ISSR ANALYSIS OF SEEDLINGS FROM CRYOPRESERVED SEEDS OF AN ANCIENT CITRUS COLLECTION IN FLORENCE

DE CARLO A.*, BENELLI C.**, LAMBARDI M.**

*) IGV/Istituto di Genetica Vegetale, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy)
**) IVALSA/Istituto per la Valorizzazione del Legno e delle Specie Arboree, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy)

Citrus, cryopreservation, ISSR, zygotic and nucellar embryos

In recent years, many studies have dealt with the cryopreservation of the genus Citrus. And, as a result, efficient procedures of vitrification or dehydration have been reported for various organs and tissues, such as shoot tips, seeds, embryonic axes, somatic embryos, ovules and embryogenic callus. Among them, the cryostorage of embryonic axes and entire seeds from polyembryonic species allows the preservation of nucellar embryos, which are genetically identical to the maternal parent. Hence, the polyembryonic seeds can be considered a valuable material for the long-term preservation of citrus germplasm. This study explored the possibility of using the cryogenic technology for the preservation of a Medicean Citrus collection, maintained at the botanical garden of the “Villa Reale di Castello” in Florence, Italy. The Citrus collection was initiated by Cosimo I de’ Medici in the XVI° century, and it includes more than 600 accessions, preserved mainly in big earthenware basins. An effective procedure of seed dehydration and direct immersion in liquid nitrogen (“one-step freezing”) was initially developed for a sample of five polyembryonic Citrus accessions, i.e., C. aurantium ‘Foetifera’, C. volkameriana, C. lumia ‘Pyriformis’, C. sinensis and the hybrid C. aurantium x C. paradisi. The initial moisture content of seeds was between 33% and 55%. Seed dehydration was obtained in open Petri dishes, exposed to sterile air flow. All the accessions showed adaptability to seed cryopreservation, after the seeds were appropriately dehydrated between 25% (C. lumia) to 15% (C. aurantium x C. paradisi). Maximum germinability ranged from 27% (C. aurantium) to 100% (C. aurantium x C. paradisi). With the only exception of C. volkameriana, only one seedling per seed was obtained after the dehydration/cryopreservation procedure. Afterwards, in order to validate the procedure for the preservation of this ancient germplasm, the ISSR (Inter-Simple Sequence Repeat) analysis was carried out to ascertain the sexual or apomitic origin of seedlings from cryopreserved seeds. The DNA was extracted from the mother plants and from the seedlings which developed after the seeds underwent to the dehydration/cryopreservation procedure. For ISSR analysis, four primers anchored at the 3’ end by 2 arbitrary and degenerate nucleotides were selected: (AG)₈YC, (AG)₈YG, (GA)₈YG (according to Fang and Roose, 1997. TAG 95:408-417), and (AC)₈YG (according to Sankar and Moore, 2001. TAG 102:206-214). The amplified fragment sizes were from 200 to 1300 bp. The number of fragments per primer ranged from 5 (with (AG)₈YC) to 12 (with (AC)₈YG). For the accessions from which only one seedling per cryopreserved seed was obtained, the molecular analysis confirmed their nucellar origin. As regards C. volkameriana, the molecular analysis showed the occasional development of also the zygotic embryos, although at a very low ratio in comparison with the nucellar embryos. With C. volkameriana, as well as with other polyembryonic Citrus
species which could have the same behaviour, this fact should be considered when using the cryopreservation technique for the long-term preservation of clonal germplasm.