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In Vitro micropropagation of *Emblica officinalis* Gaertn. cv. KRISHNA

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ABSTRACT
Seeds of the genotype Krishna were raised *in vivo* in sterilized soil rite and supplemented with MS basal liquid medium. Shoot tip and Nodal segment explants were collected from these seedlings and cultured aseptically in MS as well as WPM medium fortified with growth regulators viz., BAP, NAA, GA_3 and TDZ. The nodal segment explants showed better response than shoot tip for micropropagation of aonla. The maximum (13.67) number of multiple shoots per culture were recorded by nodal segments cultured in MS + BAP 0.25 mg/l +NAA 0.1 mg/l + GA_3 2 mg/l. However, normal rhizogenesis could not be achieved with supplement of IBA and NAA to half MS media and abnormal root induction was noticed which fails to establish during subsequent hardening.

Key words : Aonla, Micropropagation

INTRODUCTION

In general woody texas are difficult to regenerate in *in vitro* conditions. But recently some success has been achieved in leguminous tree species. (Ganga and Balkrishnamurthy, 1997). *Emblica officinalis*, a multipurpose minor tropical fruit tree is well adapted to grow in the poor soils of semi-arid tropics and thus possess good

*In-charge Modibaug, College of Agriculture, Pune-5. 1. Associate Professor, PGI, M.P.K.V., Rahuri. 2. Asstt. Professor of Botany, Biotechnology Centre, M.P.K.V., Rahuri. potential for afforestation of wastelands and degraded areas. It is one of the versatile fruit crop having social, medicinal, industrial and nutritional values hence considered as '*Amritphal*'. *In vitro* clonal propagation would go a long way to meet the everincreasing demand for quality planting materials, since the traditional vegetative propagation methods are not much efficient.

Regeneration of plantlets of aonla from endosperm, hypocotyl and from various parts of axenic seedlings have been reported. However, upto date there is no standard protocol for *in vitro* clonal propagation of this fruit crop. Therefore, the present study was undertaken to come out with optimal culture conditions for high frequency plant regeneration from nodal segment along with axillary bud and shoot tip explants of the aonla genotype Krishna.

MATERIALS AND METHODS

Present investigation was carried out at the tissue culture laboratory of the Centre of Advanced Studies in fruits, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra (India). Seeds of the genotype 'Krishna' were raised in sterilized soil rite medium supplemented with liquid MS medium. Shoot tips and nodal segments along with axillary buds (0.5 to 1.0 cm) length were collected from these *in vivo* germinated seedlings and cultured in Murashige and Skoog (MS) and Woody Plants Medium (WPM) fortified with growth regulators (BAP, NAA, IAA, IBA and TDZ) in different concentrations and combinations. The response to shoot bud differentiation, no. of days taken for shoot bud differentiation (SBD), no. of shoots produced per culture and length of shoots, no. of leaves were recorded. The experiment was conducted adopting Completely randomised block design (CRD).

RESULTS AND DISCUSSION

The results indicated that, the regeneration of shoots was achieved on basal MS and low salt WPM containing BAP, NAA, GA_3 and TDZ with different combinations and concentrations. Shoot tip

Table 1: Evaluation of shoot tips of *in vivo* grown seedlings of *Emblica officinalis* Gaertn. for *in vitro* shoot multiplication.

Sr. No.	Details of medium	Days required for SBD	Percent regeneration	Length of shoot (cm)	Average number of shoots / culture	Number of leaves
1	MS + BAP 0.25 mg/l + NAA 0.1 mg/l	9.33	86.66 (68.60)	3.97	1.00 (1.22)	6.67 (2.68)
2	MS + BAP 0.5 mg/l + NAA 0.1 mg/l	15.33	78.33 (62.26)	2.40	1.33 (1.34)	4.33 (2.20)
3	MS + BAP 0.75 mg/l + NAA 0.1 mg/l	16.67	75.83 (60.56)	2.10	1.67 (1.46)	3.67 (2.04)
4	MS + BAP 1 mg/l + NAA 0.1 mg/l	17.67	31.67 (34.24)	1.77	1.00 (1.22)	3.33 (1.95)
5	MS + BAP 0.25 mg/l + NAA 0.2 mg/l	15.00	65.00 (53.74)	2.53	1.00 (1.22)	4.67 (2.27)
6	MS + BAP 0.5 mg/l + NAA 0.2 mg/l	16.33	52.50 (46.43)	2.17	1.00 (1.22)	3.67 (2.04)
7	MS + BAP 0.75 mg/l + NAA 0.2 mg/l	17.33	40.00 (39.23)	1.90	1.33 (1.34)	3.67 (2.04)
8	MS + BAP 1 mg/l + NAA 0.2 mg/l	19.67	32.50 (34.74)	1.37	1.00 (1.22)	2.67 (1.77)
9	WPM + BAP 0.25 mg/l + NAA 0.1 mg/l	10.67	78.34 (62.26)	3.27	1.00 (1.22)	5.67 (2.48)
10	WPM + BAP 0.5 mg/l + NAA 0.1 mg/l	11.33	67.50 (55.25)	2.87	1.67 (1.46)	4.67 (2.27)
11	WPM + BAP 0.75 mg/l + NAA 0.1 mg/l	16.33	47.50 (43.57)	1.67	1.00 (1.22)	2.67 (1.77)
12	WPM + BAP 1 mg/l + NAA 0.1 mg/l	11.67	32.50 (34.75)	2.87	1.00 (1.22)	5.33 (2.41)
13	WPM + BAP 0.25 mg/l + NAA 0.2 mg/l	10.67	65.00 (53.74)	3.00	1.00 (1.22)	5.33 (2.41)
14	WPM + BAP 0.5 mg/l + NAA 0.2 mg/l	11.67	48.33 (44.04)	2.73	1.00 (1.22)	4.67 (2.27)
15	WPM + BAP 0.75 mg/l + NAA 0.2 mg/l	15.67	31.67 (34.24)	1.83	1.00 (1.22)	3.33 (1.95)
16	WPM + BAP 1 mg/l + NAA 0.2 mg/l	18.33	20.83 (27.15)	1.13	1.00 (1.22)	2.33 (1.68)
17	MS + BAP 0.25 mg/l + NAA 0.1 mg/l + GA_3 2 mg/l	13.33	72.50 (58.38)	3.17	1.33 (1.34)	5.67 (2.48)
18	WPM + TDZ 0.0011mg/l	14.00	50.00 (45.00)	2.13	3.33 (1.95)	2.67 (1.77)
19	WPM + TDZ 0.0022mg/l	13.00	65.85 (54.23)	2.43	1.67 (1.46)	4.33 (2.20)
S. E. ±		0.5014	0.7366	0.0898	0.0694	0.0803
C. D. at 5 %		1.4353	2.1085	0.2571	0.1987	0.2298

Figures in the parentheses indicates square root transformed values

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Table 2 : Evaluation of nodal segments of *in vivo* grown seedlings of *Emblica officinalis* Gaertn. for *in vitro* shoot multiplication.

Sr. No.	Details of medium	Days required for SBD	Percent regeneration	Length of shoot (cm)	Average number of shoots / culture	Number of leaves
1	MS + BAP 0.25 mg/l + NAA 0.1 mg/l	9.00	88.33 (70.06)	4.00	6.00 (2.53)	7.67 (2.86)
2	MS + BAP 0.5 mg/l + NAA 0.1 mg/l	12.33	80.83 (64.04)	3.20	2.33 (1.68)	6.67 (2.68)
3	MS + BAP 0.75 mg/l + NAA 0.1 mg/l	15.33	40.00 (39.23)	2.43	1.67 (1.46)	5.66 (2.48)
4	MS + BAP 1 mg/l + NAA 0.1 mg/l	19.33	57.50 (49.32)	1.43	2.33 (1.68)	3.67 (2.04)
5	MS + BAP 0.25 mg/l + NAA 0.2 mg/l	12.67	56.67 (48.83)	3.10	3.00 (1.86)	6.34 (2.61)
6	MS + BAP 0.5 mg/l + NAA 0.2 mg/l	15.67	60.83 (51.26)	2.43	2.00 (1.58)	3.67 (2.04)
7	MS + BAP 0.75 mg/l + NAA 0.2 mg/l	16.33	48.33 (44.05)	2.17	1.67 (1.46)	4.66 (2.27)
8	MS + BAP 1 mg/l + NAA 0.2 mg/l	18.67	38.33 (38.25)	1.63	1.33 (1.34)	3.66 (2.04)
9	WPM + BAP 0.25 mg/l + NAA 0.1 mg/l	11.00	80.83 (64.04)	3.02	2.33 (1.68)	6.66 (2.68)
10	WPM + BAP 0.5 mg/l + NAA 0.1 mg/l	11.33	68.33 (55.76)	2.93	1.67 (1.46)	6.33 (2.61)
11	WPM + BAP 0.75 mg/l + NAA 0.1 mg/l	13.67	55.00 (47.87)	2.40	1.00 (1.22)	5.33 (2.41)
12	WPM + BAP 1 mg/l + NAA 0.1 mg/l	17.67	40.00 (39.23)	1.37	1.00 (1.22)	4.33 (2.20)
13	WPM + BAP 0.25 mg/l + NAA 0.2 mg/l	14.00	75.00 (60.02)	2.27	2.00 (1.58)	5.34 (2.41)
14	WPM + BAP 0.5 mg/l + NAA 0.2 mg/l	12.33	53.33 (46.91)	2.57	1.33 (1.34)	5.34 (2.41)
15	WPM + BAP 0.75 mg/l + NAA 0.2 mg/l	18.00	42.50 (40.68)	1.27	1.00 (1.22)	3.34 (1.95)
16	WPM + BAP 1 mg/l + NAA 0.2 mg/l	17.00[j]	33.33 (35.24)	1.50	1.00 (1.22)	3.67 (2.04)
17	MS + BAP 0.25 mg/l + NAA 0.1 mg/l + GA_3 2 mg/l	12.00	82.50 (65.30)	3.40	13.67 (3.76)	6.67 (2.68)
18	WPM + TDZ 0.0011mg/l	10.67	64.16 (53.23)	2.47	3.00 (1.86)	5.34 (2.41)
19	WPM + TDZ 0.0022mg/l	12.00	76.66 (61.12)	2.80	5.33 (2.40)	5.67 (2.48)
S. E. ±		0.4588	0.7982	0.0712	0.1216	0.0715
C. D. at 5 %		1.3133	2.2848	0.2041	0.3483	0.2046

Figures in the parentheses indicates square root transformed values

Table 3 : Responsiveness of plantlets derived from explants of *in vivo* seedlings for root induction

Sr. No	Details of medium	Number of days required for root initiation	Per cent Response	Number of roots per shoot	Length of longest root (cm)	Height of seedling (cm)
1	½ MS + NAA 0.5 mg/l + IBA 0.5 mg/l	39.43	3.57 (10.73)	2.14 (1.60)	2.77	7.47
2	½ MS + NAA 1 mg/l + IBA 1 mg/l	33.85	6.42 (14.62)	2.57 (1.75)	3.31	8.48
3	½ MS + NAA 2 mg/l + IBA 2 mg/l	29.57	9.28 (17.66)	3.86 (2.08)	4.07	9.27
S. E. ±		0.4738	0.6932	0.1215	0.1419	0.1441
C. D. at 5 %		1.4607	2.1373	0.3639	0.4375	0.4443

Figures in the parentheses of column percent response indicates arcsine transformed values and figures in the column number of roots per shoot indicates square root transformed values

explant gave highest percent regeneration (86.66 %), earliest initiation (9.33 days), longest shoot (3.97 cm) and more no. of leaves(6.67) in the treatment MS + BAP 0.25 mg/l + NAA 0.1 mg/l. But the maximum no. of multiple shoots (3.33) were recorded in medium WPM + TDZ 0.0011 mg/l (Table1)

The nodal segment explants cultured in MS medium supplemented with BAP 0.25 mg/l + NAA 0.1 mg/l + GA_3 2 mg/l recorded the maximum number of multiple shoots (13.67) per culture (Table 2). The nodal segment explants recorded four times more multiple shoots than shoot tip explants. Similar to the present report shoot regeneration from shoot tips explants in MS medium supplemented with IAA or IBA or BA has been reported by Kopp's Natraja (1990) in tamarind, Papadatou *et al*. (1990) in Guava. In *S cumini*, Yadav *et al*. (1990) reported that the nodal explants produce more shoots than shoot tip explants.

A protocol for normal root induction could not be established but ½MS + NAA and IBA @ 2.00 mg/l each gave abnormal root growth and produced earliest root initiation (29.57 days), maximum no. of roots (3.86), longest length of root (4.07 cm) with callusing at distal end (Table 3). Since, these roots were abnormal i.e. spongy and thick which failed to establish and transfer for hardening. These results are in conformity with those reported by Verma *et al* (1998) in aonla and Fougat *et al* (1997) in ber cv. Gola. The transferring of shoots directly in soil rite medium pre treating with IBA solution were also failed to produce roots. Similar, results were reported by Rajmohan and Mohankumaran (1988) in jackfruit and Singh and Deka (2001) in Carambola.

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