

Investigating the effect of different induction methods on preparation of hairy roots in *Artemisia annua*, using *Agrobacterium rhizogenes*

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Background and Aims: Hairy root cultures are source for secondary metabolites production. In this study, the effect of two preparation methods of *A. rhizogenes* suspension on hairy roots induction in *Artemisia annua* was investigated.

Methods: *A. rhizogenes* strains 15834 and LBA4902 were used the root induction in *A. annua* explants. To prepare the bacterial suspensions, first single bacterial colonies were inoculated in 5 ml of LB liquid medium and were cultured on a rotary shaker (120 rpm) for 20 h and at 25°C. Then, in the first method, suspensions were directly used for infection (suspension 1) and in the second method, bacterial suspension was centrifuged (3500 rpm, for 7min), supernatant was discarded and 1 ml of LB medium was added to the remaining sediment prior to use (suspension 2). Two-week old seedlings were used as explants and were infected and were inoculated in ½ MS medium under 16 h light, at 27°C.

Results: The morphological observations, 7 to 10 days after infection showed that for strain 15834, suspension 2 induced hairy roots in 40% of the explants and suspension 1, did not induce any hairy roots. Moreover, all infected explants by both suspensions of strain 15834 were green 10 days after infection. In explants infected with both suspensions of strain LBA4902, no hairy roots were established and also necrosis was observed in 90% of the explants.

Conclusions: In suspension 2 of strain 15834, the density of bacteria used for induction was higher around the explants comparing to suspension 1 and it could cause hairy roots induction. However, for LBA4902 strain both concentrations did not induce any hairy roots and moreover, the infection caused necrosis in almost all the explants. Due to the necrosis, it could be concluded that both concentrations were too high for the strain.

Keywords: Bacterial suspension; *Agrobacterium rhizogenes*; Hairy root; *Artemisia annua*