# DIFFERENCES BETWEEN SUNFLOWER GENOTYPES TOWARD *Agrobacterium tumefaciens* INFECTION

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## Introduction

Sunflower transformation was published by Knittel *et al.* (1994) (1) and Bidney *et al.* (1993) (2).In both cases they combined the *Agrobacterium* strategy with the gene gun device to increase the transformation efficiency. With the goal to transform sunflower genotypes from Argentina, we tested the regeneration capacity under agroinfection stress and the transformation efficiency of various local genotypes (in 4 public and 8 private lines). The tissue culture and transformation strategies used were those reported by Schrammeijer *et al.* (1990) (3), with some modifications. Using *Agrobacterium* technology only, we observed a wide sensitivity range in the genotypes tested and our best result was a 4 % transgenic shoots measured under *in vitro* conditions. For the rescue of transgenic and viable plants, it is necessary to improve the explant transformation efficiency. Based in the fact that *Agrobacterium* vir genes are activated by phenolic compounds (4, 5, 6), we tested the sunflower sensitivity toward *Agrobacterium* infection between the high or low capacity of the sunflower genotypes to produce phenolic compounds (7) under stress situation.

## Materials and Methods

High phenol producers (HPP) genotypes and a low phenol producer (LPP) genotype, were exposed to *Agrobacterium* infection. Our assay schema was the following: the lines Ha 61 and Rha 276 are according to Bazzalo *et al.* (1991)(7), HPP genotypes, Ha 300B was used as LPP genotype and as control, with and without the addition of 3-5-dimetoxy 4-hidroxy-acetophenone (acph) in tree different treatments: a) Into the agrobacterias culture medium two hours prior the agroinfection. b) Supplementing the coculture medium and c) Adding to the *Agrobacterium* washing solution. In all cases the acph final concentration was 0,2 mM (Alt-Moerbe, 1988). The seeds were sterilized in 70 % ethanol (2 min.), 4 % NaOCI solution (40 min.),and rinsed in distilled sterilized water. They were allowed to imbibe overnight. We obtain the isolated apex (including the pre-existing meristematic tissues), according with (3). The explants were 3 days in coculture with the *Agrobacterium* on MS medium supplemented with 0,1 mg/l BAP.

The *Agrobacterium* strain used was LBA 4404 (pGUS.INT) (Hoeckema, 1983). B-glucuronidasa (GUS) activity was assayed using the histological test, according to Clontech, Palo Alto, California.

## Results and Discussion

The figure 1 shows the transformation efficiency and the distribution ratio (DR) of the different genotypes and treatments. The HPP genotypes showed a transformation efficiency (TE) of 92 % and 73 % (Rha 276 and Ha 61, respectively), taking the positive TE as each explant that is showing at least one transformation event. In contrast Ha 300B (LPP genotype) showed, under the same culture conditions, a TE of 52 %. Acph did not have effect onto the TE, but it affected evidently the transformation event's location. The evaluation of the event's location was made by the distribution ratio (DR), that is: the number of events observed at the apical region in relation with the ones at the explant base. For the pH genotypes and for the a) and b) treatments of LP genotypes this parameter was very low (between 0,04 and 0,24), but for the control and c) treatment in LP genotypes it was high (0,85-0,83). Evidently more experiments are necessary, but this result is a first approach to confirm the importance of the presence of phenolic compounds in the interaction *Agrobacterium*/sunflower cells. We are carrying out experiments that will allow to improve the mentioned interaction, specially at the apical region of the explant.

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