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MICROPROPAGATION OF *EMBLICA OFFICINALIS* GAERTN. THROUGH MATURE NODAL EXPLANTS

BHANU VERMA and U. KANT
Plant Pathology and Tissue Culture Biotechnology Lab., Department of Botany, University of Rajasthan, Jaipur - 302004, India

Shoot proliferation was obtained from nodal stem explants of *Emblica officinalis* Gaertn. on modified Murashige and Skoog (MS) medium supplement with BAP (3.0 - 5.0 mg/1) in combination with NAA (0.5 mg/1). These shoots thus regenerated were elongated on hormone free MS-medium. The elongated shoots were subsequently rooted on 1/2 strength MS medium containing IBA (2.0 - 3.0 mg/1). Field transfer of regenerated plants are in progress.

Keywords : *Emblica officinalis;* Micropropagation; Nodal explant.

Introduction

Emblica officinalis Gaertn. commonly known as Amla/Aonla is a tree of great economic and medicinal importance. This tree is usually propagated through seeds which show poor germination and exhibit great genetic variability. Since there is no conventional vegetative method for mass multiplication of *E. officinalis* the application of tissue culture technology is highly desirable. Several attempts have been made towards micro-popagation of forest and fruit trees[1-6]. In the present study plantlet regeneration of *E. officinalis* from mature nodal segments has been reported.

Materials and Methods

The elite trees of *Emblica officinalis* identified on the basis of quality and quantity of fruits produced, were selected for the present study. Young shoots were collected from these mature elite trees (~ 10 years old). Nodal segments excised from these shoots were washed with extran (a mild detergent) and were kept in chilled antioxidant solution containing citric acid (50 mg/1), ascorbic acid (150 mg/1) and polyvinyl pyrrolidone (500 mg/1) for one hour. The explants were then surface sterilized with 0.1% mercuric chloride solution. After several rinses in sterile distilled water they were inoculated on MS-medium [7] supplemented with BAP (3.0 - 5.0 mg/1), NAA (0.2 - 1.0 mg/1), adenine sulphate (50.0 mg/1) along with antioxidants [citric acid (100.0 mg/1), ascorbic acid (100.0 mg/1) and PVP (500.0 mg/1)]. The pH of media were adjusted at 5.8 before autoclaving at 15 psi pressure for 20 minutes. The shoot buds were developed on the above medium, elongated on hormone free MS-medium and subsequently rooted on 1/2 strength MS-medium fortified with IBA (1.0 -3.0 mg/1) and sucrose (1.5%).

All cultures were maintained at 26±2°C under 16 h photo period with a light intensity of 2000-3000 lux provided by white fluorescent tubes. Each treatment involved 50-60 explants and the experiments were repeated thrice.

Results and Discussion

Extensive experimentation were undertaken to obtain regeneration from mature nodal shoot segments of *Emblica Officinalis* Gaertn. However it was observed that mature nodal explants of this tree species showed poor response under *in vitro* conditions. This could be due to excessive *in borne* infection and

also due to severe problem of leaching of phenolics, which results in loss of nearly 90% of the cultures.

Bud break was observed in only 8-10% explants on MS-medium fortified with BAP (5.0 mg/ 1), NAA (0.5 mg/1), adenine sulphate (50.0 mg/1) together with antioxidants. A maximum of 3-4 shoots were produced per explant (Table 1).

To combat the problem of exudation of phenolics in *E. officinalis* cultures, ascorbic acid (100.0 mg/1), citric acid (100.0 mg/1) and PVP (500.0 mg/1) were added as antioxidants to the cultures medium. Ascorbic acid has been shown to act as an antioxidant[8]. The use of PVP for the removal of phenolics has been reported in teak[9], Guava[10]; *Feronia limonia*[1], and *Acacia senegal*[4].

The physiological state of the parent plant at the time of explant excision has a definite influence on the response of the buds. Explants from actively growing shoots at the beginning of the growing season generally gave best results[11]. This phenomenon was also observed in case of *E. officinalis* to the extent that no differentiation was observed in explants except in those explants which were collected and inoculated in the months of February-April and August-October which

Table 1. Effect of cytokinins on axillary bud proliferation.

Medium	:	MS + Cytokinins + additives + sucrose (3.0%)
Inoculum	:	Mature nodal stem explant
Incubation	:	At 26±2C in 16h photoperiod (2000-3000 lux) for 4 weeks.

Cytokinin/s level mg/1		No. of shoot buds per explant *Mean ± $t_{0.05}$ S.E. (X)
Kn	BAP	
0	0.control	Nil
1.0	-	Nil
2.0	-	0.33±0.539
3.0	-	0.66±0.539
4.0	-	1.30±0.573
5.0	-	2.33±0.856
6.0	-	1.66±0.539
-	1.0	Nil
-	2.0	0.66±0.539
-	3.0	1.16±0.789
-	4.0	1.83±0.789
-	5.0	3.16±0.789
-	6.0	2.16±0.789
1.0	1.0	0.66±0.539
1.5	1.5	1.33±0.539
2.0	2.0	1.66±0.539
2.5	2.5	2.16±0.789
3.0	3.0	1.83±0.426

*Values are 95% confidence limit for mean

Table 2. Evaluation of seasonal influence on bud proliferation (Month wise).

Medium	:	MS + BAP (5.0 mg/1) + NAA (0.5 mg/1) + AS (50.0 mg/10 + AA (100.0 mg/1) + PVP (500.0 mg/1) + AC (500.0 mg/1) + Sucrose (3.0%)
Inoculum	:	Mature nodal stem explant
Incubation	:	At 26±2°C in 16h photoperiod (2000-3000 lux) for 4 weeks.

Months	No. of shoot buds per (explant collected) *Mean ± $t_{0.05}$ S.E. (X)	Contamination explant factor
January	0.66±0.539	+
February	1.16±0.789	+
March	3.16±0.789	+
April	1.33±0.539	+
May	Nil	+
June	Nil	++
July	Nil	+++
August	1.50±0.573	+++
September	1.66±0.539	+++
October	0.66±0.539	++
November	Nil	++
December	Nil	++

* Values are 95% confidence limit for mean
\+ Mild/moderate
++ Considerable
+++ Maximum

is the time when the tree produces fresh axillary buds in nature (Table 2).

Shoot elongation was observed on hormone free MS-medium. These elongated shoots were rooted on 1/2 strength MS-medium supplemented with IBA (3.0 mg/1) and sucrose (1.5%).

Experiments pertaining to field transfer of *in vitro* raised plantlets are in progress.

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