

Original Research Article

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In vitro Shoot Proliferation from Nodal Segments of Indian Blackberry (*Syzygium cumini* L.)

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ABSTRACT

Keywords

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Micropropagation of Indian Blackberry (*Syzygium cumini* L.) species locally called as Jamun, has always been a challenging task. In India, Jamun tree is an underutilized minor fruit crop species possessing a high medicinal and nutritional value. An efficient protocol for rapid shoot proliferation of Jamun (cv. Rajamun) from nodal segment of locally grown mature trees has been developed. Experiments were conducted at Biotech Centre, Dr. PDKV, Akola during 2016-18. The Lloyd and McCown woody plant medium (WPM) used as a basal media supplemented with growth regulators at varying concentrations. Nodal explants were cultured on WPM supplemented with BAP (6-Benzylamino purine 8, 8.5 and 9 $\mu\text{M/l}$), Kn (Kn 8, 8.5 and 9 $\mu\text{M/l}$). WPM supplemented with BAP + Kn @ 8 $\mu\text{M/l}$ was proved to be significantly best at 5% level for high survival rate of 93.33% and required less time i.e. 32.43 days to initial shoot sprout after explants inoculated with 2.53 initial shoot multiples. The treatments for shoot proliferation were conducted on WPM supplemented with BAP @ 13, 14 and 15 $\mu\text{M/l}$, Kn @ 8, 8.5 and 9 $\mu\text{M/l}$ and NAA (Naphthalene acetic acid) @ 5 $\mu\text{M/l}$. For high frequency multiple shoot induction WPM with 13 μM BAP + 8.5 μM Kn + 5 μM NAA was proved to be significantly superior at 5% level after 4 weeks of first subculture giving 2.87 shoot multiples, followed by maximum 4.20 shoot multiples at 6 weeks stage of second subculture with significantly 3.21cm shoot length elongation at 10 weeks when supplemented on same media against 1 cm of control.

Introduction

India is a tropical country, where many minor fruit tree species are very popular and their fruits are always in high demand as the seasonal delicacies. However, most of these fruit trees are not commercially cultivated due to the poor viability of their seeds and remain underutilized for their medicinal and

nutritional value. These fruit trees provide a significant support to the livelihood of rural and tribal people as they generate additional household income. Indian Blackberry (*Syzygium cumini* L.) locally known as 'Jamun' is one of such underutilized minor fruit species having great importance in India. The use of medicinal plants plays an important role in the lives of rural poor peoples,

particularly in remote parts of developing countries, which are poorly served with health facilities. Products derived from plants or its any part that found in the tropical forests is not only useful for traditional medicine, but also often has a considerable market value. The sale of raw materials for pharmaceutical purposes can be especially important for subsistence farmers. Plant tissue culture is widely used and commercially viable technique of Plant Biotechnology. Tissue culture is more successful in soft, succulent, asexual and vegetative propagated plant species as compare to hard species. Woody plants are more difficult to propagate than herbaceous species. The currently followed methods of propagation of woody species are cuttings, grafting and layering. But these methods have been less successful with woody plants. It is due to the rapid loss of rooting ability with age of woody plant and the limited number of propagules that can be obtained in a reasonable time. The conventional methods of propagation of woody plants have limited potential for their large-scale multiplication and production. Apart from this *S. cumini* suffers from very low seed viability and poor germination in its natural habitat (Dent, 1948). The seed germinates when fresh but, after two weeks at room temperature, the same seed is not viable. Seed storage behaviour is recalcitrant; germinate well when fresh but its viability is lost within two weeks of open storage at room temperature. Due to their commercial importance and extensive use in medicine, there is a need to develop rapid and reliable methods of *in-vitro* propagation of woody plant species. It is therefore, a need to identify tree species which are capable of multiple uses, such as wood production as well as alternative products such as medicines. This would promote recognition of the value of particular species and result in their inclusion and consideration in forest management planning. Such forest trees can serve as man's medicines and many other

needs while assisting in protecting the environment upon which animals and other living creatures depend. Considering its great medicinal value, large scale cultivation of elite species of Indian blackberry is underway in many parts of Maharashtra particularly Vidarbha regions. The mass scale production of Jamun seedling of elite and economical species under government sponsored entrepreneurship program that could be possible to generate additional revenue. Therefore an attempt was made to establish a standard tissue culture protocol for in vitro shoot proliferation using nodal segment of Indian blackberry (Jamun).

Materials and Methods

The experimental work was conducted at Plant Tissue Culture laboratory, Centre of Excellence in Plant Biotechnology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Dist. Akola (M.S.) during year 2017-18. The explants (nodal segment) were collected from the campus in sterilized polythene bags and were brought in the laboratory for further processes. The explants were washed with sterilized water to remove the extra dust particles. Explants were then subjected with 1% Tween-20 detergent for 5mins, followed by 1% Bavistin (systemic fungicide) for 25mins and 1% Streptocycline antibiotic for 25mins on orbital shaker followed by repeated washing with sterilized water thrice after each sterilization treatment. The explants were further treated with 0.1% HgCl₂ solution under laminar air flow for 5-7 mins followed by washing with distilled water. To minimize the effect of phenols in explants, they were treated with antioxidants solution (0.1% ascorbic acid + 0.05% citric acid) for 20-25 mins. The experiment was laid out in Completely Randomized Design with three replications. This study was carried out to standardize media combinations for *in vitro* culture establishment protocol by using nodal

explant. The protocol showed variable response of nodal explants on nutrient media with different growth regulators combinations.

The nodal explants were inoculated on Lloyd and McCown (woody plant media, 1980) supplemented with different combinations of cytokinin's i.e. Benzyl 6 amine purine, Kn (Kn) @ 8.0 μM , 8.5 μM and 9.0 μM . Woody plant media without cytokinin hormone was treated as control summarized in table 1 and figure 1. The cultures were maintained in culture room at temperature of 25 ± 2 °C with 1000 lux or lower light generated by fluorescent tubes, automatic timers were used to regulate photoperiod. The time period was imposed at an interval of week period viz., 1st, 2nd, 3rd and 4th weeks. The response of established cultures on woody plant media with different growth regulators combinations on shoot proliferation with BAP @ 13 μM , 14 μM , 15 μM per litre, Kn @ 8.0 μM , 8.5 μM , 9.0 μM per litre and NAA @ 5.0 $\mu\text{M/l}$ were tested under time interval of week basis. The cycle of sub-culturing began from 6th stage onwards. The cultures were transferred on fresh medium after every week time. Woody plant media (WPM) without cytokinin hormone was treated as control summarized in table 1 and figure 1. The observations for shoot proliferation were observed from 6th to 10th weeks span.

Results and Discussion

Response of nodal explants on WPM with different combinations of BAP, Kn and NAA at 4th, 6th, 8th and 10th week of explants inoculation for *in vitro* shoot proliferation

The effect of growth regulators on shoot proliferation, shoot multiples and shoot length growth after 4th, 6th, 8th and 10th week after explant inoculation was attempted using nodal segment from 10 years old local mother plant. Sub-culturing after 4th week of explants establishment *in vitro* leads to enhance

maximum proliferation and quantity of shoots as summarized in table 1 and figure 1. At last, the shoot length was calculated after 10th week of subculture. The number of multiple shoots initiated after 4th week of explants inoculation was 2.87 shoots per culture of treatment T₂, when supplemented on WPM with 13 μM BAP + 8.5 μM Kn + 5 μM NAA respectively, followed by treatment T₇ exhibiting 2.60 shoot multiples provided by WPM + 15 μM BAP + 8 μM Kn + 5 μM NAA, which was followed by treatment T₁ exhibiting 2.53 shoots multiples on WPM with 13 μM BAP + 8 μM Kn + 5 μM NAA, against minimum shoot average of only 1.00 was observed in case of control treatment (T₁₀) when supplemented with plain woody plant medium (WPM) as summarized in table 1 and figure 1.

The total number of multiple shoots initiated at 6 weeks of explants inoculation were observed i.e. 4.20 shoots per culture of treatment (T₂) supplemented with WPM + 13 μM BAP + 8.5 μM Kn + 5 μM NAA, followed by treatment T₇ expressing 3.53 shoot multiples provided by WPM + 15 μM BAP + 8 μM Kn + 5 μM NAA respectively, which was followed by treatment T₆ bears 3.10 shoots containing nutrient supplement of WPM + 14 μM BAP + 9 μM Kn + 5 μM NAA against minimum shoot of average 1.73 when supplemented plain woody plant medium (WPM) as summarized in table 1 and figure 1. The total number of multiple shoots initiated at 8 weeks of explants was observed i.e. 1.93 shoots per culture of treatment T₂, (WPM+ 13 μM BAP + 8.5 μM Kn + 5 μM NAA) followed by treatment T₇ giving 1.53 shoot multiples on WPM + 15 μM BAP + 8 μM Kn + 5 μM NAA, which it was followed by treatment T₃ yielding 1.47 shoots containing nutrient supplement of WPM + 13 μM BAP + 9 μM Kinetin + 5 μM NAA against minimum shoot of average 0.20 observed in case of control treatment (T₁₀) when supplemented plain woody plant medium.

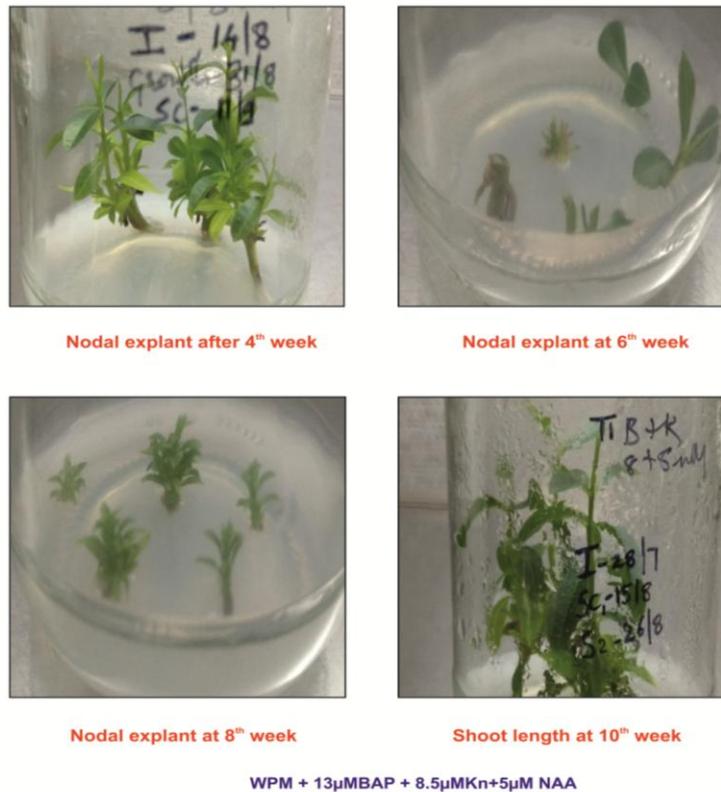
Table.1 Details of effect of nodal explants to different media combinations for *in vitro* shoot proliferation of Indian blackberry in combination of woody plant medium (WPM) with cytokines (BAP+Kn+NAA) at 4th, 6th, 8th and 10th week of explants inoculation.

Treatment details		No. of multiple shoots initiated after 4 weeks	No. of multiple shoots initiated at 6 weeks	No. of multiple shoots initiated at 8 weeks	Shoot length at 10 weeks (cm)
Treatment No.	Combinations				
1	2	3	4	5	6
T ₁	WPM + 13 µMBAP + 8.0 µMKn+ 5 µM NAA	2.53 III	1.47	0.93	0.17
T ₂	WPM + 13 µMBAP + 8.5 µMKn+ 5 µM NAA	2.87 I	4.20 I	1.93 I	3.21 I
T ₃	WPM + 13 µMBAP + 9.0 µMKn+ 5 µM NAA	2.27	3.07	1.47 III	1.12 II
T ₄	WPM + 14 µMBAP + 8.0 µMKn+ 5 µM NAA	2.27	2.53	1.33	0.57
T ₅	WPM + 14 µMBAP + 8.5 µMKn+ 5 µM NAA	2.20	2.60	1.13	0.50
T ₆	WPM + 14 µMBAP + 9.0 µMKn+ 5 µM NAA	2.33	3.10 III	1.03	0.49
T ₇	WPM + 15 µMBAP + 8.0 µMKn+ 5 µM NAA	2.60 II	3.53 II	1.53 II	0.67
T ₈	WPM + 15 µMBAP + 8.5 µMKn+ 5 µM NAA	2.47	2.53	1.20	0.73 III
T ₉	WPM + 15 µMBAP + 9.0 µMKn+ 5 µM NAA	1.60	2.00	0.60	0.40
T ₁₀	WPM without cytokines and auxin (Control)	1.00 c	1.73 c	0.20 c	0.32 c
	SE (m) ±	1.01	0.15	0.06	0.05
	CD 5%	0.32	0.44	0.18	0.13

Abbreviations: WPM – Woody Plant Medium (Lloyd and McCown, 1980), BAP- Benzyl 6 amine purine, Kinetin, NAA- Naphthalene acetic acid

Kn –

Fig.1 Response of nodal explants on WPM with different combinations of BAP, Kn and NAA at 4th, 6th, 8th and 10th week of explants inoculation for *in vitro* shoot proliferation



After 10th week of explants inoculation, the total number of multiple shoots thus obtained till this stage, the shoot length was measured and the average values were summarized in table 1 and depicted in figure 1.

The maximum average shoot length was measured i.e. 3.21cm of treatment T₂, when supplemented on WPM + BAP + Kinetin + NAA @ 13 + 8.5 + 5 µM respectively, followed by second highest treatment T₃ giving 1.12 cm average shoot length supported by WPM + BAP + Kinetin + NAA @ 13 + 9 + 5 µM duly, while third treatment was T₈ giving 0.73 cm average shoot length on WPM + BAP + Kinetin + NAA @ 15 + 8.5 + 5 µM against minimum average of 0.32 cm shoot length observed in case of control treatment. Out of all treatments combination, treatment 1 i.e. WPM with BAP + Kn @ 8 µM/l was proved to be significantly

best at 5% level for high survival rate of 93.33% with required 32.43 days for initial shoot sprout and 2.53 initial shoot multiples after 4th week span respectively summarized in table 1 and depicted in figure 1.

However, for high frequency multiple shoot proliferation and shoot elongation, media supplemented with 13µM BAP + 8.5µM Kn + 5µM NAA was proved to be significantly superior at 5% at 4 weeks of first subculture giving 2.87 shoot multiples, followed by maximum 4.20 shoot multiples at 6 weeks stage of second subculture with significantly 3.21cm shoot length at 10 weeks of duration duly.

The relevant work for shoot multiplication was substantially increased after 3rd week of nodal segment inoculation highlights the successful establishment of culture of Indian blackberry

suggesting positive association between BAP and NAA with 17.7 μ M BAP and 1.3 μ M NAA by third sub-culture on multiplication medium (Yadav *et al.*, 1990; Anand *et al.*, 1999; Rathod *et al.*, 2004; Remashree *et al.*, 2007, Choudhri *et al.*, 2013; Naaz *et al.*, 2017). The identical results suggest positive association of WPB with BAP, Kinetin and NAA in other crops like *M. alternifolia*. Oliveira *et al.*, (2010); Guava Meghwal *et al.*, (2010), tea tree (*Melaleuca alternifolia* Cheel) Jala *et al.*, (2014). The parameters optimized during the present experimentation has practical relevance during standardization of high shoot proliferation of Jamun elite species through tissue culture approach with slight modification.

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References

- Chaudhary, B. and Mukhopadhyay, K., (2012). *Syzygium cumini* (L.) Skeels: a potential source of *Nutraceuticals*. 2(1):46-53.
- Choudhari, N.A., G.S. K Swamy and Jagadeesh, R.C., Prabhuling and Basavarajappa, H.R., (2013). Micropropagation studies in Jamun. *Int. J. of Applied Biotechnol.* 3(1): 781-788.
- Dent, T. V. (1948). Seed storage with particular reference to the storage of seeds of Indian Forest Plants. *Indian For. Res. (N.S.) Silviculture*, 7:1-134.
- Hemant Sharma and Vashistha, B.D., (2015).

- Plant tissue culture: a biological tool for solving the problem of propagation of medicinally important woody plants- A review. *Int. J. of Adv. Res.* 3(2): 402-411.
- Jadhav, N.M. and Deodhar M.A. (2015). and *In vitro* propagation of aromatic woody plant *Mesua ferrea*. *J. of plant sci. and res.* 2 (1):120.
- Jala, A. and Chanchula, N. (2014). Effect of BA and NAA on micropropagation of tea tree (*Melaleuca alternifolia* Cheel) *in vitro*. *Thai J. of Agril. Sci.* 40:37-43.
- Lloyd, G.B. and McCown, B.H. (1980): Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use of shoot-tip culture. *Proc. Int. Plant Prop. Soc.* 30: 421-427.
- Mathew Mary K. and Molly Hariharan, (1989). *In vitro* shoot regeneration in *Syzygium aromaticum*. *Ann. of Bot.* 65, 277-279.
- Prabhuling, G., Rashmi, H. and Babu A.G., (2017). *In vitro* regeneration of jamun (*Syzygium cumini* L.). *Int. J. of Sci. and nature.* 8(4): 902-907.
- Purohit, K. and Daradka, J., (2000). Antidiabetic activity of black plum seeds. *Hamdard.* 43:331.
- Rathore, V., Shekhawat, N.S and Singh, R.P., Rathore, J.S. and Dagla, H.R., (2004). Cloning of adult tree of Jamun (*Syzygium cumini*). *Indian J. of Biotech.* (3):241-245.
- Remashree, A.B., Varghese Thomas, Raghu, A.V., Nabeesa, E. and Neelakandan, N., (2007). Micropropagation of *Syzygium cumini* (Linn.) Skeels. A Multipurpose Tree. *Res. J. Botany* 2 (4):208-213.

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