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EMBRYO CULTURE AND DEVELOPMENT OF SEEDLINGS IN DIFFERENT *CITRUS SPECIES*

Bikramjit Singh Gill, Savreet Sandhu and Sukhdev Singh

Department of Horticulture, Khalsa College, GNDU, Amritsar-143 002, Punjab.

E-mail: singh_kotla@yahoo.co.in

ABSTRACT: The citrus industry is considered to be a major fruit industry hence it needs to be improved to cater to the diverse needs of consumers and crop breeders. Genetic manipulation through conventional techniques in this genus is invariably a difficult task for plant breeders as it poses various biological limitations comprising long juvenile period, high heterozygosity, sexual incompatibility, nucellar polyembryony and large plant size that greatly hinder cultivar improvement. The demands for elite rootstock material are continuously increasing for fruit production and to fulfill such demands application of *in vitro* propagation techniques is one of the successful alternative particularly in case of citrus crops. One of the essential requirements for the successful application of plant propagation technology in agriculture is its capacity to regenerate elite plantlets. The process of embryo culture is a suitable method of micropropagation and has the potential of mass propagation commercially. Keeping in mind these things experiment on "Embryo culture and development of seedlings in different *Citrus species*" was conducted. The seeds were extracted from the developing fruits from the trees growing in the college nursery and were sterilized. The embryos of six *Citrus species* were cultured to obtain the stock plants. The germination ranged from 71.5 to 96.0 per cent and the embryos were inoculated on the basal Murashige and Skoog medium. *Citrus limon* gave the maximum (96 per cent) germination and *Citrus sinensis* resulted in minimum (71.5 per cent) germination. It was concluded from the experiment that *in vitro* propagation has been a great potential tool to overcome problems related with the field culture for citrus species. These advances in biotechnology have generated new opportunities for citrus genetic improvement. Therefore, development of efficient embryo culture protocols is necessary for conservation and genetic improvement of citrus.

Keywords : *Citrus sp., embryo culture, germination.*

Citrus is highly popular crop among the masses and is commercially cultivated for its processing quality, fresh consumption and aromatic flavour. Despite its cultivation on large areas, citrus plantation still has some problems such as slow growth, long juvenility, insect pests, diseases, alternate bearing, pre and post harvest losses, large number of seeds per fruit, short season of supply and short storage life etc. Traditional genetic plant improvement offers limited scope for the production of new varieties of scion and root stocks and the new varieties produced so far were originated form natural selection and mutations. Advances in the field of biotechnology have generated new opportunities for citrus genetic improvement. One of the essential requirements for the successful application of plant propagation technology in agriculture is the capacity to regenerate elite plantlets (Karwa and Chikhale, 6). The demand for elite rootstock material are continuously increasing and to fulfill such demands, application of *in vitro* propagation technique is the only alternative way. *In vitro* propagation has therefore been a great potential tool to overcome problems related with the field culture for such species. The process of embryo culture is a suitable method of micropropagation and has the potential of mass propagation commercially. Usefulness of citrus rootstocks for the improvement of canopy architecture, fruit production, quality and tolerance to biotic and a-biotic stresses of citrus crops is well known. However these rootstocks, propagated by growing open-pollinated seeds, are highly nucellar and produce true to type plants. Depending upon the rootstocks, generally 1 to 40% zygotic seedlings

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are produced, which must be culled from seed beds to maintain clonal uniformity. All citrus cultivar selections are usually grafted to selected rootstock seedlings. Some potentially valuable rootstocks produce few or no seed and thus seed shortage of such popular rootstock occurs periodically. Further, the demand of quality planting material of important rootstocks in ample number necessitates for their *in vitro* propagation. During the past years, micro-propagation techniques have been widely used for several plant species. Also, the plant regeneration in citrus species has been reported by various workers. Tissue culture technique could be used for propagation of citrus rootstocks and thus, the number of plants produced would not be limited by their seed supply, rather more uniform disease free and quality plant populations might be produced. In spite of micro-propagation of citrus genotypes reported by several workers, a very few reports of *in vitro* multiplication of citrus rootstocks Pectinifera, Troyer citrange, Cleopatra mandarin and Rough lemon are available in the literature.

The citrus industry is considered to be a major fruit industry hence it needs to be improved to cater to the diverse needs of consumers and crop breeders. Several attempts are made to improve Citrus species by using various *in vitro* techniques. Citrus varieties are propagated sexually through seeds, while most of the commercial varieties are propagated by various asexual methods (Chaudhary, 2). Genetic manipulation through conventional techniques in this genus is invariably a difficult task for plant breeders as it poses various biological limitations and large plant size that greatly hinder cultivar improvement. Micro propagation is an important asexual method that can be used for the production of virus – free roots tock plants (Roistacher *et al.,* 8). An efficient embryo culture protocol is a perquisite for clonal propagation of citrus species. Embryo culture can be used to rapidly expand the area under citrus cultivation as embryogenesis provides opport-unities of raising true to type plants.

MATERIALS AND METHODS

The study was carried out during 2009-10 in the Department of Horticulture, Khalsa College, Amritsar. The freshly extracted seeds from fruits of 5 citrus species viz. Baramasi lemon, Mosambi, Rangpur lime, *Pectinifera,* Jatti Khatti and a hybrid viz. *Carrizo*. The developing fruits were picked from the fruit trees growing in the field.

After extracting the seeds were washed with detergent and surface sterilized with 0.1 per cent HgCl2 (Mercuric chloride) for 8-10 minutes followed by 2-3 washings with sterilized distilled water. The testa of seeds was removed with the help of a needle and forceps. The embryos were removed and their size was measured with the help of a graph paper. Single embryo was inoculated in each culture tube containing 20 ml of MS (Murashige and Skoog) basal medium. All the experimental manipulations were carried out strictly under sterile conditions in a laminar flow cabinet (Klenzaids Bombay) and the cultures were maintained in air conditioned room at a temperature of 25 ± 2°C.

RESULTS AND DISCUSSION

(a) Germination in relation of *Citrus species*

The results of culturing of embryos in five citrus species and a hybrid have been divulged in Table 1. The data indicates that the germination ranged from 71.5 to 96.0 per cent when the embryos were inoculated on basal MS medium. The maximum (96.0 per cent) germination was recorded in *Citrus limon* and the minimum in *Citrus sinensis* (71.5 per cent). The cultured embryos showed the signs of germination after 3-4 days and emergence of the radicle within 5-7 days followed by unfolding of the cotyledons and elongation of the plumule. The embryo derived seedlings of *Citrus species* were normal in the growth and showed good root and shoot formation along with the appearance of leaves after 20-25 days of inoculation. The earlier findings of Ali and Mirza (1), Costa *et al.* (3) and Das *et al*. (4) also supported the present results.

Table 1: Germination percentage on basal MS medium through embryos in *Citrus species.*

S. No.	Name of *Citrus species*	No. of seeds	No. of seeds germinated	Germination/ regeneration (per cent)	Time taken for germination (days)	Ranking of *Citrus species* for germination of embryo
1.	*Citrus limon*	178	171	96.0	5-6	1
2.	*Citrus sinensis*	196	140	71.5	10-12	6
3.	*Citrus jambhiri*	106	97	91.5	6-8	2
4.	*Citrus limonia*	85	69	81.2	7-9	5
5.	*Citrus pectinifera*	96	80	83.3	5-8	4
6.	*Poncirus trifoliata* × *Citrus sinensis*	50	43	86.0	5-6	3

Table 2: Germination in relation to embryo size.

S. No	Citrus species	Embryo size (mm)	No. of embryos inoculated	No. of embryos germinated	Germination (%)
1	Citrus limon	3.0-5.0	17	12	70.5
		5.1-7.0	22	19	86.3
		7.1-8-0	23	22	95.6
		8.1-9.0	113	113	100.0
2	Citrus sinensis	3.0-5.0	15	4	26.0
		5.1-7.0	35	20	57.2
		7.1-8.0	60	49	81.6
		8.1-10.0	80	67	83.7
3	Citrus jambhiri	3.0-5.0	9	4	44.4
		5.1-7.0	18	16	88.8
		7.1-8.0	19	17	89.5
		8.1-9.0	60	60	100.0
4	Citrus limonia	3.0-4.0	7	2	28.5
		4.1-5.0	10	4	40.0
		5.1-6.0	11	8	72.7
		6.1-7.0	57	54	94.7
5	*Citrus pectinifera*	3.0-4.0	9	3	33.3
		4.1-5.0	12	8	66.6
		5.1-6.0	15	12	80.0
		6.1-6.5	60	57	95.0
6	*Carrizo*	4.0-5.0	5	2	40.0
		5.1-7.0	10	8	80.0
		7.1-8.0	10	8	80.0
		8.1-11.0	25	25	100.0

(b) Germination in relation to embryo size

Embryos of different sizes (<3 mm to 11 mm), cultured on MS basal medium showed great variation in germination ranging from 26.0 to 100.0 per cent in the cultivars selected (Table 2). The regenerated plants were normal in growth alongwith the formation of roots. The embryos ranging form 5 mm to 7 mm showed 94.7 per cent germination. The highest germination (100 per cent) was registered in embryos measuring from 8mm to 11 mm in case of *Citrus limon, Citrus jambhiri* and *Carrizo* while the embryos ranging from 5 mm to 6.5 mm in case of *Citrus pectinifera* showed 95 per cent germination. Plants produced were healthy and vigorous which were used for further experimental studies. It was noted that the embryos ranging form 5 mm to 7 mm produced 2-3 seedlings while 4 mm to 6 mm sized embryos only generated secondary embryoids in *Citrus pectinifera.*

The germination was high in all the species (71.5 per cent to 96.0 per cent) when embryos were cultured on basal MS medium. *Citrus sinensis* showed lesser germination as compared to the other species. Such genotypic differences in germination of seedlings from mature embryos have also been reported in different species of *Citrus* by Dubey and Rishi (5). The embryos showing less germination may be due to some developmental abnormalities of the embryos. In another experiment, embryos of different size ranging from less than 3 mm to 11mm were cultured. Embryos larger than 8 mm showed 100 per cent germination in *Citrus limon* and in *Carrizo* orange. High germination per cent for large sized embryos may be due to their capacity to produce hormones in sufficient quantity that promoted embryo development. The earlier findings revealed that the embryos smaller than 2 mm did not showed any rooting whereas 1-2 embryos had poor rooting. The experimental study revealed that germination was directly related to the embryo size, larger the embryos, higher the germination. Similar results have been reported in 'Morton' (Katyal, 7). In the present investigation embryo smaller than 2 mm lacked germination possibly due to the fact that the excised embryos were from mature fruit. The results of the present investigation on embryo culture show that larger the embryo size, higher the germination. Apart from this genotypic differences also influenced embryo germination. Seedlings derived from embryos smaller than 2 mm lacked rooting.

There is a need to culture immature embryos at different stages of their development so as to work out the minimum size of the embryos having reasonable capability to develop into seedlings for producing viable inter-specific and inter-generic hybrids.

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