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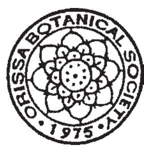

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Micropropagation of *Syzygium cumini* (Linn.) Skeels through *in vitro* culture of seedling derived shoot tips

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ABSTRACT

Shoot tip explants from *in vitro* grown seedlings were cultured on Murashige and Skoog's (MS) medium supplemented with different cytokinins alone or in combination with auxins for multiple shoot induction. Benzylamino purine (BAP, 1.0mg/l) was most effective for multiple shoot proliferation. *In vitro* regenerated shoots were best rooted on ½ MS medium supplemented with 1.4 mg/l Indole-3-butyric acid (IBA). About 95 % of *in vitro* derived plantlets were successfully acclimatized, hardened and established in natural condition.

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1. Introduction

Syzygium cumini, commonly known as Jamun in Hindi and black plum in English, is a member of the family Myrtaceae. It has great importance in the food as well as wood industry and is useful in social forestry programme (Anonymous, 1992). The tree has a great economic importance since most of the parts including the bark, leaves, seed and fruits are used as alternative medicine to treat various diseases (Chaudhary and Mukhopadhyay, 2012). In traditional system of medicine it is extensively used against diabetes and sore throat (Schossler *et al*., 2004). The tree (aerial parts like stem, bark, leaves, flower, fruit, seed) is rich in phytochemicals like glycoside jambolin, anthocyanins, tannins, terpenoids, gallic acid and various minerals (Chaudhary and Mukhopadhyay, 2012). Its bark is used as astringent in dysentery; seeds are antidiabetic; fruits are used to treat cough, diabetes, dysentery, inflammation (Swami *et al*., 2012).

Syzygium cumini suffers from very low seed viability and poor germination in its natural habitat (Dent, 1948). Multiplication in Jamun is also carried out through budding and grafting, but to obtain a scion or bud it requires a fresh shooting period and also population maintained is very low and budding showed less success (Choudhri *et al*., 2013). Tissue culture has emerged as a science with a vast potential for human welfare ranging from large-scale plant production in horticulture and forestry, human health, plant protection as well as environmental protection (Anis and Ahmad, 2016). However woody plants are generally not easy to culture due to constraints of episodic growth pattern, their recalcitrant nature, complex vegetative life cycle, and phenolic exudation. Furthermore, their regeneration and multiplication is not easy under *in vitro* condition, especially when explants are taken from mature trees. They often secrete substances into the medium in response to wounding or excision which inhibits the growth and development of explants *in vitro* (Naaz *et al*., 2014). A few tissue culture works has been done in *Syzygium cumini*. Hence in the present study attempts has been made for mass multiplication of this plant using shoot tip explants from *in vitro* seedlings.

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2. Materials and Methods

Seeds of *Syzygium cuminii* were collected during July. Then they were brought to the laboratory and thoroughly washed with running tap water for about an hour. The seeds treated with 5% teepol (v/v) for 5 minutes and then washed with distilled water. The seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for about 3-5 minutes followed by thorough wash with sterilized distilled water for 3-4 times to remove traces of $HgCl_2$. Seeds were then inoculated on half and full strength of Murashige and Skoog's (1962) basal medium (MS) with and without agar (0.8%). Shoot tips from two weeks old seedlings were excised aseptically and cultured on MS medium alone or supplemented with different concentrations (0.2 to 1.5 mg/l) and combinations of growth regulators (6 benzylamino purine or BAP, Kinetin or Kn, Indole-3-acetic acid or IAA, Indole-3-butyric acid or IBA, α-Napthalene acetic acid or NAA). The *in vitro* grown shoots were transferred on to half and full strength of MS basal medium without or supplemented with auxins (1.0 -1.8 mg/l of IBA, IAA or NAA) for root induction. All the cultures were maintained at 25 $\pm$ 2^0C with 16 h photoperiod at 55% relative humidity. For proper growth and development the cultures were frequently transferred onto fresh medium at fifteen days intervals.

For each treatment, ten replicates were taken and each experiment was carried independently and repeated thrice. The cultures were kept under regular observation and data were recorded at 7-day interval. The percentage of response, mean number of shoots, mean shoot length, mean number of roots and standard error of mean (S.E.M) for each treatment were calculated.

3. Results

3.1 *In vitro seed germination*

Germination of seeds was observed in all the media tested but the best response (90%) was observed on ½ MS liquid medium. The seeds took 5-6 days for germination and after two weeks 4 shoots (due to polyembryony) developed (Fig. 1).

3.2 *Multiple shoot induction*

The shoot tips were carefully excised from *in vitro* grown seedlings and transferred to MS media devoid of any growth regulators or MS supplemented with different concentrations and combinations of growth regulators. The explants failed to show any morphogenetic response on growth regulator free MS medium. Shoot tips cultured on MS media supplemented with different concentrations of cytokinins (0.5 to 1.5 mg/l of BAP or Kn) alone or in combination with lower concentrations of auxins (0.2 and 0.5 mg/l of IAA, IBA or NAA) responded differently (Table 1).

Among the different concentrations and combinations of growth regulators tested, best response was observed on MS medium supplemented with 1.0 mg/l BAP (Table 1). BAP was found to be more effective than Kn. It was also observed that addition of auxins to the cytokinin supplemented medium reduces the percentage of response and number of shoots per explant. Shoot buds started developing after one week of inoculation on MS medium supplemented with 1.0 mg/l BAP (Fig. 2) and more number of shoot buds were developed after two weeks (Fig. 3). Elongation of shoots and development of more shoots were observed after three weeks of culture (Fig. 4). An average of 14.4 ± 0.2 shoots having an average of 2.2 ± 0.1 cm shoot length per explant was recorded after four weeks of culture (Table - 1, Fig. 5).

3.3 *In vitro rooting*

The *in vitro* regenerated shoots (more than 2cm) were carefully separated and transferred to rooting medium. Full strength of MS medium with or without auxins and ½ MS medium without auxins did not respond for rhizogenesis but showed callusing at the base. After four weeks of culture it was noticed that ½ MS basal media supplemented with IBA produced better response than the media supplemented with IAA or NAA. Among all the concentrations tested 1.4 mg/l IBA showed best response for rooting (Table – 2). Half - strength MS medium supplemented with 1.4 mg/l IBA produced an average of 4.6 ± 0.2 roots with an average length of 4.2 ± 0.2 cms (Table 2; Fig. 6).

3.4 *Acclimatization*

After four weeks of maintenance in the rooting medium the plantlets with well developed roots were carefully removed from the culture tubes and washed thoroughly by sterilized double distilled water to remove any remains of medium. The well rooted plantlets were transferred to thermo cool glass containing a sterile mixture of garden soil, sand and compost in the ratio of 1:1:1 (Fig. 7). They were irrigated with MS liquid medium at two days interval for fifteen days and kept in the culture room at 25 ± 2^0 C with 16 h photoperiod at 55% relative humidity. Gradually they were irrigated with ½ MS liquid medium and then by plain water and transferred to less humid conditions in order to acclimatize the plants to normal atmospheric conditions. About 95 percent of the regenerated plants survived after hardening procedure (Fig. 8). No morphological difference was found in between the natural and *in vitro* grown plants.

Table 1

Effect of plant growth regulators on multiple shoot formation from shoot tip derived from *in vitro* seedling of *Syzygium cumini*

Plant growth regulators (mg/l)					% response	Shoots/explant	Shoot length in cm
BAP	Kn	IAA	IBA	NAA		Mean* ± S.E.M	Mean* ± S.E.M
0.5					63	9.2 ± 0.2	1.4 ± 0.1
1.0					80	14.4 ± 0.2	2.2 ± 0.1
1.5					66	11.3 ± 0.1	1.7± 0.2
	0.5				46	6.1 ± 0.1	1.0 ± 0.1
	1.0				66	9.6 ± 0.2	1.5 ± 0.2
	1.5				50	7.4 ± 0.2	1.2 ± 0.1
1.0		0.2			70	12.1 ± 0.1	1.8 ± 0.1
1.5		0.5			60	8.6 ± 0.2	1.3 ± 0.1
	1.0	0.2			56	7.3 ± 0.1	1.3 ± 0.2
	1.5	0.5			43	5.7 ± 0.1	1.1 ± 0.1
1.0			0.2		66	9.5 ± 0.2	1.2 ± 0.1
1.5			0.5		56	6.6 ± 0.1	1.0 ± 0.1
	1.0		0.2		53	5.8 ± 0.1	1.1 ± 0.2
	1.5		0.5		40	3.7 ± 0.2	0.9 ± 0.1
1.0				0.2	73	10.7 ± 0.3	1.6 ± 0.1
1.5				0.5	60	7.8 ± 0.2	1.4 ± 0.1
	1.0			0.2	56	6.2 ± 0.1	1.3 ± 0.1
	1.5			0.5	46	4.2 ± 0.1	1.0 ± 0.2

*Mean values given in the table are the average of three independent experiments each with 10 replicates with the standard error

Table 2

Effect of auxins on root induction of *in vitro* shoots

Auxins (mg/l)			% rooting	Number of roots/shoot	Root length in cm
IBA	IAA	NAA		Mean* ± S.E.M	Mean* ± S.E.M
1.0			80	2.8 ± 0.1	2.3 ± 0.2
1.2			83	3.7 ± 0.2	3.5 ± 0.3
1.4			96	4.6 ± 0.2	4.2 ± 0.2
1.6			90	3.2 ± 0.2	3.1 ± 0.2
1.8			73	2.4 ± 0.1	2.2 ± 0.1
	1.4		76	2.3 ± 0.1	3.2 ± 0.2
	1.6		83	2.7 ± 0.2	2.8 ± 0.1
	1.8		70	1.8 ± 0.1	1.8 ± 0.1
		1.4	70	2.2 ± 0.2	2.7 ± 0.1
		1.6	60	1.6 ± 0.1	2.1 ± 0.2
		1.8	53	1.2 ± 0.1	1.6 ± 0.1

*Mean values given in the table are the average of three independent experiments each with 10 replicates with the standard error

Fig. 1: Germination of seed on ½ MS medium after 2 weeks of culture, Fig. 2: Shoot tips developed after one week on MS + 1.0 mg/l BAP, Fig. 3: Multiple shoots regeneration from shoot tips after two weeks on the same medium, Fig. 4: Multiple shoots after three weeks of culture, Fig. 5: Multiple shoots proliferation after four weeks of culture on MS + 1.0 mg/l BAP, Fig. 6: Rooting on ½ MS medium + 1.4 mg/l IBA, Fig. 7: Regenerated plant acclimatized on a potting mixture of 1:1:1 of soil, sand and compost, Fig. 8: *In vitro* regenerated plants growing in natural condition.

4. Discussion

In the present study it was observed that MS medium supplemented with BAP induced multiple shoots in shoot tip explants derived from two weeks grown *in vitro* seedlings. In contrast Yadav *et al*. (1990) also studied *in vitro* micropropagation through culture of shoot tip and node from 10-15 day-old seedling of *Syzygium cumini* using MS medium supplemented with BA singly and in combination with NAA, IAA or IBA. Jain and Babbar (2000) studied multiple shoot development from epicotyl segments of *in vitro* seedling of *Syzygium cumini* using MS medium supplemented with 1.0 mg/l BA. In this study we also observed that MS medium supplemented with only BAP 1.0 mg/l was best for induction of multiple shoots from shoot tip and it corroborates the findings of Jain and Babbar (2000).

In the present study root induction was observed on ½ MS medium supplemented with IBA, IAA and NAA. However best response was observed on half strength of MS medium supplemented with 1.4 mg/l IBA. IBA was also reported to have favoured root induction in earlier reports of *S. cumini* (Remashree *et al*., 2007; Choudhri *et al*., 2013; Vidwans *et al*., 2015). IBA induced rooting was also reported in other fruit trees including pomegranate (Naik *et al*., 1999). Rathore *et al*. (2004) also reported best rooting on half-strength MS + 10.0 µM IBA. However addition of 0.1% activated charcoal to the medium was found to be essential. Contrary to our work Jain and Babbar (2000) and Jain and Babbar (2003) reported rooting on Knop's medium supplemented with 1.0 mg/l IAA.

5 Conclusion

The present work describes a protocol for mass multiplication of *Syzygium cumini*, an economically important species, using shoot tip explants from *in vitro* grown seedlings. This method of plant regeneration has the potential to meet the demand of quality planting materials of the species for commercial utilization.

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