

TOWARDS THE REGENERATION AND GENETIC TRANSFORMATION OF *CYNARA CARDUNCULUS* L.

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Artichoke (*Cynara cardunculus* var. *scolymus* Fiori) is a perennial plant belongs to the Asteracea family. The Mediterranean basin is the centre of origin of this species and the leading producing countries are France, Spain and Italy in which is cultivated as vegetable for its edible young flower heads and as medicinal plant. The *in vivo* propagation by using offshoots, *ovoli* and crown segments is used as source to obtain clonal plant. However, the two main factors that hinder the vegetative propagation are the low rate of multiplication and the potential of disseminating disease. The plant tissue culture techniques such as micropropagation and adventitious regeneration might avoid these problems. In addition, the *in vitro* regeneration procedure is also a prerequisite for the application of various biotechnological techniques such as genetic transformation. In this study, we addressed the effects of growing regulators on massive propagation and artichoke organogenesis. An undertaken procedure for genetic transformation has been reported. The genotypes of artichoke assayed in this work were: C3SAB8; C3SABLB; Violetto di Ferrara; Caio; Tizio and Violetto di Romagna. Plants were growing in medium containing Murashige and Skoog salts, B5 vitamins, 3% sucrose and 0,8% Plant agar (POC). For massive propagation, shoots were grown on POC medium added with 0,01% ascorbic acid and 2.2 mM BAP. Shoots explants were cultivated for 4 weeks in a growth chamber under a photoperiod of 16h light/ 8h dark at 24±1°C and then transplanted onto a POC medium. The ability to produce axillary shoots at the base of artichoke plants was detected in the medium without any growth regulator with an average of one shoot per plants. On the other hands, the presence of cytokinin in the medium induced the regeneration of axillary shoots, which was the highest in the cv Violetto di Romagna. To assay the *de novo* artichoke organogenesis, leaf explants, collected from one-month-old plants, were cultivated onto POC medium added with 2,4D at several concentrations. Leaf explants between 6-8 weeks onto this medium shown only the formation of callus. In absence of any growth regulator the explants did not survive. As the content of 2,4D increase in the medium the number of explants that produce callus increased, reaching the maximum frequency in presence of 2,4-D 5mM. The *de-novo* callus was initially soft and translucent that turned brown during the cultivation. New proliferation areas were observed and were distinguished for their compactness and yellow colour and were considered as embryogenic calli. So far, we have not able to obtain artichoke plants from this embryogenic callus. Proliferating meristems, induced by growth regulators, were assayed as an alternative target for gene delivery. Therefore, we have co-cultivated young artichoke shoots with *Agrobacterium tumefaciens* strains p35SGUS-INT and then transferred onto ASI medium. After 6 weeks a PCR analysis was carried out on genomic DNA extracted from multiple buds. This study reports the first approach to manipulate the genome of artichoke by *A. tumefaciens*.