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Challenges of *in vitro* conservation of Citrus genetic resources

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Motivation and Aim: To preserve *in vitro* collections of plant genetic recourses including elite cultivars of tree crops, it is necessary to use vegetative buds and meristems as explants that are genetically identical to the stock plant. However, the stage of aseptic culture initiation of woody vegetative buds is associated with such challenges as a high contamination rate *in vitro*, a slow growth, and the appearance of secondary-infected explants. The main challenges in creating *in vitro* backup collection of Citrus cultivars are a high degree of fungal contamination of vegetative buds and the subsequent decrease in the growth potential of plantlets *in vitro*. It is well known, woody plants, in particular Citrus species, are in close cohabitation with fungal microorganisms. Surface sterilization of explants does not relieve tissue from internal infection, on the cultural medium the hyphae of the fungus leave the plant tissues and proliferate on culture media, which inhibits the development of explants *in vitro* [1]. In this regard, the aim of our work is to study the effectiveness of various tools of decontamination of vegetative Citrus explants for initiation *in vitro* culture and development of a reliable *in vitro* conservation.

Methods and algorithms: Pre-cultivation techniques, pre-treatment of cuttings with fungicides, gradual sterilization, addition of antibiotics to the nutrient medium, as well as micro-grafting were tested for establish efficient tissue culture initiation of elite lemon cultivars of collection FGBSI RRIFSC (Sochi, Russia). The studies were carried out on *Citrus limon* (L.) Burm cultivars. Axillary buds with shoot segment of 0.5–0.7 cm long were taken as explants.

Results: The highest rate of sterile explants 32–42 %, was obtained by pre-cultivation the cuttings in a incubating chamber at 22 °C, followed by pretreatment with fungicides, using gradual sterilization and adding tetracycline 400 mg/l to the culture medium. However, after the third passage of subculture and conservation, the viability of the plantlets was reduced, leaves dropped and plantlets died within 6 months of in vitro conservation. Using *in vitro* micrografting technique, it was possible to overcome the problem of viability losses of bud explants, but this method is extremely labor- and time-consuming, only 1 of 300 manipulations was successful.

Conclusion: Thus, reliable preservation of valuable citrus genotypes in collection *in vitro* is currently problematic due to close cohabitation with fungal microorganisms and low efficiency of micropropagation as a whole.

References

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