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Short Communication

Clonal micropropagation of Indian gooseberry (*Emblica officinalis* Gaertn.)

Maneesh Mishra, Rajesh Pati, Ramesh Chandra and C. Kole[1]

Biotechnology Lab., Central Institute for Subtropical Horticulture, Rehmankhera, P.O. Kakori, Lucknow 227 107

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Indian gooseberry (*Emblica officinalis* Gaertn.), commonly known as aonla, belongs to the family Euphorbiaceae and is well known for its medicinal and therapeutic properties. Non-availability of genuine planting material and desired number of shoots for grafting or budding is the main limiting factor in the establishment of Indian gooseberry orchard. The use of micropropagation approach for accelerating the production of clonal stock of commercial cultivars in Indian gooseberry has not been a success. Mishra *et al.* [1] tried to propagate Indian gooseberry through *in vitro* shoot tip culture. However, micropropagated plants could not be acclimatized due to lack of functional roots. Therefore, we modified our protocol to develop a complete micropropagation system with high frequency shoot proliferation and efficient acclimatization. About 14 year old, bearing tree of *Emblica officinalis* cv. NA-6 was chosen for the study. The tree was pruned to stimulate new growth. The long shoots were excised, defoliated and trimmed to three centimeter keeping at least one axillary bud from 10-15th nodal segments of shoots as per protocol of Mishra and Pathak [2]. The shoots were first washed under running tap water and then kept in to a solution containing 0.1% Carbendazime (Bavistine) + 60.75 mM Rifampicin with two drops of tween-20 for one hour. The washed shoot buds were further surface sterilized with 0.1% HgCb for 8 minutes aseptically followed by 5-6 washing with sterile distilled water. Sterilized shoots were inoculated on MS medium supplemented with differential level of GA_3 (0.0, 1.44, 2.88 and 4.33 mM) and constant regime of Kinetin (13.9 mM) and Glutamine (342.11 mM) to examine the proliferative ability of shoot buds. Twenty-five explants were used in each treatment. The pH of the media was adjusted to 5.75 prior to autoclaving. Two-to-three cm long *in vitro* raised microshoots were cultured on MS and 1/2 MS basal medium containing IBA (0.0, 39.36 and 49.20 mM) and NAA (0.0, 5.37, 10.74 and 16.11 mM) in various combinations with 0.1% activated charcoal. The cultures were kept in dark for first 48 h and finally shifted to light. After 45-50 days, the rooted plantlets were thoroughly washed with sterile distilled water and were transferred to glass bottles (450 ml Vol.) containing autoclaved sand + soil + FYM (1:1:1). A total of 24 plantlets of similar age and size were inoculated in bottles. All bottles were supplemented with 1/2 MS nutrient salt solution and 3.40 mM Paclobutrazol. Initially, the culture bottles were incubated in the growth room under 40 $\mu molm^2\ s^{-1}$ light at 25°C temperature for about 15 days, then were shifted to plant growth chamber where high illumination (100 $\mu molm^2\ s^{-1}$) and high humidity (70-80%) was maintained. After 25-30 days, the plants were shifted to shade net house (50% shade intensity) provided with misting. The caps of the bottles were loosened gradually and the plants were shifted to pots. Cultures were incubated in growth room at 25 ± 2°C temperature for 16 h photoperiod with light at 40 $\mu molm^2\ s^{-1}$ at 55% relative humidity. Autoclavable, transparent glass bottles (450 ml volume) each containing 25 ml of nutrient media with two explant per culture bottle were used in the experiments. In the beginning, explants were inoculated on MS medium fortified with 0.4 mgl^{-1} kinetin for bud induction. The medium was gelled with 0.8% agar and supplemented with 3% sucrose. The data obtained from each character were analyzed by using the completely randomized design and two ways factorial analysis.

A perusal of data (Table 1) clearly revealed that GA_3 was critical for proliferation of shoots. Shoot explants inoculated on MS medium supplemented with 4.33 mM GA_3 + 13.9 mM Kinetin + 342.11mM Glutamine gave the highest shoot proliferation (13.33 indeterminate shoots/culture) followed by 2.88 mM GA_3 + 13.9 mM Kinetin + 342.11 mM Glutamine (10.33 indeterminate shoots/culture). Results clearly indicated that microshoots cultured on MS medium supplemented with 39.364 mM IBA + 5.37 mM NAA rooted profusely (9.67 roots per explant). However, microshoots inoculated on 1/2 MS containing 49.2 IBA + 10.74 NAA mM produced the lengthiest roots (3.04 cm) without any callus phase having fibrous root architecture, whereas MS + 39.36 IBA + 5.37 mM NAA produced the thickest roots (0.133 mm root diameter) lacking root hairs. All microshoots cultured on MS medium, irrespective of hormonal regime employed, produced small amount of callus at the shoot base, which affected the quality of feeder root. Such

[1]Present address: Department of Horticulture, Pennsylvania State University, Pennsylvania, USA

Table 1. Mean effect of bioregulants on *in vitro* proliferation of Indian gooseberry

Treatment (mM)	No. of explants inoculated	No. of explant responded for growth	Length of shoots (cm)		No. of shoots		No. of leaves
			Indeter-minate	Deter-minate	Indeter-minate	Deter-minate	
MS	25	15	0.800	0.000	6.000	0.000	2.333
MS+$GA_3$1.443+Kinetin 13.9+Glutamine342.11	25	19	0.967	0.067	7.667	0.667	3.667
MS+$GA_3$2.88+Kinetin 13.9+Glutamine342.11	25	21	1.167	0.167	10.333	1.000	4.333
MS+$GA_3$4.33+Kinetin 13.9+Glutamine342.11	25	23	1.633	0.400	13.333	3.000	5.667
SEm±			0.10	0.05	0.89	0.46	0.51
CD (0.05)			0.231	0.115	2.055	1.062	1.21

plants do not acclimatized well. Twenty four micropropagated plants having well developed root as well shoot system were inoculated on autoclaved Soil + Sand + FYM. Around 72.22% plants could be acclimatized. *In vitro* oxidative browning and in borne contaminations were the two major problems in establishing aseptic cultures of *Emblica officinalis*. Young shoots excised from new flush followed by treatment with 100mg/l PVP (Polyvinylpyrollidon) solution for 60 minutes under agitated condition and frequent subculturing was found to be effective in controlling problem of oxidative browning. Soon after *in vitro* bud induction, a determinate shoot appears with leaves, which only bears flowers. However, indeterminate shoots are mandatory for further multiplication. Therefore, role of GA_3 for elongation of indeterminate shoot is of paramount importance. High frequency shoot proliferation (13.33 shoots/culture) was observed under the influence of 4.33 mM GA_3 + 13.9 mM Kinetin + 342.11 mM Glutamine. In some woody species GA_3 has been used in shoot proliferation medium to improve shoot elongation [3]. Usually, GA_3 inhibits shoot bud formation. However, once shoot buds were formed it did not inhibit their further development. *In vitro* leaf fall was the major problem in micropropagating Indian gooseberry. Incorporation of glutamine (342.11 mM) could solve this problem. Amino acids such as glutamine, alanine, arginine, proline even polyamines have found to play important role in senescence of leaf [4]. It can be concluded that 1/2 MS (49.20 mM IBA + 10.74 NAA mM) proved to be better for *in vitro* rooting of Indian gooseberry (Table 2). The cultures were kept in the dark for first 48 hours and then transferred to light for normal growth of roots. Micropropagated plantlets of Indian gooseberry grown on coconut husk substrate showed *in vitro* leaf fall whereas they grew well under mixture of soil, sand and compost. The plants are being evaluated.

Table 2. Influence of bioregulants on *in vitro* rhizogenesis in Indian gooseberry

Bioregulants (mM)	No. of explants inoculated	No. of explant responded	No. of roots/ plant	Average length of roots (cm)	Average root wt. (g)	Root diameter (mm)	Days taken for rooting
MS+IBA 39.36+NAA 5.37	15	15	9.67	1.94	0.0160	0.0200	6.33
MS+IBA 39.36+NAA 10.74	15	15	3.67	2.45	0.0200	0.0167	7.00
MS+IBA 39.36+NAA 16.11	15	15	6.30	2.17	0.0051	0.0267	7.00
MS+IBA 49.20+NAA 5.37	15	15	3.67	2.96	0.0170	0.0367	5.67
MS+IBA 49.2+NAA 10.74	15	15	3.67	2.58	0.1880	0.0233	6.67
MS+IBA 49.2+NAA 16.11	15	15	3.67	1.99	0.0183	0.0233	7.00
1/2MS+IBA 39.36+NAA 5.37	15	15	3.33	2.83	0.0046	0.1330	7.67
1/2MS+IBA 39.36+NAA 10.74	15	15	4.33	1.82	0.0798	0.0167	8.67
1/2MS+IBA 39.36+NAA 11.16	15	156	3.67	2.10	0.0117	0.0467	8.67
1/2MS+IBA 49.20+NAA 5.37	15	15	1.00	2.73	0.0058	0.0233	7.33
1/2MS+IBA 49.20+NAA 10.74	15	15	4.00	3.04	0.0098	0.0333	7.33
1/2MS+IBA 49.20+NAA 16.11	15	15	1.00	1.33	0.0020	0.0200	8.67
SEm±			0.88	0.51	-	0.0038	-
CD (0.05)			1.82	1.056	NS	0.0080	NS

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