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Micropropagation of *Syzigium cumini* (Linn.) Skeels. A Multipurpose Tree

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Abstract: An efficient protocol for the *in vitro* propagation of the valuable medicinal plant, *Syzygium cumini* L. is described through axillary bud proliferation. Multiple shoots were induced from mature nodal explants cultured on Woody Plant Medium (WPM) supplemented with combinations of Benzyl Adenine (BA; 8.8 μM), Kinetin (9.3 μM) and Naphthalene Acetic Acid (NAA; 5.37 μM) produced an average of 35-40 shoots by 6-7 weeks after inoculation. The shoots were rooted in WPM supplemented with 4.9 μM Indole-3-butryic acid (IBA). *Ex vitro* rooting was also successful in this species. Plantlets established in the field showed 80% survival and exhibited identical morphological characteristics as the donor plant.

Key words: *Syzygium cumini*, micropropagation, mature nodes, nodal explant, *ex vitro* rooting

INTRODUCTION

Syzygium cumini (Linn.) Skeels (Myrtaceae) is a large evergreen tree distributed through out the tropical regions. This tree is of real value in apiculture. The flowers of this species have abundant nectar and are source of fine quality honey. The leaves are served as fodder for livestock and as food for silkworms. The bark of the tree contains tannin and is much used in tannin industries. Apart from this lot of medicinal uses has been reported in this species. Medicinally, the bark is stated to be astringent, digestive, anthelmintic, constipating, stomachic and antibacterial. It is useful in diabetes, leucorrhoea, fever, gastropathy, stomachalgia and dermatopathy. The leaves are antibacterial and are used for strengthening the teeth and gums. The fruits and seeds are sweet, acrid, sour, tonic and cooling and are used in diabetes, diarrhoea, pharyngitis, splenopathy, urethrorrhea and ringworm (Warrier *et al*., 1994). The anti diabetic properties of the seeds have been clinically checked (Purohit and Daradka, 2000). *S. cumuni*, has great importance in the food as well as wood industry and is useful in social forestry programme (Anonymous, 1992). Wine and vinegar are also made from the fruit.

Syzygium cumini suffers from very low seed viability and poor germination in its natural habitat (Dent, 1948). Propagation through stem cuttings is an alternative, but not feasible for obtaining large quantity of planting materials. In this context it is necessary to standardise a suitable protocol for clonal propagation of this species. Forest trees in general have proved to be difficult for mass propagation by tissue culture. Earlier *in vitro* propagation of this plant through callus cultures (Remashree *et al*., 2003) and from nodal explants of seedlings (Yadav *et al*., 1990) were reported. The present investigation was to develop a mass multiplication procedure through *in vitro* culture using nodal explants of a mature tree with superior traits like growth and stress tolerance within a short duration. To the best of our knowledge, this is the first report on rapid mass multiplication of *S. cumini* from mature nodal explants.

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MATERIALS AND METHODS

Nodal explants were colleted from the mature plants grown in the Botanical Garden of the Calicut University, Kerala, India. Explants were treated with 1% (v/w) Teepol detergent for 5 min followed by thorough wash with running water. Thereafter, the explants were surface sterilized with 0.1 (w/v) Mercuric chloride (0.1%) for 5 min. The sterilized explants were thoroughly washed with sterile distilled water. The surface sterilized explants were cultured on Woody Plant Medium (WPM) (Llyod and Mc Crown, 1980) supplemented with sucrose (30 g L^{-1}), BA (0.44-17.7 μM), KIN (0.46-23.2 μM) and NAA (5.37-16.1 μM) either alone or in combination. The media were adjusted to pH 5.8 before gelling with agar (8 g L^{-1}). The cultures were incubated at 23±2°C and 16 h photoperiod under an irradiance of 45 μmol $m^{-2}sec^{-1}$ supplied by cool white fluorescent light (1600 Lux).

For rooting, *in vitro* shoots (2-3 cm long) were transferred to rooting medium (WPM with 3% sucrose and 0.7% agar) containing various concentrations of IBA (0.049-24.6 μM) or NAA (0.053-26.8 μM). For *ex vitro* rooting, shoots without roots were dipped in 2.46-9.8 μM IBA for 1 min and planted in cups containing sand and hardened at 70-80% RH and 28+2°C. Shoots with well-developed roots were rinsed with water to remove adhering culture medium from the roots and transferred to pots containing a mixture of garden soil and sand (1:1).

All experiments were repeated thrice with 12 replicates each. Standard errors of means were calculated and statistically significant mean differences were determined by the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

The nodal explants responded with 75% bud break within two weeks in the media containing woody plant salts supplemented with different concentrations of BA and NAA. Woody Plant Medium was effective than MS basal medium (data not shown) for giving favorable responses and hence it was used for multiplication trials. The superiority of WPM for *in vitro* responses was reported earlier (Raghu *et al*., 2006; Nirmal Babu *et al*., 2003) in medicinal plants. WPM medium contains different concentrations of BA (0.44-17.7 μM), Kin. (0.46-23.2 μM) and NAA (0.53-10.7 μM) were used to establish the optimum concentration for shoot bud initiation and elongation (Table 1). Combination of 8.8μM BA and varying concentration of NAA (0.53-10.7 μM) showed increase in the number of shoots upto 12 per culture but the shoot length was not favourable in these combinations (Table 1, Fig. 1A and B). Exogenous applications of cytokinin and auxin have been known to be important for shoot induction and elongation of many plant species *in vitro* (George, 1993). Of all cytokinins and auxins, BA and NAA have been used most commonly for shoot induction (Tripepi, 1997; Nasiruddin *et al*., 2003; Shiau *et al*., 2005; Usha *et al*., 2007). Cytokinins commonly stimulate shoot proliferation and inhibit shoot elongation, particularly BA (Brassard *et al*., 1996). The frequency of multiplication and elongation of shoots were increased when medium supplemented with combinations of BA (8.8 μM), NAA (5.3 μM) and Kin (9.3 μM) (Table 1, Fig. 1C). The shoots elongate up to 5-6 cm in length within 30-40 days. The shoots were healthy and showed well developed leaves (Fig. 1C). Medium supplemented with Kin resulted in elongated shoots. Other similar observations were found in medicinal plants like *Gymnema sylvestre* (Komalavalli and Rao, 2000), *H. antidysenterica* (Raha and Roy, 2001) and *Tinospora cordifolia* (Raghu *et al*., 2006). The cultures were raised upto 4th subcultures in the medium containing of BA (8.8 μM), Kin (9.3 μM) and NAA (5.3 μM), which was optimum for shoot multiplication (Table 2). Maximum number of shoots (1:40) were produced in WPM containing 8.8 μM BA, 5.37 μM NAA and 9.3 μM Kin. (Table 1 and 2).

Table 1: Effect of growth regulators on number and length of shoots from the nodal explants of *S. cumini*

Plant growth regulators (µM)					
BA	NAA	Kinetin	Percentage of response	Mean No. of shoots	Average length of shoots (cm)
0.44			20±1.26	0.0	0.0
2.2			40±1.36	0.0	0.0
4.4			60±0.28	1.1±0.2[a]	0.2±0.4[a]
8.8			75±0.42	2.0±0.6[ab]	0.6±0.3[a]
13.3			50±0.97	0.0	0.0
17.7			30±1.78	0.0	0.0
8.8	0.53		40±0.98	2.2±1.3[ab]	0.25±0.1[a]
8.8	2.6		50±1.34	6.3±1.5[d]	0.6±0.34[a]
8.8	5.3		80±1.38	12.1±1.8[ef]	1.5±0.56[b]
8.8	10.7		60±0.57	6.4±0.7[d]	0.6±0.45[a]
8.8	16.1		50±0.30	4.7±0.5[c]	0.6±0.12[a]
8.8	5.3	0.46	60±1.45	13.0±0.9[f]	1.0±0.78[ab]
8.8	5.3	2.3	60±0.34	18.3±1.3[g]	2.3±1.22[c]
8.8	5.3	4.6	70±1.67	22.1±1.9[h]	2.6±1.78[c]
8.8	5.3	9.3	70±1.87	35.7±0.4[i]	5.6±2.6[e]
8.8	5.3	13.6	60±0.98	10.4±0.9[e]	4.8±2.65[de]
8.8	5.3	18.6	50±1.76	2.5±1.7[ab]	4.2±1.89[d]
8.8	5.3	23.2	20±0.78	2.4±1.0[ab]	3.2±1.43[cd]

Data were recorded 45 days following transfer of the shoots to MS medium after each subculture. Treatment means followed by same letter(s) within columns are not significantly different from each other ($p<0.05$); comparison by LSD test

Table 2: Effect of different hormonal combinations on *S. cumini* during subcultures. S2* 2nd subculture, S3* 3rd subcultue, S4*-4th subculture. Observation taken after 6th week of subculture

No. of subcultures	Growth regulators (µM)			No. of shoots/node	Average length of shoots (cm)
	BA	KIN	NAA		
S2*	8.8	2.3	5.3	10±0.4[a]	2.3±1.22[a]
	8.8	4.6	5.3	15±0.2[b]	3.6±0.78[b]
	8.8	9.3	5.3	25±1.8[c]	4.5±0.6[c]
S3*	8.8	2.3	5.3	13±0.8[a]	2.4±1.3[a]
	8.8	4.6	5.3	25±1.8[b]	3.3±1.4[ab]
	8.8	9.3	5.3	35±1.8[c]	4.8±0.3[c]
S4*	8.8	2.3	5.3	18±0.5[a]	2.8±1.1[a]
	8.8	4.6	5.3	28±1.7[b]	4.1±0.5[b]
	8.8	9.3	5.3	40±0.9[c]	5.8±0.8[c]

Treatment means followed by different letter(s) within each subculture are significantly different from each other ($p<0.05$) comparison by LSD

For rooting, excised shoots were transfer to, WPM supplemented with NAA and IBA. Basal callus was found in medium containing NAA at 26.8 µM with single root within three to four weeks time (Fig. 1D). IBA (0.049-24.6 µM) produced 70% of rooting within 12 days of culture. Maximum number of roots was induced in 4-4.9 µM IBA within 12 days (Fig. 1E). For *ex vitro* rooting two months old *in vitro* shoots were subjected to an external pulse treatment by dipping in IBA (2.46-24.6 µM) and planted in sand maintained at 100% humidity induced rooting within 15-12 days (Fig.1 I). Maximum number of roots were obtained in 9.8 µM IBA. In *ex vitro* rooting, roots were developed from the internodes as well as from the axils of leaf. The explant with a minimum size of two nodes was enough for the induction of *ex vitro* rooting. This method can be used as an alternative to *in vitro* rooting. Nirmal Babu *et al*. (2000) reported the effect of IBA on *ex vitro* rooting in curry leaf tree.

In vitro rooted plants were transferred to plastic containers filled with non-sterile river sand and covered with polythene bags to provide high humidity (Fig. 1F). The plantlets began to produce new leaves and healthy growth within 22-30 days. But *ex vitro* rooted plants have undergone hardening and rooting procedure in a single step. Within 30-40 days both *in vitro* and *ex vitro* rooted plants were ready for transfer in to field (Fig. 1G and J).

Fig. 1: Micropropagation of *Syzygium cumini*. (A) Mature nodal explant with shoot initiation in WPM containing BA (8.8 μM), and NAA (5.3 μM), (B) and (C) Multiple shoot induction and shoot elongation in the WPM medium containing BA (8.8 μM) and NAA 5.3 μM and Kin 9.3 μM, (D and E) Root induction in WPM with 4.9 μM IBA, (F) Hardened plant in cups, (G) Two months old hardened plantlets in poly bags, (H) Field transferred plantlet after six months, (I) *Ex vitro* root induction using 9.8 μM IBA and (J) *Ex vitro* rooted healthy plants in polybags

In the field, both the *in vitro* and *ex vitro* rooted plants showed 100% survival rate and healthy growth (Fig. 1H).

In conclusion, here we report a successful protocol for micropropagation and *ex vitro* rooting of *Syzigium cumini* from mature nodal explants. By using this protocol it is projected that one can achieve a multiplication rate of thousands of plantlets per node annually. More over *ex vitro* rooting of *in vitro* developed shoots reduce one phase of growth in culture. This increases the efficacy of micropropagation with reduced cost and time for production of plantlets.

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