

APPLICABILITY OF THE DROPLET VITRIFICATION METHOD TO OLIVE SOMATIC EMBRYOS

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Somatic embryos of olive have been successfully cryopreserved using the droplet-vitrification method on aluminium foil strips (1) after dehydration in PVS2 for 30 min. Although acceptable recovery rates have been obtained (2) and cultures morphology apparently did not change, the influence of this cryopreservation protocol on the somatic embryogenesis process is unknown.

The aim of this investigation was to assess the influence of the droplet-vitrification method on the different phases of somatic embryogenesis and to evaluate its applicability to somatic embryos of different genotypes.

The results obtained revealed that the genotype plays a key role in the maintenance and maturation phases and determines the regeneration potential of the embryogenic lines. Cryopreservation only affected some specific aspects, such as the proliferation capacity in maintenance medium, germination of newly developed embryos and length of the shoots obtained. Nevertheless, a statistically significant interaction between genotype and cryopreservation was appreciated in different parameters related to cultures proliferation. The influence of cryopreservation on cultures morphology during the maturation phase and embryo germination was also genotype-dependent.

Thus, the regeneration potential was not significantly affected by cryopreservation and the quality of the plantlets obtained was similar in both types of cultures, although slightly longer shoots were obtained in plants derived from cryopreserved explants.

In relation to the applicability of the droplet-vitrification method to olive somatic embryos of different origin, response to cryopreservation significantly varied depending of the genotype, with recovery rates ranging from 0 to 60%. The appearance of cultures established from frozen somatic embryos was similar to that of the corresponding controls.

In conclusion, our investigations show that droplet-vitrification is a reliable procedure for preserving the viability, embryogenic competence and regeneration capacity of olive somatic embryos. Nevertheless, additional optimization of this method is required in order to improve the recovery rates obtained in some of the genotypes tested.

(1) Panis B, Piette B & Swennen R (2005) *Plant Sci.* **168**, 45-55. (2) Sánchez-Romero C & Bradai F (2013) in *Abstract Book X Reunión SECIVTV*, pp 56.