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In vitro propagation of *Emblica officinalis* from nodal segment culture

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Micropropagation studies in Aonla were carried out to develop a protocol for mass multiplication of true to type plantlets. Nodal segments of *Emblica officinalis* were cultured on twenty different fortifications of MS medium. Analysis of variance exhibited highly significant differences among different culture media combinations. The basal MS medium fortified with 4.0 mg.l^{-1} BAP and 0.5 mg.l^{-1} NAA was found to be more responsive for shoot proliferation efficiency (47.65%), number of shoot(s) per explant (3.20) and average shoot length (1.43). Rooting was high on MS basal medium supplemented with 2.0 mg.l^{-1} IBA and 0.5 mg.l^{-1} BA (17.20%) *in vitro*.

Key words: *Emblica officinalis*, nodal segment culture, *in vitro*, morphogenesis

Abbreviations: BA- 6-benzylaminopurine; IBA-Indole-3-butyric acid; MS- Murashige and Skoog medium; NAA- α-Naphthalene acetic acid; 2,4-D- 2,4-dichlorophenoxyacetic acid

Introduction

Aonla *(Emblica officinalis* syn. *Phyllanthus emblica*) is an important fruit crop of commercial significance due to its high medicinal and nutritional value. Aonla is the richest source of vitamin C among all fruits after Barbados Cherry. Availability of genuine planting material is main limiting factor in the establishment of Aonla orchards. Aonla has long been raised by seed or through approach grafting, but from seed it does not ensure true to type plants because of cross-pollinated nature. Aonla is being propagated through budding. Owing to erect tree habit, large numbers of branches are not available for approach

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grafting. Moreover, the success in vegetative propagation in different varieties ranges from 25-80 per cent (Ram, 1982). The non-availability of desired number of shoots for grafting or budding is another bottleneck problem for multiplication, which is labour expensive and economically not viable due to slow rate of multiplication and field survivability. The use of biotechnological approaches especially micropropagation offers a unique alternative for massive multiplication of true to type plantlets.

During the last year, an array of efforts has been made to establish regenerable culture system in Aonla. For this purpose, various explants have been used to produce regenerable cultures via *in vitro* morphogenesis such as nodal segments (Mishra *et al*., 1998; 1999; Verma and Kant, 1999; Rahman *et al*., 1999; Mishra and Pathak, 2001), cotyledon and cotyledonary nodes (Verma and Kant, 1999), shoot tips (Rahman *et al*., 1999; Verma and Kant, 1999), and hypocotyls (Verma and Kant, 1999). However, the regeneration frequencies in all above experiments were found considerably low. The major reason for low regeneration frequency is unsuccessful establishment of proficient culture system due to contamination of explants and oxidation of phenolic compounds released by cellular disruption attributed to wounding (Dalal *et al*., 1992). Phenol accumulation is a serious problem generally faced in culturing the adult tissue of woody species. Due to release of such compounds in to culture medium resulted in mechanical injury leading to stimulation of metabolism of phenolics (Mishra *et al*., 1998; 1999; Verma and Kant, 1999). Moreover, regeneration potential is depended upon the hormonal composition of the culture medium (Mishra *et al*., 1999; Verma and Kant, 1999), nature of explants (Rahman *et al*., 1999; Verma and Kant, 1999) and other physical factors.

The objectives were to develop a system of *in vitro* regeneration of Narendra 7 (NA-7) variety of commercial importance of India, by means of the culture of nodal segments. This study was conducted to understand the possible role of plant growth regulators in the multiple plant regeneration via *in vitro* morphogenesis.

Materials and methods

The present investigation was carried out at the Horticultural Biotechnology Laboratory, KNK College of Horticulture, Mandsaur, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) during the session 2007-2008. Narendra Aonla 7 (NA-7), being the most prolific cultivar of Aonla, was chosen for the study.

The indeterminate shoots from top or mid portion of canopy were collected from 4-5 years old tree planted at Sewage Farm, KNK, College of Horticulture, Mandsaur (M.P.) in distilled water. Top 1-10 nodal segments were

rejected being very soft and tender whereas the nodal segments from 10-20 th were trimmed to 1-2 cm length and determinate shoots attached with nodes were removed as per method described by Mishra *et al*. (1999). These explants were washed under the running tap water for 30 minutes to remove adhering dirt particles. Then, nodal segments were placed into double distilled water containing 2% Tween 20 (v/v) for 10 min to remove the adhering fine particles. The cleaned segments were then treated with 70% (v/v) ethanol for 1 min followed by treatment with different concentrations of Bavistin® (BASF, Germany). These segments were then dipped in to aqueous solutions of two different surface sterilizing agents i.e. $Ca(OCl)_2$ and $HgCl_2$ in different concentrations and combinations for diverse durations with initial vacuum of 100 psi (Table 1). Finally the nodal segments were washed 4-5 times with sterile double distilled water.

Culture media combinations were short listed on the basis of work conducted by various scientists as well as preliminary experiments conducted in this laboratory (data not presented). A preliminary experiment of two different fortifications of basal media viz: MS (Murashige and Skoog, 1962) and WPM (Lloyd and McCown, 1980) were tested to find out better *in vitro* response. Apart from MS/WP basal macro and micro salts, vitamins, and agar powder, three different auxins, namely 2,4-D, NAA and 2,4,5 T (alone) and four diverse cytokinins viz. BAP, kinetin, zeatin and TDZ (alone) in varying concentrations were added to fortify media for culturing the nodal segments. Inoculation of sterilized explant in culture medium, releases brown to black leachates in medium occurred immediately after 4-8 hours which influences *in vitro* morphogenesis up to a great extent as reported earlier by various scientists. In order to check the phenol leaching from cut surface of explant, media was fortified with four different antioxidants/ phenol binding agents i.e. ascorbic acid, citric acid, PVP and activated charcol in varying concentrations and combinations (Table 2). During the course of preliminary experiments, it was observed that an auxin as well as a cytokinin alone is not adequate for inducing morphogenesis in higher frequencies. Therefore, for final experiment basal MS medium was fortified with different concentrations and combinations of plant growth regulators (BAP and Kn in combination with NAA and 2,4-D), 30.0 g l^{-1} sucrose and 7.5 g l^{-1} agar powder (Table 3). All culture media was autoclaved at 121^0C under 1.1 kg cm^{-2} for 20 min after adjusting the pH to 5.6 ± 0.1 with 1 N KOH. Readymade MS/ WP basal medium and plant growth regulators were procured from Hi-media Laboratories, Mumbai, India.

Cultured baby food bottles, 1-2 pieces of nodal segments were plated and sealed with Lab film (Parafilm®) which were incubated under complete darkness at 25 ± 2°C for one week. Later *in vitro* cultured explants were

incubated at 16 hours photoperiod regime at 1200 lux photosynthetically active radiation, 25 ± 2 ^{o} C temperature and 70% relative humidity. The regenerated plantlets were transferred to test tubes containing MS rooting medium supplemented with different concentrations and combinations of plant growth regulators (IBA, NAA, BAP and Kn alone as well as IBA in combination with BA and kinetin), 15.0 g l^{-1} sucrose and 7.5 g l^{-1} agar powder (Table 4). Observations were recorded for percentage of shoots proliferating explants, average number (s) of shoots/ explant, mean shoot length and *in vitro* rooting percentage to find out the significance of different culture media combinations. For this purpose Completely Randomized Design (CRD) was adopted. Each treatment was consisting of two replications. Per replication approximately 100-120 explants were excised and cultured on each media. All data were recorded in percentage the arc-sine transformation made before analysis and analyzed as per method.

Results and discussions

The higher percentage of aseptic culture was recorded with 2% bavistin in combination with 0.2% $HgCl_2$ (79.21%), closely followed by 2% bavistin in association with 0.1% $HgCl_2$ (75.26%) for 10 minutes but overall survival rate was found very poor (30-35%). The best survival rate was recorded with 1% bavistin in combination with 0.2% $HgCl_2$ treatment for 10 minutes (72.45%) and the percentage of aseptic culture was noted also higher (73.54%) (Table 1). Consequently, this combination for surface sterilization was applied throughout the experiment.

The antioxidants and absorbents were added in culture medium to reduce the browning of medium due to leaching of phenolics. During present investigation, ascorbic acid, citric acid, PVP, and activated charcoal were added into culture medium alone as well as in combinations. In present study, explants subjected to medium fortified with a combination of ascorbic acid, citric acid, PVP, and activated charcoal showed comparatively better survival and reduced explant browning than the ascorbic acid, citric acid, PVP, and activated charcoal alone. Data presented in Table 2 revealed that the highest survival rate was achieved when explants were treated with 25.0 mg l^{-1} each of ascorbic acid, citric acid, PVP in combination with 3.0 g l^{-1} activated charcoal closely followed by 50.0 mg l^{-1} each of ascorbic acid, citric acid, PVP and 3.0 g l^{-1} activated charcoal. The application of 50.0 mg l^{-1} each of ascorbic acid, citric acid, PVP in combination with 3.0 g l^{-1} activated charcoal significantly reduced the browning of explants (36.45%) as well as enhanced the survival of explants in higher frequencies (47.56%). The reason may be attributed to complete blockage of outlet for phenolic compounds and thereby the establishment of

explant in the media. The nutrient might have been absorbed by the side surface of nodal shoot. Parallel results were obtained by Sharma (1984) in *Citrus* species and by Mishra *et al.* (1999) and Verma and Kant (1999) in Aonla.

Explants from NA-7 aonla cultivar were cultured on various modification of MS medium. The first response of cultured nodal segments was similar after 7-10 days and mostly independent from explant and culture media. All explants became swollen and no callus proliferation was observed during first few days. After 10 days of culture callus initiation was observed from a few cultured nodal segments. From nodal section, callus proliferation started from the portion that was in contact with the medium and spread upward. However, the frequency of callus formation was found very low. In most cases, direct single shoot proliferation was observed frequently from the nodal portion instead of callus. However, in some instances, shootlets were developed directly from the meristematic zones in clusters (multiple plantlets) from cultured nodal segments (Fig. 1).

In nodal segment cultures, the nodal segment excised from top 1-10 nodes could not induce higher bud break, which may be due to tender nature of buds. The exposure of explants with sterilants/antioxidants may kill these soft buds. Maximum bud break was observed from 10-15 nodes, possibly due to juvenile state and capacity to withstand the toxic effect of sterilants/antioxidants. These results are in accordance to the findings of Tran Than Van (1973) and Mishra *et al*. (1999) who found higher morphogenic response followed by plantlet regeneration that appears to be highly dependent on position of explant. Studies have revealed that lower or mid portion of the branch are easier to establish *in vitro* than upper part of the branch (Bonga, 1987).

For growth regulators large variations have been found in preliminary experiments, where some combinations led to morphogenesis from cultured explants, while other produced only callus with high or low growth rate - not all the cells within an explant or callus raised from them went on to form organs or embryoids. Also, not all explant responded equally to conditions congenial for morphogenesis. Moreover, it was observed that an auxin as well as a cytokinin alone is not adequate for inducing morphogenesis in higher frequencies. Therefore, for final experiment basal MS medium was fortified with both types of plant growth regulators (an auxin as well as a cytokinin) in varying concentrations and combinations.

The analysis of variance revealed that the mean sum of squares due to different culture media combinations were highly significant at 1% probability level for shoot proliferation efficiency and average number of shoot (s) per explant. It indicates the presence of considerable amount of variability amongst the different culture media combinations. Effects of different culture media combinations are presented in Table 3. Although, shoot proliferation was

recorded on all twenty culture media combinations, their frequencies varied amongst different culture media combinations. Among different twenty culture media combinations, culture media MS4BN (47.65%) and MS3BN (43.56%) were found remarkably superior for shoot proliferation from nodal segments. Next responding group of culture media included MS2BN (39.46%), MS4BD (38.45%), MS4KN (38.14%) and MSBN (36.50%). Nutrient media MS5KD (11.77%), MSKD (13.41%), MS5KN (17.44%) and MS5BD (18.26%) were low responding in this regard. In nodal segment culture, culture media MS4BN and MS3BN (containing a higher concentration of a cytokinin with lower concentration of NAA) produced shoots in higher frequencies than culture medium containing kinetin in same concentration suggested that BAP is better for shoot proliferation than kinetin. This finding contradicts to the findings of Mishra *et al*. (1999) who found kinetin doing better instead of BAP for shoot proliferation from nodal segment culture of Aonla.

Among the different culture media combinations, MS4BN (3.20) and MS4KN (3.12) were found significantly superior closely followed by nutrient media MS4BD (2.84) and MS4KD (2.42) for proliferating higher number (s) of shoots per explant. Culture media MS3BN (1.91), MS3KN (1.80), MS3BD (1.76), MS3KD (1.65) and MS2BN (1.47) were next to them. Remaining culture media combinations were at par with each other and MS5KD (0.88) proved to be the lowest performer. Total number of shoots per explant was also recorded higher on culture media supplemented with higher concentration of BAP in combination with lower concentration of NAA, which suggested that higher concentration of a cytokinin is required for this purpose.

The shoot of higher length was recovered from culture media MS4BN (1.43 cm) closely followed by MS4BD (1.30 cm). Culture medium MSKD (0.71 cm) produced shoots of minimum length. However, statistically all culture media combinations performed equally since no significant difference was found among the treatment combinations

Maximum number of *in vitro* rooting was exhibited by culture media MS2I.5B (17.20%) closely followed by MS2I.5Kn (15.45%). The treatment of 2.0 mg l^{-1} IBA was found to be significantly superior over all other treatments with respect to maximum rooting. Culture media combinations MS2I (14.45%), MSI.5B (13.28%), MSI.5Kn (12.46%) and MSI (11.24%) were the next performing group. Auxins promoted adventitious root development on intact plant cells as well as excised stems. Of these, IBA was the most effective one than any other growth regulator in most of the cases apparently because it is not destroyed by IAA oxidase or other enzymes and therefore persists longer. The above results are in conformity with the earlier findings of Rana and Singh (2002) and Kumar *et al*. (2001) with *Citrus* species. A group of 7 culture media

combinations MS.5N, MS3N, MSB, MS2B, MS3B, MS2Kn and MS3Kn did not produce roots *in vitro*. Longer incubation of these microshoots in the media caused complete leaf fall. Callusing near cut end was observed with NAA. This finding is in accordance with Mishra *et al*. (1999).

During the present study, it was found that under appropriate conditions nodal segments of Aonla as in number of other fruit crops give rise to low to moderate number of shoots. These explants can be cultured to obtain multiple plants. A system for plant regeneration in Aonla is thus available, which has immediate potential for breeding and biotechnological studies. The reason for low frequency of regeneration and *in vitro* rooting is not clear and needs further study, which can be probably enhanced by modifying culture medium by supplementing appropriate proportions of different plant growth regulators and other culture media ingredients.

Table 1. Effect of surface sterilizing and antifungal agent on recovery of aseptic culture.

Treatments	Concentration (%)	Exposure Time (in minute)	Aseptic Culture (%)	Survival of explants (%)
$Ca(OCl)_2$	10	10	12.65 ^{h} (20.79)	25.32 ^{f} (30.18)
$Ca(OCl)_2$	10	15	14.36 ^{h} (22.16)	30.18 ^{f} (33.22)
$Ca(OCl)_2$	10	20	30.34 ^{fg} (33.25)	44.21 ^{de} (41.67)
$Ca(OCl)_2$	15	10	24.68 ^{g} (29.73)	49.16 ^{cd} (44.50)
$Ca(OCl)_2$	15	15	29.45 ^{g} (32.76)	55.23 ^{c} (48.02)
$Ca(OCl)_2$	15	20	38.45 ^{ef} (38.26)	62.46 ^{b} (52.26)
$Ca(OCl)_2$	20	10	35.38 ^{f} (36.48)	33.46 ^{f} (35.31)
$Ca(OCl)_2$	20	15	41.34 ^{e} (39.99)	44.18 ^{e} (41.60)
$Ca(OCl)_2$	20	20	44.20 ^{e} (41.64)	25. 43 ^{f} (30.21)
$HgCl_2$	0.1	5	35.36 ^{f} (36.46)	59.78 ^{b} (50.69)
$HgCl_2$	0.1	10	49.89 ^{d} (44.94)	61.16 ^{b} (51.49)
$HgCl_2$	0.2	5	46.46 ^{de} (42.96)	64.56 ^{b} (53.51)
$HgCl_2$	0.2	10	63.43 ^{c} (52.85)	67. 49 ^{b} (55.25)
Bavistin + $Ca(OCl)_2$	1+10	20	37.46 ^{f} (37.68)	47.26 ^{d} (43.43)
Bavistin+ $Ca(OCl)_2$	1+15	20	51. 26 ^{d} (45.72)	58.42 ^{bc} (49.90)
Bavistin +$Ca(OCl)_2$	1+20	20	53.45 ^{d} (46.98)	27.15 ^{f} (31.26)
Bavistin+ $HgCl_2$	1+0.1	10	68.40 ^{bc} (55.88)	65.56 ^{b} (54.14)
Bavistin+ $HgCl_2$	1+0.2	10	73.54 ^{b} (59.10)	72.45 ^{a} (58.42)
Bavistin + $HgCl_2$	2+0.1	10	75.26 ^{ab} (60.24)	34.26 ^{ef} (35.72)
Bavistin+ $HgCl_2$	2+0.2	10	79.21 ^{a} (63.09)	29. 18 ^{f} (32.62)
CD (0.05)			**9.61**	**10.02**

$Ca(OCl)_2$- Calcium hypochlorite, **$HgCl_2$** - Mercuric chloride

* Values within column followed by different letters are significantly different at 5% probability level.

Table 2. Effect of antioxidant and absorbing agent on browning and survival of explants.

Treatments	Concentration (mg. l^{-1})	Explant browned (%)	Survival of explants (%)
Ascorbic acid	25.0	83.55 ^{ab} (66.09)	13.26 ^{g} (21.14)
Ascorbic acid	50.0	79.53 ^{b} (62.14)	15.42 ^{fg} (22.95)
Ascorbic acid	100.0	73.29 ^{b} (58.92)	23.86 ^{e} (29.19)
Ascorbic acid	150.0	69.56 ^{c} (56.53)	25.37 ^{e} (30.23)
Citric acid	25.0	87.26 ^{a} (69.22)	9. 56 ^{g} (17.93)
Citric acid	50.0	84.45 ^{a} (66.85)	14.26 ^{g} (22.08)
Citric acid	100.0	79.26 ^{b} (63.00)	21.45 ^{f} (27.54)
Citric acid	150.0	73.24 ^{b} (58.89)	22.24 ^{f} (28.02)
PVP	25.0	77.35 ^{b} (61.77)	19.56 ^{f} (26.14)
PVP	50.0	71.67 ^{b} (57.88)	22.27 ^{ef} (28.13)
PVP	100.0	57.18 ^{cd} (49.13)	29.45 ^{de} (32.82)
PVP	150.0	49.42 ^{de} (44.68)	38.37 ^{c} (38.24)
Activated charcoal	1000.0	84.35 ^{a} (51.85)	12.45 ^{g} (20.55)
Activated charcoal	2000.0	74.26 ^{b} (59.55)	19.65 ^{f} (26.26)
Activated charcoal	3000.0	68.16 ^{c} (55.68)	27.36 ^{e} (33.98)
Activated charcoal	5000.0	70.26 ^{bc} (56.97)	25.18 ^{e} (30.03)
AA+CA+PVP+AC	25.0+25.0+25.0+3000.0	41.65 ^{ef} (40.18)	53.88 ^{a} (47.25)
AA+CA+PVP+AC	50.0+50.0+50.0+3000.0	36.45 ^{f} (37.12)	47.56 ^{ab} (43.58)
AA+CA+PVP+AC	100.0+100.0+100.0+3000.0	34.26 ^{f} (35.80)	44.34 ^{bc} (41.73)
AA+CA+PVP+AC	150.0+150.0+150.0+3000.0	29.35 ^{f} (32.77)	37.29 ^{cd} (37.61)
CD (0.05)		**13.252**	**7.855**

.AA - Ascorbic Acid, **CA** - Citric acid, **PVP**- Polyvinylpyrrolidone, **AC** - Activated charcoal

* Values within column followed by different letters are significantly different at 5% probability level

Table 3. *In vitro* response of cultured nodal segments on various modification of MS medium.

Culture medium	Growth regulators mg l^{-1}				% Response	No. of shoots/ explant	Mean shoot length (cm)
	2,4-D	NAA	BAP	Kn			
MSBD	0.5	-	1.0	-	24.32^{f}(29.50)	1.14^{e}(6.15)	0.82 ^{a} (5.19)
MS2BD	0.5	-	2.0	-	27.81^{e}(31.37)	1.38^{e}(6.79)	1.04 ^{a} (5.80)
MS3BD	0.5	-	3.0	-	32.84^{cd}(34.93)	1.76^{d}(7.60)	1.16 ^{a} (6.11)
MS4BD	0.5	-	4.0	-	38.45^{c}(38.30)	2.84^{ab}(9.72)	1.30 ^{a} (6.53)
MS5BD	0.5	-	5.0	-	18.26^{g}(25.23)	0.98^{e}(5.63)	0.83 ^{a} (5.22)
MSBN	-	0.5	1.0	-	36.50^{c}(37.13)	1.22^{e}(6.29)	0.91 ^{a} (5.47)
MS2BN	-	0.5	2.0	-	39.46^{bc}(38.89)	1.47^{d}(7.03)	1.14 ^{a} (6.01)
MS3BN	-	0.5	3.0	-	43.56^{ab}(41.28)	1.91^{cd}(7.92)	1.25 ^{a} (6.41)
MS4BN	-	0.5	4.0	-	47.65^{a}(43.65)	3.20^{a}(10.30)	1.43 ^{a} (6.79)
MS5BN	-	0.5	5.0	-	29.52^{de}(32.88)	1.11^{e}(6.02)	0.87 ^{a} (5.34)
MSKD	0.5	-	-	1.0	13.41^{g}(21.42)	1.04^{e}(5.85)	0.71 ^{a} (4.82)
MS2KD	0.5	-	-	2.0	18.36^{fg}(25.24)	1.26^{e}(6.42)	0.75 ^{a} (4.96)
MS3KD	0.5	-	-	3.0	24.37^{ef}(29.53)	1.65^{d}(7.38)	0.96 ^{a} (5.59)
MS4KD	0.5	-	-	4.0	27.40^{e}(31.47)	2.42^{bc}(9.00)	1.21 ^{a} (6.28)
MS5KD	0.5	-	-	5.0	11.77^{g}(19.99)	0.88^{e}(5.38)	0.74 ^{a} (4.93)
MSKN	-	0.5	-	1.0	25.45^{e}(25.23)	1.11^{e}(6.02)	0.80 ^{a} (5.13)
MS2KN	-	0.5	-	2.0	27.64^{e}(31.66)	1.39^{de}(6.79)	1.04 ^{a} (5.80)
MS3KN	-	0.5	-	3.0	31.85^{d}(34.28)	1.80^{d}(7.70)	1.16 ^{a} (6.11)
MS4KN	-	0.5	-	4.0	38.14^{c}(38.09)	3.12^{a}(10.14)	1.27 ^{a} (6.42)
MS5KN	-	0.5	-	5.0	17.44^{g}(24.53)	0.98^{e}(5.63)	0.77 ^{a} (5.03)
Mean					**28.67(31.37)**	**1.63(7.18)**	**1.00(5.70)**
CD (0.05)					**(6.945)**	**(0.545)**	**(0.726)**

* Values within column followed by different letters are significantly different at 5% probability level.

Table 4. Effects of plant growth regulators on *in vitro* rooting of shootlets.

Culture medium	Growth regulators mg L^{-1}				% Response
	IBA	NAA	BAP	Kn	
MS.5I	0.5	-	-		8.46^{cd}(16.87)
MSI	1.0	-	-	-	11.24^{bc} (19.46)
MS2I	2.0	-	-	-	14.45^{ab} (22.30)
MS3I	3.0	-	-	-	5.43^{de} (13.46)
MS.5N	-	0.5	-	-	0.00^{e} (0.00)
MSN	-	1.0	-	-	3.16^{e} (10.10)
MS2N	-	2.0	-	-	4.95^{e} (12.75)
MS3N	-	3.0	-	-	0.00^{e} (0.00)
MS.5B	-	-	0.5	-	2.45^{e} (9.00)
MSB	-	-	1.0	-	0.00^{e} (0.00)
MS2B	-	-	2.0	-	0.00 e (0.00)
MS3B	-	-	3.0	-	0.00 e (0.00)
MS.5Kn	-	-	-	0.5	3.48 e (10.62)
MSKn	-	-	-	1.0	4.44 e (12.08)
MS2Kn	-	-	-	2.0	0.00 e (0.00)
MS3Kn	-	-	-	3.0	0.00 e (0.00)
MSI.5B	1.0	-	0.5	-	13.28 b (21.23)
MS2I.5B	2.0	-	0.5	-	17.20 a (24.47)
MSI.5Kn	1.0	-	-	0.5	12.46 b (20.61)
MS2I.5Kn	2.0			0.5	15.45 a (23.11)
Mean					5.82(10.80)
CD (0.05)					3.75

* Values within column followed by different letters are significantly different at 5% probability level.

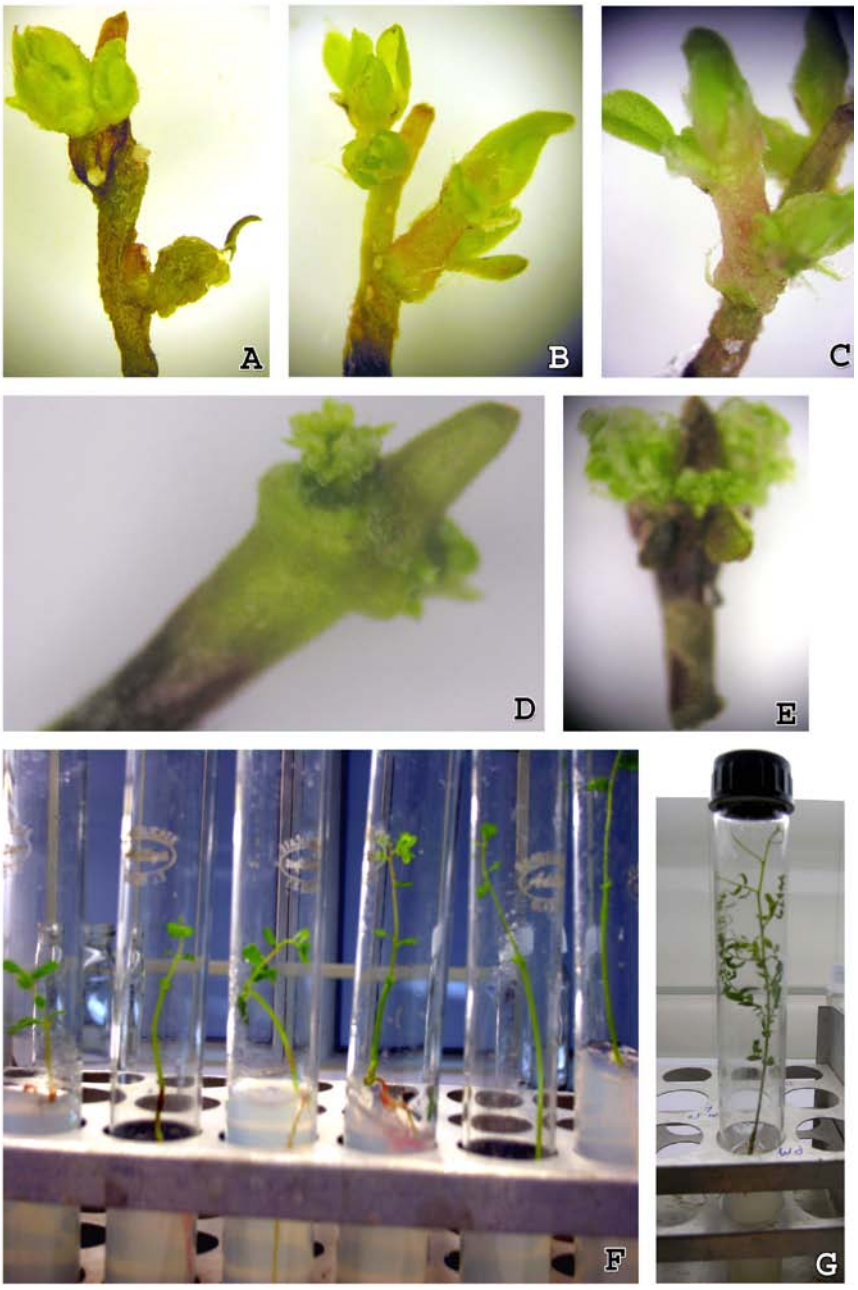

Fig. 1. Plant regeneration from nodal segments of *Emblica officinalis*: **A-C.** Shoot initiation after 25-30 days in culture; **D-E.** Multiple shoot initiation after 25-30 days in culture; **F.** Shootlets after 40-45 days of culturing; **G**. Gammo-rhizogeensis *in vitro* after 60-75 days of culturing.

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