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In-vitro propagation of Rangpur lime (*Citrus limonia*)

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Abstract
The present study was undertaken with an objective to develop a protocol for micro propagation of Rangpur lime (*Citrus limonia*) through shoot tip. Shoot tip of 1 to 2 cm were collected and culture in Murashige and Skoog (MS) medium. The treatment $HgCl_2$ 0.1% for 10 min was found to be best sterilization treatment with respect to survival culture percent and aseptic culture percent. BAP 2mg/l was found to be best treatment for establishment media with respect to maximum sprouting, minimum days taken for sprouting with highest number of shoots/explant. IBA 2mg/l + NAA 0.1mg/l + activated charcoal 1000mg/l was found to be significantly superior over all other treatments with respect to maximum root initiation percentage, days to root initiation, highest number of roots.

Keywords: Micropropagation, *Citrus reticulata*, BAP, $HgCl_2$

Introduction
Citrus is one of the important fruit crop and is preferred due to its low acidity and high juice content. Vegetative propagation methods like budding, cutting, layering are practiced during limited period of the year. Moreover, these methods tend to propagate pathogens present in mother explants. By using tissue culture techniques, thousands of genetically identical elite plants can be vegetative propagated in short time span and limited space with year round availability. Rangpur lime (*Citrus limonia*) is susceptible to a number of diseases like Phytophthora rot (fungaldisease), greening (bacterial) disease and psorosis, exocortis (viral diseases). Propagation through nuclear tissue of fertilized or unfertilized ovules is tedious. Moreover, ovules at right stage of development are available only for a very short period during the year. Juvenile characteristics and delayed bearing are disadvantages in production of true to type and virus free plants. The present study was conducted with an objective to develop a protocol for Rangpur lime micro propagation through shoot tips.

Materials and Methods
Shoot tip explants of Rangpur lime (*Citrus limonia*) were collected from Citrus Nursery, at AICRP in citrus, DR.PDKV, Akola. Experiment was conducted at the Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidhypeeth, Akola. The explants were thoroughly washed under running tap water for 15-20 minutes properly. The explants were further treated with 0.1% bavistin for 20 minutes on rotator-shaker. This facilitates proper action of sterilant chemical on the explants taken. The explants were then washed properly with double distilled water (DDW). The explants were than subjected to different concentrations of $HgCl_2$ i.e. 0.05%, 0.1% and 0.2% for different duration 5 min, 10 min, and 15min. The explants were dipped in 70% ethyl alcohol for 30 seconds and finally rinsed thrice with double distilled water. Murashige and Skoog (MS) medium with different concentrations of BAP i.e. 1mg/l, 2mg/l and 3mg/l, (Table No. 2) 3% w/v sucrose and 0.8% w/v agar was took for (Fig. 1) inoculation of explants. The pH of the media was adjusted to 5.8 before autoclaving. Culture tubes/flasks after inoculation were incubated in the incubation chamber by maintaining 25±2 °C temperature with 16/8 hours day/night regime with 300-3200 lux light intensity supplied through white fluorescent tubes. Different concentrations of IBA i.e. 1mg/l, 2mg/l and 3mg/l were used with combination of NAA 0.1 mg/l and 1000mg/l activated charcoal for shoot proliferation in different concentrations in establishment medium. The rooted plantlets were carefully removed from agar media and washed in a shallow tray containing sterile water to remove adhering agar. They were then transferred to pots containing sand: soil: compost (1:1:1). The sterilized pots were kept under plant growth chamber with punched polythene covering top of the plants the plants were kept under such condition for nearly 25-30 days. Then pots were kept under polyhouse conditions for nearly two weeks for

hardening and then transferred to the field. The experiment was subjected to analysis of variance using completely randomized design (CRD). 3 replications were taken for each treatment (control counted as treatment) comprising 30 units. In $HgCl_2$ treatment the percentage data was subjected to arcsine transformation before statistical analysis.

Results and Discussion

It was observed from Table No. 1 that the highest aseptic culture percentage of 91.10% was for Rangpur lime (*Citrus limonia*) shoot tips with treatment 0.2% $HgCl_2$ for 15 min. and highest survived culture percentage of 96.66% was recorded for Rangpur lime (*Citrus limonia*) shoot tips with treatment of 0.05% $HgCl_2$ for 5 min.

In surface sterilization of explant both survived culture percentage and aseptic culture percentage are equally important; in this study it was revealed that both are directly proportional to each other. Therefore the optimum surface sterilization treatment for Rangpur lime (*Citrus limonia*) should be 0.1% $HgCl_2$ for 10 min, as it showed 86.66% aseptic culture percent and 84.43% survived culture percent.

The toxic effects of $HgCl_2$ have been reported earlier by several investigators. Increase in exposure time of the above surface sterilizing agent drastically affected explants survival. One probable reason for the death of explants when exposed to surface sterilizing agent for longer duration may be due to heavy metal concentration of mercury present in $HgCl_2$, proving detrimental for the survival of explants.

In low concentration of $HgCl_2$ the explant survival percentage was high but also increases the contamination in culture due to insufficient concentration of surface sterilizing agent ($HgCl_2$) to kill microorganisms present on explant. Therefore the concentration of $HgCl_2$ which showed balance in both survival and aseptic parentage were acceptable for surface sterilization of Rangpur lime (*Citrus limonia*) which is 0.1% $HgCl_2$ for 10 min. The results obtained are in conformity with Rana and Singh (2002) [5], Symal *et al*., (2007) and Upadhyay *et al*., (2010) [8]. They used $HgCl_2$ as surface sterilizing agent for citrus explants.

In present investigation revealed that the BAP concentration were standardized for shoot initiation in Rangpur lime (*Citrus limonia*). treatment of BAP @ 2 mg/l showed highest number of shoots per explant i.e. 4.66 shoots it took 15.67 days for shoots proliferation after shoot initiation as per described in table 2 and showed in Fig. 2.

Highest number of explant response to shoot initiation for every 10 explant per replication i.e. 9.66 it took minimum 9.66 days for shoots initiation as per described in table 3.

Table 1: Standardization of surface sterilization treatment for *Citrus limonia* with different concentration of $HgCl_2$

Concentration of $HgCl_2$	Exposure time (in minute)					
	5 min		10 min		15 min	
	Percent of aseptic culture after 15 days	Percent of survived culture after 30 days	Percent of aseptic culture after 15 days	Percent of survived culture after 30 days	Percent of aseptic culture after 15 days	Percent of survived culture after 30 days
Control	00	00	00	00	00	00
0.05%	11.10	96.66	28.86	93.33	56.66	90
0.1%	58.86	91.10	86.66	84.43	88.86	78.86
0.2%	63.33	86.66	87.76	72.20	91.10	55.53
CD at 5%	5.42	4.79	4.61	5.58	5.80	5.28
SE (M)	1.66	1.47	1.42	1.71	1.78	1.62

Table 2: Effect of BAP concentration on number of shoots per explant and days to shoot proliferation in Rangpur lime (*Citrus limonia*)

BAP concentration in mg/l in MS media	Number of shoots per explant	Days required for shoot proliferation after shoot initiation
Control	1.66	25.33
1	2.66	19.33
2	4.66	15.67
3	3.33	17.33
CD at 5%	0.85	1.84
SE (M)	0.26	0.57

Inhibitory effects of high concentration of BAP on explant have been reported earlier by several investigators. Due to high concentration of BAP in MS media explant metabolic activity get influence by imbalance of growth regulator concentration in explant, therefore in high concentration of BAP the number of shoots per explant gets reduced.

The obtained results were found in similarity with Kamble *et al*., (2005), Rathore *et al*., (2006), Haripyaree *et al*., (2011) and Upadhyay *et al*., (2010) [8].

Table 3: Standardization of BAP concentration for shoot initiation in *Citrus limonia*

Treatments	concentration of BAP (mg/L) in MS media	No. of explant inoculated per replication	Response to shoot tip initiation	Days for shoot initiation
T1	0.0	10	3.33	27.66
T2	1.0	10	6.33	14.66
T3	2.0	10	**9.66**	**9.66**
T4	3.0	10	8.66	11.33
CD at 5%			1.67	3.06
SE (M)			0.52	0.94

It is evident from Table No. 4 that the treatment IBA (2.0mg/l) + NAA (0.1 mg/l) + activated charcoal (1000 mg/l) was found to be significantly superior over all other treatments for shoot tips. This best treatment gave maximum root initiation of 86.66% minimum of 15.66 days took for root initiation, highest number of 3.66 roots. The above results have some resemblance with the earlier findings of Rana and Singh (2002) [5], Upadhyay *et al.*, (2010) [8] and Kumar *et al.*, (2001) who have reported good roots initiation results with IBA and NAA combinations. Auxins promote adventitious root development on intact plants as well as excised stems. Of these, IBA is 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. Auxins stimulate cell expansion by cell wall loosening after a short time following exposure, in addition, auxins promote protein synthesis by controlling gene expression as a response after a long time interval. Activated charcoal may also induce the formation of adventitious roots in some species. Its presence in the medium reduces the light supply to *in-vitro* regenerated shoots and helps in improvement of absorption of growth regulators in cultures. The complete plantlets with well-developed roots were transferred to plastic jars filled with mixture of sand: soil: compost (1:1:1) before transferring to field directly.

Table 4: Effect of IBA on root initiation percentage, days to root initiation, number of roots

IBA concentration in mg/l in MS media	Root initiation	Days to root initiation	No. of roots
00	00*	00	00
1 mg/l+NAA0.1mg/l+1000mg/l Activated charcoal	38.00%	21.33	1.33
2 mg/l NAA0.1mg/l+1000mg/l Activated charcoal	86.66%	15.66	3.66
3 mg/l NAA0.1mg/l+1000mg/l Activated charcoal	60.33%	22.00	1.66
CD at 5%	2.20	1.99	0.66

*Figure in parentheses show arc in transformation value.

Fig 1: Inoculated shoot tip of *Citrus limonia*.

Fig 2: Proliferated shoots of *Citrus limonia*.

References

1. Bhansali R, Arya HC. Differentiation in explant of Citrus paradisi Macf. (Grapefruit) grown in culture. Indian J Exp. Biol. 1978;16:409-11.
2. Kour Kiran, Bakshi, Parshantand Kher, Ravi. Studies on micropropagation of rough lemon. Indian J Hort. 2007;64:454-55.
3. Kumar Krishnan, Dhatt AS, Gill MlS. In vitro plant regeneration in sweet orange (*Citrus sinensis* L. Osbeck) cv. Mosambi and Jaffa. Indian J Hort. 2001;58:208-11.
4. Parthasarathy VA, Barua A, Nagaraju V, Parthasarathy U. Quadratic response of Citrus species to cytokinins and comparative efficacy on morphogenetic characters of in-vitro proliferated shoots. Indian J Hort. 2001;58:336-41.
5. Rana J, Singh Ranvir. /rr-v/froclonal propagation of Kagzi lime (*Citrus aurantlfolia* Swingle) through shoot tips. Prog. Hort. 2002;34:27-34.
6. Starrantino A, Carus A. /n-v/fro culture for Citrus micro propagation. Acfa Hort. 1988;227:444-46.
7. Syamal MM, Upadhyay Sujata, Biswas Sangita. /n-v/froclonal propagation of Kagzi lime (*Citrus aurantifolia* Swingle) Indian J Hort. 2007;64:84-86.
8. Upadhyay S, Shyamal MM, Ltoo H. Micro-propagation of Sweet Orange cv. Mosambi through shoot tips and nodal segments. Ind. J Hort. 2010;67:21-25.