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OPTIMIZATION OF AN IN VITRO EMBRYO RESCUE PROTOCOL FOR BREEDING SEEDLESS TABLE GRAPES (VITIS VINIFERA L.) IN ITALY

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Seedlessness is one of the most valuable agronomic traits in grapevine (*Vitis vinifera* L.) traditionally preferred for dried raisins production and recently appreciated also for fresh table consumption. Breeding for seedless grapes is traditionally based on “seedless × seeded” and “seedless × seedless” hybridization or crossing genotypes with different ploidy levels. Conventional breeding to obtain viable progeny from intraspecific crosses between seedless cultivars is cost-expensive and time/space-consuming. Moreover, the procedure presents some technical constraints as hybrid viability is severely reduced by embryo abortion in the early berry development, leading to underdeveloped seeds or seed traces and very low percentages of seedless F1 progenies (0 to 16%). Recovery rate of viable embryos can be significantly increased and the time necessary to regenerate drastically reduced, by applying an *in vitro* embryo rescue protocol preventing immature embryos death in the stenospermocarpic berries firstly by medium plating fertilized ovules to allow embryo growth beyond the stage of abortion, and then by opportunely culturing the newly developed embryos until germination and plantlet formation. The technique partially overcomes traditional limitations, but it strictly depends on several variable factors. In the present work, both genetic and methodological issues were addressed to optimize a three-step *in vitro* protocol for the regeneration of viable hybrids from crosses between stenospermocarpic table grape cultivars. The influence of parental genotypes (six “seedless × seedless” crosses), ovule sampling time (30, 40, 50 days after pollination – DAP), and extent of embryo germination induction (4, 6, 8 weeks) were assessed on

ovule fertilization, embryo development and germination, rooting, and plantlet formation to establish the best rescue time for each hybrid. Our protocol was successfully applied to all the hybridization events but sampling time (berry ripening stage for immature ovule isolation) and crossing genotypes were crucial factors affecting the efficiency of final hybrids viability. Among the three rescue steps, embryo formation and germination were fundamental to determine the recovery rate of viable plants. Moreover, the extent of embryo induction could also be opportunely tuned (prolonged not over 8 weeks) to optimize space and time for gaining the best recovery efficiency. Our optimized protocol included immature ovule isolation at 40 DAP and embryo germination induction for 8 weeks. As for genotypes, the most efficient embryo germination was recovered from hybrids of Thompson, Superior, and Regal cultivars, whereas the highest percentage of viable plants was derived from 50-DAP ovules of Luisa × Thompson progeny. Such an optimized protocol could be useful to improve future breeding programs for grape seedlessness and make embryo rescue a more efficient and predictable method compared to conventional breeding.