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Short communication

Acclimatization of *in vitro* generated *Citrus* plantlets

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Citrus is the leading tree fruit crops of the world. The genus citrus includes more than 162 species belongs to the Order Geraniales family Rutaceae and sub family Aurantoideae. mandarin (*Citrus reticulata* Blanco.) sweet orange (*C. sinensis* (L) Osbeck), grapefruit (*C. Paradisi* Macf.), pummelo (*C. Grandis* Osbeck), acid lime (*C. aurantifolia* Christm) and lemon (*C. limon* (L) Burn F.) etc. are commercially grown for fruits in the world. Rough lemon (*C. jambhiri* Lush, Carrizo (*C. Carrizo*) and Cleopatra (*C. reshnii* Tanaka),karma khata (*C.karna*) , trifoliate orange (*Poncirus trifoliata*) and sour orange(*C.oarantium*) are widely used for root stock in different parts of the country. The rootstocks have prominent effect on growth, productivity, fruit quality and longevity of the tree on one hand and influence the susceptibility of the trees to various insect-pest and diseases on the other hand. In India, more than 80 percent citrus plants are being raised on rough lemon. A good citrus rootstock should have high degree of poly-embryory, compatibility with large number of scion, adaptability to particular soil, tolerance to cold, drought and wind, tolerance to viruses especially tristiza, exocortis (Chadda,1970) and ring spot, fungal diseases and nematodes. However, no single rootstock can fulfill all the criteria. Hence in present study three rootstocks viz. rough lemon (*C. jambhiri* Lush, carrizo (*C. carrizo*) and cleopatra (*C. reshnii* Tanaka) were selected. The rough lemon is the most widely used poly embroyonic rootstock for various scion cultivars all over the world. It imparts resistance to tristiza and exocortis viroied and is tolerant to salt and drought. Carrizo citrange, a hybrid between (*C. sinensis* cv Washington Naval x *Poncirus trifoliata* (L) is poly embryonic and cold hardy and tolerant to tristiza and foot rot. Cleopatra is a mono embryonic rootstock, dwarf, tolerant to tristiza, exocortis, drought and salt.

After Mexico, India is the leading producer of citrus fruits with an area of 923 lakh hectares with production of 8608 lakh tones annually (Anonymous, 2). In India, it ranks third in production after Banana and Mango. Among citrus crops, mandarin orange (Kinnow Mandarin, Nagpur, Khasi, Darjling) covers largest area followed by sweet orange (Musambi, Pineapple, Blood Red and Jaffa) and Acid lime. In India, citrus is being grown in Punjab, Rajasthan, Haryana, Himachal Pradesh, Jammu & Kashmir, and Utter Pradesh. In south India, Wynad, Nilgiri, Polney and Shevry hills are major mandarin growing belts while, hills of Meghalaya (Khasi, Dusha, Garo, Jaintea), Mizorum, Tripura, Sikkim and Arunachal Pradesh have predominance in mandarin cultivation.

In citrus, the infection of viruses and related pathogens have received attention due to decline of citrus trees and it was mainly attributed to viruses (Tristiza, Psorosis, Greening Ring spot virus, Exocortis, Gumosis, Xyloporosis, Bacteria etc.), fungi (Phytopthora) and Bacterial canker. Beside other factors like rootstock incompatibility, poor management, malnutrition, marginal soils, and irrigation water also affect citrus production, Tristiza virus has wiped out the citrus industry in many countries. For example, in Argentina and Brazil where the citrus industry expanded after first World War, Tristiza destroyed about 30 million trees. Similar situation was also reported from Spain, Japan, and United States. The estimates indicate that Tristiza destroyed about a million trees in India (Fraser *et al.,* 3 and Ahlawat, 1).

Most of the citrus are highly cross pollinated plant so that the maintenance of genetic make up of genotype in successive generation is not possible. So that the production of uniform large number of plants are not possible through seed. Hitherto, the most of the root stocks are polyembryony plant which produces true to type and disease free seedlings but identification of these seedlings are too complicated (Rangan, 7). The nucellar embryos resemble similar characters as parent. Tissue culture technology offers an advantage over conventional methods of propagation (seed) in producing large number of true to type plants from healthy plant with in a short period of time. Nucellar seedlings are true to type and free from any diseases. In this perspective studies were taken out to standardize the protocol for micro propagation of rough lemon from nodal segments of nucellar seedlings. The survival percent of *in vitro* generated plantlets were less in field condition if they are transferred directly with out acclimatization, in this

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perspective studies were carried out to standardize the protocol for acclimatization of *in vitro* generated citrus plantlets.

The rooted plantlets of different citrus species were carefully removed from the culture tubes and their roots were thoroughly washed under running tap water and cleaned with fine brush to remove adhered agar. The plantlets were covered with cotton wetted with half strength MS medium for 24 hours in culture room (Plate 1). The plantlets were treated with Bavastin @ 0.2 percent for 10 minutes to prevent fungal contamination. These plantlets were transferred to pots containing sterilized soil, vermiculite, and perlite in equal proportion. These pots were kept in green house at 90 percent humidity with temperature 26±2^{0}C. The humidity was continuously lowered within 8-10 weeks up to 60 percent. During this period the plantlets were irrigated with Hoglands solution at three days interval for one month. After that, these were irrigated with Hoglands solution and simple water at an interval of 2-3 days alternately. The observations were recorded for number of plantlets survival after 15, 30 and 60 days of planting in pots and incubated in green house.

Plate 1. Preparation of plantlets for hardening.
A, B = Plantlets; C,D = Plantlets in pot

The data recorded on survival percent of plantlets in pot in greenhouse after 60 days of transfer from culture room, are presented in table 1. The perusal of data reveal that the survival percent of kinnow was 82.50 percent, Carrizo 83.00 percent, Rough lemon 90.00 percent and Cleopatra 75.00 percent (Table 1). The maximum damage of plantlets after 60 days was recorded in Cleopatra. *In-vitro* propagated plantlets were successfully acclimatized by proper handling of plantlets. The higher survival in all the three genotypes may be due to higher number of roots, high porosity, cation exchange capacity (CEC) and water holding capacity of potting medium. The lowest survival of platelets was observed in Cleopatra and it may be due to lower number of roots. According to Gill *et al.,* 5 and Baruah *et al.*, 4) survival percent of *in-vitro* plantlets is directly related to number of roots. The results of the present study are in line with earlier reporters, Singh *et al.* (8) who reported that 60 percent plantlets of khasi mandarin established in soil. Perez-Molphe- Balch and Ochoa-Alejo (6) reported 85 percent survival rate in Musambi, Singh *et al.* (9) reported that 80 percent of Rough lemon plantlets established in the soil.

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Table 1. Percent Survival of plantlets of different citrus species.

Percentage of plantlets survival after (in days)	Kinnow	Carrizo	Rough lemon	Cleopatra
15	85.00(67.21)	85.50(67.62)	92.50(74.11)	82.50(65.27)
30	82.50(65.27)	83.00(65.65)	90.00(71.57)	75.00(60.00)
60	82.50(65.27)	83.00(65.65)	90.00(71.57)	75.00(60.00)

*Figures given in parentheses are angular transformed values.

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