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Short Communication

Clonal propagation of *Citrus jambhiri* Lush through nucellar embryo culture

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Citrus jambhiri Lush (Family Rutaceae) is native to India and is used as a rootstock to commercially important citrus trees like *Citrus reticulata* and *Citrus sinensis*. The seedlings have normally slow growth and majority of them do not attain appropriate size within a normal budding period for transplanting and one has to discard such under developed seedlings for budding [1].

One of the essential requirements for the successful application of plant propagation technology to agriculture is the capacity to regenerate elite plantlets. Micropropagation of commercially important Citrus species has been reported by many workers [2, 3] but the *in vitro* studies for *C. jambhiri* are not yet reported. With above views a protocol for clonal propagation of *C. jambhiri* has been developed using nucellar embryo as a starting material.

Large and healthy fruits of *C. jambhiri* were selected from a local orange orchard from disease resistant trees. Seeds were isolated and washed thoroughly under tap water. Seeds were decoated and nucellar embryos were removed and surface sterilized by immersing in 0.1% $HgCl_2$ (w/v) followed by 4 washes with sterilized double distilled water. These embryos were placed on surface of a full strength Murashige and Skoog's [4] basal medium with sucrose (30,000) and phytagel (0.25%). Plant growth regulators were added at different concentrations and combinations according to the experimental requirements. The tubes were incubated at 27 ± 2° C, 65% relative humidity and 16:8 h photoperiod at 1500 lux. Treatments were replicated 5 times and 10 plants were used for each replication. Cultures were examined and observations for induction of shoot and multiple shoot formation were recorded in the form of statistical data [5]. The percentage of explants forming shoots and the mean number of shoots per explants were determined for each treatment. The shoots thus obtained were transferred to auxin rich MS medium for root induction. Number of roots developed per shoot was noted 4 weeks after transfer.

Two weeks after the nucellar embryos were inoculated, shoot induction was noted in the tubes. All the newly formed shoots developed into plantlets (each with 6-8 leaves and 3-4 roots) after being transferred onto basal medium supplemented with IBA for 30 days. The plants were ready for further use as a rootstock for *in vitro* micrografting technique or to be transferred to green house.

Without any subculture on hormone free basal medium 17.16 percent of nucellar embryo explants of *Citrus jambhiri* formed an average of 1.03 shoots in eight weeks (Table 1). However when basal media was fortified with BAP at 2.22 µM concentration, average number of shoots formed was noted to be 18.20. This concentration induced a higher percentage of explants forming shoots and shoot number per explant than the hormone free treatment. Further when NAA was introduced in the media the number of shoots formed was found to be declining. Effect of the cytokinins are said to vary with cultivars but Mohanty *et al.* [6] did not find any response of *Citrus sinensis* for shoot proliferation in MS + BAP and 2,4-D. However, Gill and Gosal [7] reported maximum shoot regeneration in MS media supplied with BAP 1mg l^{-1} and GA_3 2 mg l^{-1}. BAP is effective in inducing *in vitro* morphogenesis in shoot regeneration and proliferation [8] of several horticultural crops. In this paper we show that BAP alone is found to be effective in multiple shoot induction from nucellar embryo explants of *Citrus jambhiri*. The shoots were transferred to MS medium supplemented with IBA alone or IBA and BAP for rooting. A maximum of 88.50 percent rooting was noted. Similar to present investigation, Sandra and Morehart in 1988 [9] reported that increasing IBA concentration had no effect on induction of number of roots instead it reduced total root length and promoted callus production. On the other hand Starrantino and Caruso [10] rooted citrus

Table 1. Effect of BAP and NAA on shoot formation from nucellar embryo explants of *Citrus jamhhiri* Lush

BAP (μM)	NAA (μM)	Percentage of explants with shoots	Average number of shoots per explant
0.00	0.00	17.16±0.42	1.03±0.03
0.44	0.00	38.06±0.33	4.30±0.23
2.22	0.00	74.50±0.30	18.20±0.49
4.44	0.00	50.63±0.09	7.70±0.05
2.22	2.69	53.00±0.34	11.5±0.35
4.44	2.69	41.33±0.83	7.2±0.58

Table 2. Effect of BAP and IBA on *in vitro* rooting of *Citrus jambhiri* Lush

BAP(μM)	IBA (μM)	Percent rooting	Average number of roots per shoot
0.00	0.00	0.00	0.00
0.00	2.46	65.68±0.54	2.90±0.05
0.00	4.92	88.50±0.66	6.70±0.03
1.11	2.46	70.50±0.75	4.50±0.25
1.11	4.92	46.00±0.11	2.00±0.63

* Values in ± denotes S.D.

rootstocks like Carrizo, Troyer and CPB4475 in MS media nutrified with NAA 1 mg l^{-1}. The *in vitro* morphogenesis stimulated by the same explant on different hormone treatments is quiet different. Therefore the culture requirements and media composition for specific selected plants needs to be identified and optimized.

In the present investigation a reliable protocol via multiple shoot formation and *in vitro* rooting of the shoots for *Citrus jambhiri* was established. The micropropagated plantlets thus obtained can be used for micrografting.

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