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IN VITRO REGENERATION IN *EMBLICA OFFICINALIS* FROM JUVENILE ROOT-DERIVED CALLUS

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Effect of NAA, BAP used singly and in combinnations were studied in seedling derived root explant of *Emblica officinalis* (Aonla) It was observed that BAP support shoot proliferation and multiplication. BAP used alone was found to be better than NAA alone or combination with BAP. Effect of subculturing was also observed in root-shoot junction explant of this important medicinal plant. It was noticed that with each progressive subculturing, percentage shoot proliferation increases. Within one week all explants produced callus. Differentiated micro shoots produced roots when inoculated on half strength MS media supplemented with 2.0 mg/l IBA.

Key words : *Emblica officinalis*, *in vitro*, regeneration
Abbreviations: NAA: Naphthelene acetic acid; BAP: Benzyl Amino Purine; PGR: Plant Growth Regulator

Emblica officinalis is an important horticultural species suitable for semi arid land and saline soil. The fruits of the plant are a rich natural source of vitamin C. The fruit of this plant are used in treatment of several diseases and disorders such as hemorrhage, chronic dysentery, diabetes, jaundice and cough etc. It is used in preparation of various important ayurvedic medicines and in several Indian cuisines. It is an important constituent in herbal cosmetic industry. Seed germination percentage is not good enough to supply the demand of market. Hence tissue culture interventions for this species will help in bridging the gap between demand and supply of plants.

Micropropagation of this species from mature tree explants has been reported by Sehgal and Khurana (1985), Verma and Kant (1999), Tyagi and Govil (1999) and Sharda and Mukundan (2006). Here we report for the first time the regeneration through juvenile seedling derived root explant and root-shoot nodes in *Emblica officinalis*.

MATERIAL AND METHOD

Healthy seeds were procured from CAZRI, Jodhpur. Seeds were dipped in distilled water and those which floated were discarded. Only submerged seeds were used for *in vitro* germination. Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes followed by 4 washings with sterile distiled water. These were inoculated on hormone free MS (1962) media. The seeds germinated within one week. Forty days old seedlings were used as source of explant.

MS media supplemented with phyto-hormones was used throughout and prepared using the standard laboratory procedures. The pH of the media was adjusted to 5.8 using 1.0N NaOH and 1.0N HCl. Forty milliliter media was

Table1: Effect of plant growth regulators on seedling derived root segments (after 8 weeks); orientation of explant: horizontal; No. of explant per treatment: 10

S. No	PGR (mg/L)	Percentage explant showing callus	Callus growth	Percentage explant producing shoots	Total no. of shoots from 10 explants
1	1.0 BAP	100.00 %	Slow	66.67 %	55
2	2.0 BAP	55.56 %	Slow	100.00 %	58
3	0.5 NAA	100.00 %	Moderate	0.00 %	0
4	1.0BAP+ 0.5 NAA	100.00 %	Moderate	75.00 %	10

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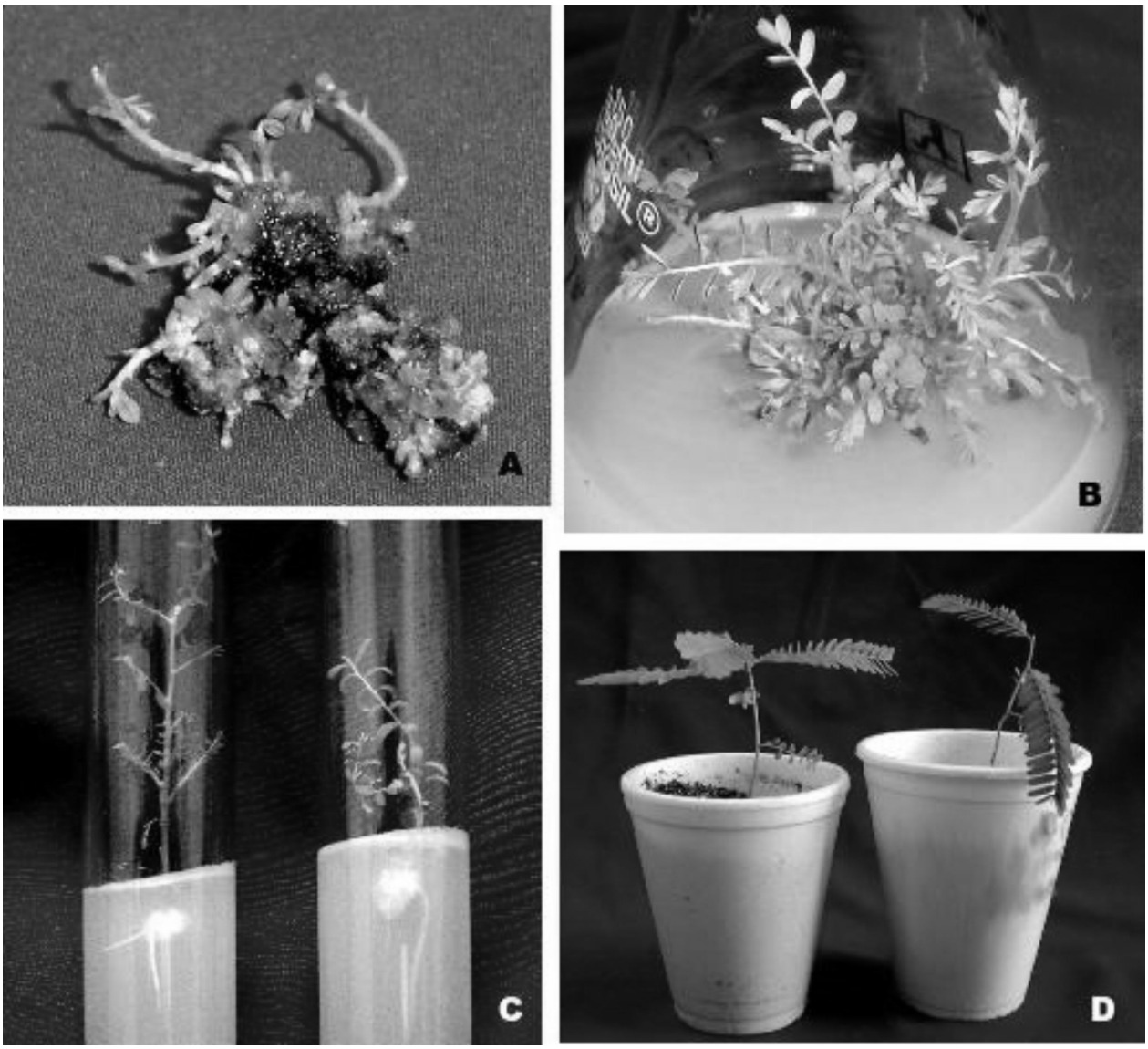

Figure 1A-D.: *Emblica officinalis in vitro* regeneration- A: Shoot morphogenesis from root derived callus; B: Shoot proliferation and elongation; C: Rooting in elongated shoots; D: Plants undergoing hardening.

Table 2: Effect of subculture on seedling derived root-shoot node (after 8 weeks), orientation of explant: horizontal

Subcultures	Percentage explant showing callus	Percentage explant producing shoots	Shoot buds from 10 explants	Mean shoot length (cm)
1	100 %	12.5 %	1	3.0
2	100 %	75.0 %	43	0.4
3	100 %	87.5 %	45	1.0
4	100 %	100.0 %	54	0.8

dispensed per flasks. Non absorbent cotton plug were used throughout the experiments. The media was autoclaved at 15psi pressure and 121ºC for 15-20 minutes. The cultures were incubated in the culture chamber having 26± 2ºC temperature and 16 hours photoperiod with 2000 lux intensity of light.

Seedling derived root segments were inoculated on MS media supplemented with various concentration of NAA and BAP used alone and in combinations. These explants were kept horizontally on the surface of the medium. The explant spanning the junction of the root and the shoot (root-shoot junction explant) were inoculated on MS media supplemented with NAA (0.5 mg/l) and BAP (5.0 mg/l). This media was fortified with PVP, citric acid and ascorbic acid at a concentration of 100 mg/l each as per

Verma (1996). The root-shoot nodes were kept in vertical orientation, with root part dipped in the medium. In this experiment effect of consecutive subculturings was also studied. Under consecutive subculturing regime the explants were subcultured on fresh media after every four week interval.

RESULT AND DISCUSSION

Out of all the plant growth regulators and the combinations tried out, BAP was found to be more effective for shoot proliferation when used alone compared to NAA used alone or in combination with BAP. However, no significant difference between effect of 1.0 mg/l BAP and 2.0 Mg/l BAP on shoot multiplication was observed. Five to six fold shoot multiplication has been achieved in this protocol starting from *in vitro* derived root explants.

At higher concentrations of BAP callus induction percentage was found to be lower than the rest of the media used (as given in table 1). Fast callus growth rate was also observed on MS media supplemented with NAA rather than that supplemented with BAP. It is due to the fact that auxins support callus induction and callus growth.

Callus induction was observed in all root shoot nodes within four weeks. The shoot morphogenesis percentage was found to increase with each subculturing (Table 2). A maximum of upto 5 fold multiplication achieved by fourth subculturing stage (Fig1A). Microshoot elongation occurred on the same medium (Fig 1B).

All elongated micro shoots were inoculated on half strength MS media supplemented with IBA (2.0mg/l). Maximum rooting percentage of 50% was achieved (Fig 1C). Plants have hardened well under green house conditions (Fig 1D).

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