ASYMMETRIC PROTOPLAST FUSION BETWEEN PR107 AND REYAN8-79 BY USING PEG

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Abstract

The genetic improvement of rubber tree using traditional sexual hybridization is a time consuming and laborious task. Somatic hybridization through protoplast fusion is an alternative approach to improve rubber tree, for somatic hybrids had been obtained successfully in many other plant species, and protoplast regeneration systems for several rubber clones had been already established. An attempt was made to perform asymmetric protoplast fusion between two Hevea clones, PR 107 and Reyan 8-79, by using polyethylene glycol (PEG). Protoplasts derived from embryogenic suspension cells of PR107 were used as donor protoplasts and treated with ultraviolet (UV) irradiation (380 μW/cm²) for 30, 60, 90, 120 and 180 s. Protoplasts derived from embryogenic suspension cells of Reyan 8-79 were used as recipient ones and treated with iodoacetamide (IOA) at concentrations of 1, 2, 3 and 4 mM for 15 min. PEG at 10, 20, 30 and 40% (w/v) were respectively tested to obtain a higher frequency of binary fusion. Results showed that the cell division of donor protoplasts was largely inhibited by UV irradiation for more than 90 s, and that the cell division frequency of recipient protoplasts was decreased to less than 5% by 3 mM IOA. A maximal binary fusion frequency (about 20%) was obtained when the concentration of PEG was 30%. The donor protoplasts exposed to UV for 90 s and 120 s were respectively fused with 3 mM IOA-treated recipient protoplasts by using 30% PEG to produce somatic hybrid cells. The fusion products obtained were then transferred onto feeder layer culture. Visible cell colonies from these two fusion combinations were produced 28 and 35 d after feeder layer culture, and their formation frequencies were 146 ± 12 and 23 ± 5 per 10⁵ protoplasts, respectively. They were transferred onto callus inducing medium for multiplication culture for one month, and then transferred onto differentiation medium for somatic embryogenesis. A total of 112 somatic embryos were produced from the cell colonies, but they failed to germinate as they turned brown or vitrified during their further culture.

Keywords: Hevea brasiliensis, embryogenic cell suspensions, protoplast fusion, feeder layer culture, somatic embryogenesis, callus, rubber