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Somatic embryogenesis and *Agrobacterium*-mediated transformation of satsuma mandarin

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Cultivar improvement of satsuma mandarin (*Citrus unshiu* Marc.) by sexual hybridization has been extremely constrained due to mainly generative sterility and polyembryony and genetic transformation has been expected to become an alternative tool. However, satsuma mandarin has known as one of most recalcitrant species for genetic transformation. In order to develop the genetic transformation system in this crop, we investigated some factors affecting the induction of somatic embyos and plant regeneration such as the weight of calli cluster, agar and lactose concentration, and plant growth regulators. The highest induction of adventitious embryos resulted from 40% of 30, 40, and 50 % in percoll concentration, 1.2 % of 0.8, 1.2, and 1.6 % in agar concentration and 7% of 5, 7, and 9% in lactose concentration, and 0.1mg/L BA and 0.01mg/L NAA of the combination of 0, 0.1, 0.5, and 1.0mg/L BA and 0 and 0.01mg/L NAA. The genetic transformation of embryogenic calli was conducted by *Agrobacterium*-mediation. After bacteria inoculation and co-cultivation, the calli were transferred on selective adventitious embryo induction medium and the hygromycin-resistant embryos were further transferred to selective regeneration medium. The putative transgenic shoots were grafted on trifoliate orange and acclimatized. Gene transfer was confirmed by PCR and southern blot analysis.

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