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Micropropagation studies in jamun (*Syzygium cuminii* L.)

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Abstract

In vitro propagation of the jamun (*Syzygium cuminii* L.) can be achieved by using single nodal explants taken from the seedlings. Single nodal explants cultured on half strength MS along with 2mg/l BAP + 3% sucrose + 3% activated charcoal was proved to be better for shoot proliferation. The shootlets were successfully rooted on 1/4th strength MS along with 3% sucrose and 2.5 mg/l IBA. The rooted shootlets were then hardened on vermiculite (32.5 % survival) for six weeks and then planted in the polybags and transferred to the field.

Key words: Activated charcoal, *In vitro* propagation, Media strength, Nodal explants, Per cent survival, Shootlets, Shoot proliferation, Vermiculite.

1.2 Introduction

Jamun (*Syzygium cuminii* L.) a dryland horticultural crop of Myrtacea family.

It is an important minor and important fruit of India. It is widely grown from Indo-Gangetic plains in the north to Tamil Nadu in south, and in many parts of the India (Singh and Shrivastava, 2000). Jamun gained its importance due to its nutritive value and of its medicinal properties. Fruits are tasty, good source of iron, minerals, sugars and proteins. The vinegar prepared out of juice extracted from slightly unripe fruit is stomachic, carminative, diuretic, while fruit syrup is remedy for diarrhea (Thaper, 1958). The seed powder has anti-diabetic properties and is a lotion for the cure of ringworm (Dustur, 1952). In jamun multiplication 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. Tissue culture is a technique which offers an alternative procedure for the vegetative propagation of various plant species. This technique so far had proved successful in many woody plants like eucalyptus (Burger, 1987), and fruit plants like kiwi (Hassan *et al*., 2000) and guava (Jaiswal & Amin, 1992; Amin & Jaiswal, 1987). However it is often difficult to culture explants derived from mature trees due to the constraints of episodic growth pattern, their recalcitrant nature and the high incidence of microbial contamination. The often high levels of polyphenol exudation, virtification, low response level and difficulty in root induction are some of the constraints which hinders micropropagation studies in perennial woody fruit crops (Bonga, 1992). Present investigation was an alternative to develop a mass multiplication procedure through *in vitro* culture using nodal explants of jamun seedlings.

1.3 Material and Methods

The investigations on micropropagation studies in jamun (*Syzygium cuminii* L.) were carried out at Department of Crop Improvement and Biotechnology, Kittur Rani Channamma College of Horticulture, Arabhavi (University of Agricultural Sciences Dharwad), Karnataka during 2009-10 by using explants from 5 years old jamun tree and 11 months old jamun seedlings. A twig of 3-4 nodes was excised from the mature tree when in shooting stage and a twig of 5-6 nodes was excised from 11 months old seedlings. The twigs were surface sterilized with mercuric chloride (0.1 %) in a sterile container and taken to the laboratory, then washed repeatedly for 4-5 times with distilled water and twigs were cut into the single node (1 cm), double node (3-5 cm) and apical portion. The nodes at distal ends were discarded due to its tender nature. The explants were then kept in a solution of 3 per cent sucrose with 100 mg/l ascorbic acid under refrigerated condition for 1 day. On the next day the explants were washed 4-5 times and treated with 0.2 per cent (w/v) bavistin (Fungicide) + streptomycin sulphate (Bactericide) 100mg/l, for 20 minutes then washed with distilled water for 5-6 times and again in LAF (Laminar Air Flow chamber) the explants were surface sterilized with mercuric chloride (0.5 %) for 11 minutes and washed 4-5 times, then the explants were ready to culture.

The cultures were incubated in an AC room at a temperature 25±2 ^{0} C, RH of 60 per cent, under a photoperiodic regime of 16 hrs light and 8 hrs dark cycles. For light purpose a florescent tube of 1600 flux, of 36 W / 6500 K of Surya Company was used. The observations like per cent establishment, per cent mortality, mean number of shoots per expalnt, mean shoot length (cm), mean number of leaves per shoot, per cent rooting and per cent survival during hardening were recorded and analyzed by completely randomized design (CRD) as described by Panse and Sukhatme (1978).

1.4 Result and discussion

MS at full strength increased the number of shoot (8.8), length of the shoot (2.62 cm), mean number of leaves per shoot (7.07) (Table 1), as also reported by Muralikrishna (1988) while comparing White's, B5 and MS medium on *in vitro* shoot proliferation of pomegranate, grape, and guava, found MS medium as the best. In the present study it was found that the treatment with full strength MS medium showed on par results with half strength MS and half and full strength WPM (Woody Plant Medium). In this case the use of MS at half strength was better because it was a low salt media and cost effective and there was a good control over phenolic browning. Similar results were also noticed by Adiga (1996) where in half strength medium found to be the best in jackfruit.

In the present study BA, neither NAA, nor their combinations showed any significant influence on shoot proliferation (Table 2). Where as optimum response was recorded by Jain and Babbar (2000) on the medium containing 1 mg/l BA with an average of 8.6 shoots per explants in jamun (*Syzygium cuminii*). In contrary to results in the present investigation revealed that both BA and NAA and their interactions at all lower concentrations improved the shoot proliferation but to know significant difference further study will be needed with higher concentrations and with other cytokinins.

In vitro rooting usually follows treatment with an auxin. In the present experiment auxins like IBA and IAA were used to induce rooting *in vitro*. The highest rooting (100%) and significantly more number of roots (5.5) were associated with 2.5 mg/l IBA (Table 3). The roots were longer (5.7 cm) when IBA was used @ 1 mg/l. The mean number of secondary roots was higher (4.0) with IAA 5 mg/l+ IBA 5 mg/l. Early rooting (13 days) was noted in IBA at 25 mg/l (Table 3, Fig.1). It was found that when IBA was used as a source of auxin, the *in vitro* root production was higher compared to the IAA. Similar results were also noticed by Drew *et al* (1993) in *Carica papaya* L. where in root initiation was higher using IBA than IAA, and NAA. In the present study the longer root length was noticed in IBA 25 mg/l this might be due to early rooting where as more number of roots was noticed at 2.5 mg/l and this might be due to development of shorter roots and more in numbers.

The per cent survival was highest initially in all the potting media but gradually declined till the fifth week and remained constant afterwards without

any further decline (Table 4, Fig.2). This suggests that plants require about five weeks adapting themselves to typical plant water control mechanisms, especially the stomatal regulation and development of proper vascular connections between the shoot and the roots for better establishment of the plantlets. After six weeks of hardening, highest extent of survival (35.7%) was recorded with vermiculite, and there was no response when perlite and sand was used as a potting media. At the beginning perlite has given higher per cent survival and followed by sand but later from 3 to 5th week there was a twofold decrease in the survival per cent. This might be due to that perlite and sand though more porous in nature but water holding capacity is very low compared to other potting media and summer season which leads to the higher moisture loss and with low relative humidity buildup causing death of the explants. Whereas vermiculite and coco pith were more porous with higher water holding capacity. Due to higher moisture retention and higher humidity buildup there is good per cent survival in vermiculite and coco pith. Overall per cent survival showed only 35.7 per cent and this might be due to the hot summer season and lack of improper relative humidity buildup. Then the plantlets are planted in polybags and transferred to the field.

Table 1. Influence of basal media and their strength on mean number of shoots per explant, shoot length and mean number of leaves in jamun seedlings

Treatment	Mean number of shoots per explant	Mean shoots length (cm)	Mean number of leaves per shoot
T1-Half strength MS Media	7.0	1.68	6.23
T2-Full strength MS Media	8.8	2.62	7.07
T3-Half strength WPM Media	6.2	2.42	5.63
T4-Full strength WPM Media	6.8	2.20	4.86
SEm±	1.8	0.26	0.53
C.D (0.01)	NS	NS	2.30

Observations are recorded at 7th week after the transfer to the media.
NS- Non significant MS-Murashige and Skoog media WPM- Woody Plant Media

Table 2. Effect of BA and NAA, on response of nodal explants to shoot proliferation

Treatment	Mean number of shoots per explant	Mean length of shoot (cm)	Mean number of leaves per shoot
T1-BA 1 mg/l	6.25	2.10	4.475
T2-BA 0.5 mg/l	6.75	2.02	4.507
T3-NAA 2 mg/l	5.50	1.90	4.175
T4-NAA 2.5 mg/l	6.50	2.55	5.950
T5-BA 1 mg/l+ NAA 2 mg/l	7.00	1.80	3.957
T6-BA 0.5 mg/l+ NAA 2 mg/l	6.50	1.85	4.025

T7-BA 1 mg/l+ NAA 2.5 mg/l	8.50	1.82	7.500
T8-BA 0.5 mg/l+ NAA 2.5 mg/l	8.00	2.02	3.955
SEm±	2.12	0.28	2.129
C.D (0.01)	NS	NS	NS

Observations are recorded at 7th week after the transfer to the media.
NS- Non significant BA-Benzyladenine NAA- Napthalene acetic acid

Table 3. Effect of IAA and IBA on *in vitro* production of roots in jamun

Treatment	Per cent rooted	Mean number of roots	Mean root length (cm)	Mean number of secondary roots	Days taken for rooting
T1-IAA 2.5 mg/l	85 (67.23)	3.0	2.60	2.5	48.16
T2-IAA 5 mg/l	93 (74.75)	2.5	2.05	1.0	30.60
T3-IAA 15 mg/l	87 (68.90)	2.0	1.46	1.0	27.08
T4-IAA 25 mg/l	84 (66.90)	1.5	3.95	1.0	17.00
T5-IBA 2.5 g/l	100 (84.76)	5.5	3.06	2.5	45.08
T6-IBA 5 mg/l	95 (77.24)	1.0	3.45	1.0	19.00
T7-IBA 15 mg/l	97 (80.11)	1.5	2.75	1.5	21.10
T8-IBA 25 mg/l	93 (74.33)	1.5	5.70	1.5	13.00
T9-IAA 5 mg/l+ IBA 5 mg/l	100 (84.76)	1.5	4.81	4.0	18.10
T10-IAA 10 mg/l + IBA 10 mg/l	93 (74.68)	3.5	1.6	4.5	14.10
SEm±	1.00	0.30	0.15	0.18	0.900
C.D (0.01)	4.10	1.21	0.61	0.74	3.660

The values given in parenthesis are arc sine transformed values ($Sin^{-1}\sqrt{X/100}$).
Observations are recorded at 8th week after the transfer to the media.
IAA-Indole-3-acetic acid IBA-Indole-3-butyric acid

Table 4. Effect of potting media on extent of survival of *in vitro* plantlets during hardening in jamun

Treatment	Survival (%)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
T1-Vermiculite	90 (73.69)	90 (71.59)	73 (58.69)	44 (41.54)	35.5 (36.56)	35.5 (36.56)
T2-Perlite	95 (77.17)	87 (68.86)	44 (41.54)	27 (31.29)	00.0 (00.64)	00.0 (00.64)
T3-Cocopith	88 (69.73)	85 (67.25)	53 (46.71)	37 (37.45)	21.5 (27.62)	21.5 (27.62)
T4-Sand	70 (56.79)	42 (40.39)	20 (26.55)	00 (00.64)	00.0 (00.64)	00.0 (00.64)
SEm±	01.44	00.54	00.37	00.23	00.07	00.07
C.D (0.01)	06.22	02.33	01.59	00.99	00.31	00.31

The values given in parenthesis are arc sine transformed values ($Sin^{-1}\sqrt{X/100}$)

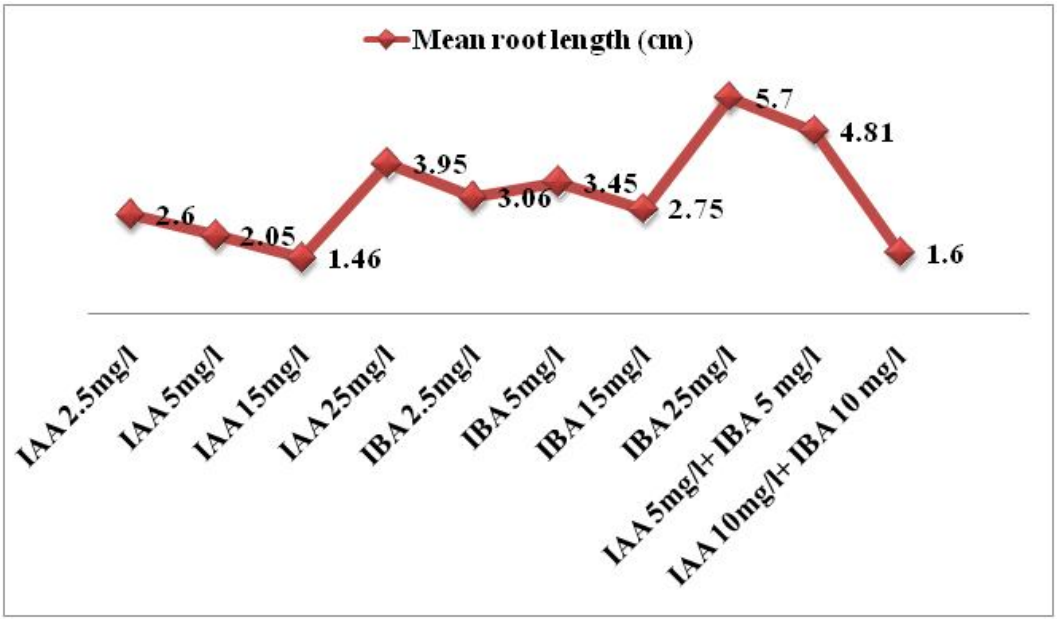

Fig. 1 Effect of IAA and IBA on mean root length in jamun

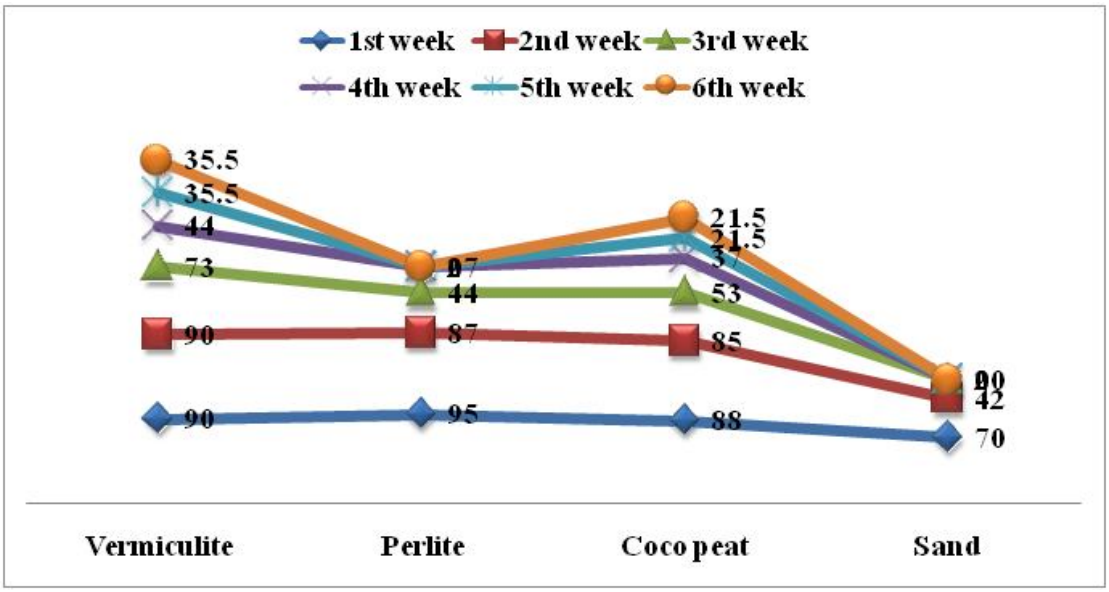

Fig. 2 Effect of potting media on extent of survival of *in vitro* plantlets during hardening in jamun

1.5 Conclusion

Jamun (*Syzygium cuminii* L.) can be easily propagated by *in vitro* using single nodal segment from the seedlings cultured on half strength MS along with

2mg/l BAP + 3% sucrose + 3% activated charcoal was proved to be better for shoot proliferation. The shootlets were successfully rooted on 1/4th strength MS along with 3% sucrose and 2.5 mg/l IBA and hardened on vermiculite (32.5 % survival) for six weeks and then planted in the polybags and transferred to the field.

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