Massive Micropropagation of Chilean Strawberry

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During the 20th century, cultivated strawberry (*Fragaria ×ananassa* Duch.) replaced Chilean wild strawberry [*Fragaria chiloensis* (L.) Duch.] as a crop in Chile, even though the latest has a greater flavor and aroma and resistance to biotic traits (Crock et al., 1982; Daubeny and Pepin, 1965; Hancock et al., 1999; Shank and Baritt, 1974, 1984). However, the cultivated strawberry has higher fruit size, yield and a longer period of harvesting, due to the development of improved cultivars and the use of higher levels of inputs. The Experimental Research Center of INIA in Cauquenes, has a large germplasm collection of this specie (Cameron et al., 1993). From this collection, superior genotypes have been selected based on their high yield and fruit quality. To reach a commercial level of production, it is necessary to produce a high number of plants. Clonal propagation through axillary bud proliferation has been used commercially (Boxus, 1998; Rosati, 1993), controlling the number of subcultures (Boxus et al., 2000; Damiano et al., 1995; Jungnickel, 1988) to avoid genetic instability. The objective of this study was to develop an efficient micropropagation protocol to propagate selected clones of Chilean wild strawberry (Fig. 1).

Materials and Methods

Selected wild strawberry plants, grown in the greenhouse, served as mother plants for the excision of axillary bud. The surface sterilization of runner-tips to control fungal contamination consisted of an immersion in 70% alcohol, use of an antioxidant and sodium hypochlorite (25%) for 15 min and rinsing of materials in sterile and distilled water. Axillary buds aseptically-isolated from runner tips and crowns (Fig. 1A), were cultured on agar-solidified MS nutrient medium modified with a low mineral content, and supplemented with IBA (1.0 mg·L⁻¹), BA (0.1 mg·L⁻¹) and GA₃ (0.1 mg·L⁻¹) and incubated at 25 °C with 16 h photoperiods. Shoots differentiated were transferred to MS-agar medium, supplemented with IBA (0.1 mg·L⁻¹), BA (0.5 mg·L⁻¹) and GA₃ (0.1 mg·L⁻¹), and incubated under same conditions mentioned above, in order to induce mass proliferation of shoots (Fig. 1B). Plantlets

(a) Axillary buds

(b) Multiplication

(c) Plantlet elongation

(d) Rooting

(e) Pre-acclimation

(f) Field establishment

were transferred to the MS-agar medium with a BA concentration of 0.1 mg·L⁻¹ for elongation and rooting (Fig. 1C and D). Rooted plants were transferred to a substrate under greenhouse conditions with a mist irrigation system and 20 to 25 °C (Fig. 1E) and transferred to the field (Fig. 1F).

Results and Discussion

Results indicated that micropropagation of wild chilean strawberry can be done effectively using the protocol describe above. The changes introduced in this procedure allowed to reduce in 50% the multiplication, rooting and acclimation period and reduce the cost of chemicals and labor during this work. Specifically, 1) the sterilization protocol was very effective in removing contaminants from plant material; thus, the contamination rate was <5%; 2) the establishment of the buds took 4 weeks and generated 80% of the healthy plantlets; 3) the multiplication stage lasted 3 weeks and obtained an average rate of 15 plantlets per bud; with less than eight subcultures; 4) The elongation and rooting stages took about 2 and 3 weeks, respectively, and the survival rate under greenhouse conditions was 95%. Finally, this procedure allowed to produce 10.000 plants for field evaluation and commercial purposes.

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Fig. 1. Stages of in vitro micropropagation in *Fragaria chiloensis*.

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