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Enhancing shoot recovery from transgenic avocado somatic embryos

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Use of biotechnological tools in avocado (*Persea americana* Mill.) is hampered by difficulties in obtaining mature somatic embryos with an acceptable germination capacity. Use of semi-permeable cellulose acetate membranes on top of maturation medium has improved the quality of obtained embryos and their germination rate; however, in the case of transgenic embryos the conversion rate is still rather low. In this investigation, a protocol for recovery of transgenic plants has been developed. Mature avocado somatic embryos, over cellulose acetate membranes, were obtained and induced to germinate following the protocol described in Palomo-Ríos (2012). Some transgenic embryos developed buds ≤ 2 mm, which failed to elongate. For shoot recovery, cotyledons were partially removed and the embryonic axis cultured over 4 weeks in MS medium supplemented with either 1 mg/l BA, 1 mg/l TDZ or 1 mg/l BA and 1 mg/l TDZ. Highest shoot recovery was obtained in medium supplemented with 1 mg/l BA and 1 mg/l TDZ, 53.6±6.7% versus 23.2±5,7% and 39.6±6.4% in media supplemented with 1 mg/l BA or 1 mg/l TDZ, respectively. Afterwards, sprouted embryos were cultured over 4 additional weeks in MS medium supplemented with 1 mg/l BA. In some cases, resulting shoots could be induced to proliferate in GD medium (Gamborg et al., 1968) supplemented with 0.3 mg/l BA, while in other cases, they had to be recovered through micrografting onto in vitro germinated seedlings as described in Pliego-Alfaro and Murashige (1987). MS medium supplemented with 1mg/l BA and solidified with 6 g/l Sigma A-1296 agar, was used for micrografts. Shoots derived from micrografts were either induced to proliferate in GD medium with 0.3 mg/l BA or further grafted and cultured in liquid MS medium supplemented with 0.1 mg/l BA, using perlite as substrate. After 8-12 weeks, micrografts could be transferred to ex vitro conditions.

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Key words: *Persea americana*, transgenic plants, micrografting, somatic embryogenesis