SCREENING OF SOME CITRUS ROOTSTOCK GENOTYPES FOR SALINITY TOLERANCE

By

ASHUTOSH ANIRUDDHA MURKUTE

Centre for Rural Development and Technology

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To

My Parents

Mrs. Usha and Prof. A.R. Murkute

CERTIFICATE

This is to certify that the thesis entitled "Screening of some citrus rootstock genotypes for salinity tolerance" submitted by *Mr. Ashutosh Aniruddha Murkute* has been prepared under my guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The research reports and results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

(Dr. Satyawati Sharma)

Centre for Rural Development and Technology
Indian Institute of Technology Delhi,
Hauz Khas, New Delhi 110016,
INDIA

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ABSTRACT

Soil salinity is one of the major environmental impediments limiting crop yield and quality. Citrus, a major fruit crop of the India and subtropical and tropical world is grouped as a glycophyte. Though, the salinity tolerance do exist to a shorter extent in some of the genotypes of citrus, a nutritional and biochemical metabolism would be helpful to plant breeders and geneticists to form a policy to evolve salt tolerant cultivars/ hybrids. Citrus, being commercially propagated by shield budding; the study of different rootstocks for salinity tolerance forms an important aspect of citriculture. The Arbuscular mycorrhizal fungi are thought to increase salt tolerance of the plants under saline conditions in some crop plants. Hence the research on its role in citrus is also imperative. The biological manipulation of plants by tissue culture means is promising, environment friendly and energy efficient approach. The technique also reduces the labour and space requirement for evaluating and selecting large number of genotypes within a short span of time irrespective of season. Considering the facts the present investigation was carried out in two phases viz. *in vivo* and *in vitro* studies.

The *in vivo* studies were conducted with four citrus rootstock genotypes viz. *Citrus jambhiri*, *Citrus karna*, *Poncirus trifoliata* and *Poncirus trifoliata* x *Citrus sinensis*. The mature fruits of selected citrus species were obtained from the Citrus orchard of Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi. The seeds were extracted and sown freshly in the earthen pots having potting mixture composed of well decomposed FYM, sand and soil (1:1:1). Initially the effect of salt (NaCl) was observed on different germination parameters i.e. germination percent, time of germination and true leaf emergence. In another experiment

the three-month-old seedlings of about similar size of selected citrus genotypes were taken for the experiment. The seedlings were then transferred to plastic containers (20 cm depth and 20 cm. mouth diameter) having same potting mixture composition. The soil based mycorrhizal inocula was applied (@ 300 spoes/100 g soil) in root zone while transplanting the seedlings. The seedlings were allowed to establish for a week and then the salinity gradient was developed by regular irrigation of salt water with NaCl (0, 50, 100 and 150 mM). The experiment was replicated thrice.

The physical parameters viz. plant height, leaf number, stem diameter, the root infection by Arbuscular Mycorrhizal fungi (AM fungi) and spore count in the rhizospere soil were recorded. The proline, total proteins and sugars estimations were done using the tip portions (stem and leaves) of fresh plant samples, while the chlorophyils estimation was done by using tender leaves only. The macronutrient and micronutrient analysis was done by using the oven dried whole plant samples using standard protocols.

Based on the results of citrus species in the *in vivo* studies, *C. jambhiri* and *C. karna* were taken for *in vitro* studies. The different phytohormones and explants were standardized for shoot and root differentiation. The standardized hormones' combination was used for the *in vitro* screening of citrus species for salinity tolerance. The salinity gradient was developed by using NaCl in different concentrations (0, 50, 75, 100, 110, 120, 130mM NaCl). The well developed salt tolerant plantlets were shifted for hardening and further to waste land for field evaluation. The different nutritional and biochemical parameters were also studied in salt tolerant plants.

The results of *in vivo* study indicated that all the physical parameters including seed germination were affected with increasing salinity. Proline, total proteins, sugars,

phosphorus, potassium, sodium and chloride accumulation increased and chlorophyll contents, calcium, magnesium, copper and manganese decreased significantly with increasing salinity. The AM colonization decreased significantly under salt stress; however, it increased the phosphorus and chlorophylls under controlled condition and chloride and sugars concentration in plants irrespective of salinity.

In case of *in vitro* study, among the different phytohormones and explants the combination of growth substances 1.0 mg/l BAP + 0.5 mg/l NAA + 40 mg/l Adenine Sulphate was proved best for higher explant response, days required for shoot initiation, shoot number/ explant and shoot length in both the genotypes. The nodal segment explant gave significantly higher number of shoots/ explant than shoot tip explant. The rooting was easily achieved with combination of NAA (0.5 mg/l) and IBA (0.5 mg/l).

Results on *in vitro* screening for salinity tolerance revealed that all the physical parameters viz. shoot differentiation response, days required for shoot differentiation, shoot number/explant and shoot length were severely affected due to salt stress. Similarly all rooting parameters were also significantly affected by salinity. *C. jambhiri* was found to resist salinity quite well as compared to *C. karna*. The nutritional and biochemical parameters were also found affected significantly with the salt tress and showed similar results as that of *in vivo* studies. The protein profile of salt tolerant saplings substantiated formation of some new unknown proteins (57 kDa, 46 kDa and 23 kDa) as a response to stress. The change to heterotrophic condition from an autotrophic, the survival of plantlets was reduced during acclimatization. The field studies revealed the better performance of *in vitro* salt tolerant saplings over control.

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