot formation from cotyledonary callus of *Syzygium cuminni* L.

Concentration of BAP (mgl^{-1})	Culture showing shoots formation (%)			
	Concentration of NAA (mgl^{-1})			
	0	0.05	0.25	0.50
0	-	-	-	-
0.5	60 bB (25 aA)	75 bA (50 aA)	45 aC (30 bB)	45 aC (10 aD)
1.0	70 aB (20 aB)	85 aA (60 aA)	35 bC (25 aC)	40 aC (05 bD)
2.0	45 cA (15 bC)	50 cA (25 bA)	15 cB (15 cB)	19 bB (02 bD)

Figures within parenthesis represent the number of shoots per culture.

Mean in a column followed by same letter (capital in row and small in blocks) are not significantly different (P = 0.05) according to Duncan's multiple range test.

Table 2 : Effect of auxins on rooting of shoots of *Syzygium cuminii* after 4 weeks of culture.

MS + Auxin (mgl^{-1})		Rooted shoots (%)	Number of roots/shoots	Root length (cm)
Basal		-	-	-
IBA	0.5	70 b	1.5 c	0.8 b
	1.0	105 a	2.0 b	1.0 a
NAA	0.5	60 b	1.5 c	1.0 b
	1.0	85 a	2.5 b	0.9 a
IBA + NAA				
0.5	+ 1.0	95 a	7.0 a	0.5 c
1.0	+ 1.0	90 a	6.0 a	0.5 c

Means in a column following by same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.