

## Effect of *Agrobacterium rhizogenes* Strains on Hairy Root Induction in Different Explants of Alfalfa

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### Introduction:

Alfalfa (*Medicago sativa* L.) is one of the plants that has a long history in food and medicinal uses in traditional medicine. In nature, the production rate of secondary metabolites is low and it takes a long time to produce them. Since the chemical synthesis of natural plant products is not easily possible, the use of biotechnological methods for the rapid and mass production of secondary metabolites and medicinal substances seems necessary. *Agrobacterium*-mediated transformation is an efficient technology for induction of hairy roots in plants. The transgenic hairy root system is a promising source in the production of secondary metabolites in plants with high medicinal value. In the present study, the effect of *Agrobacterium rhizogenes* strains and explant type on hairy root induction in alfalfa was investigated.

### Materials & methods:

A factorial experiment was conducted in a completely randomized design with three replications in Islamic Azad University, Sabzevar Branch. The studied factors were *Agrobacterium rhizogenes* strains at four levels (A13, 318, 15834 and A4) and explant type at two levels (trifoliate leaf and hypocotyl). After disinfection, alfalfa seeds were

cultured in solid MS culture medium without any growth regulators. The culture containers were transferred to the growth room with a temperature of 25°C and a photoperiod of 16 hours with a light intensity of 3000 lux. After seed germination and seedling growth, hypocotyl pieces and the first trifoliolate leaf were used as explants. LB culture medium was used for bacterial culture. After co-cultivation and removal of bacteria, the explants were transferred to MS culture medium in order to investigate hairy root induction. The characteristics studied included the percentage of hairy root induction, the number of hairy roots in the explant, the length and dry weight of hairy roots. In order to confirm the transformation of hairy roots, PCR technique was used with specific primers for *ro/B* gene replication. The extracted DNA was amplified by PCR using *ro/B* gene specific primers. The statistical analysis of the data was done using SAS software and graphs were made by Excel software. Comparison of average data was done using the least significant difference (LSD) test at the 5% level.

### **Results & discussion:**

The results of the analysis of variance showed that the effect of explant type, bacterial strain, as well as the interaction effect of explant type and bacterial strain on the percentage of hairy root induction, the number of hairy roots in the explant, the length and dry weight of hairy roots were significant. The percentage of hairy root induction in leaf explants (50%) was significantly higher than the percentage of hairy root induction in hypocotyl explants (24.99%). The highest percentage of hairy root induction was obtained in leaf explants inoculated with bacterial strain A4, which was significantly higher than other strains of *Agrobacterium rhizogenes*. Inoculation with bacterial strain 318 caused the lowest induction of hairy roots in leaf explants and hypocotyl. Bacterial strains A13 and 15834 produced the highest number of hairy roots per leaf and hypocotyl explants, respectively. The lowest number of hairy roots in leaf explants was produced under inoculation with 318 strain. The highest and lowest hairy root length in leaf explants, were observed under inoculation with bacterial strains A4 and 318, respectively. In hypocotyl explants, there was no significant difference between strains of *Agrobacterium rhizogenes* in terms of number and length of hairy roots. The dry weight of hairy roots produced in leaf explants was significantly higher than the dry weight of hairy roots in hypocotyl explants. The highest dry weight of hairy roots in leaf explants was obtained in the condition of inoculation with A4 strain, which was significantly higher than other bacterial strains. In hypocotyl explants, the highest root dry weight was obtained under the conditions of inoculation with A13 strain. In this research, different explants showed different responses in terms of hairy root production. It has been reported that the success of hairy root induction through *Agrobacterium rhizogenes* depends on various factors such as species, type and age of plant tissue. The bacterial strain used and the concentration of the bacterial suspension for inoculation are other effective factors in the transformation process and hairy root induction. The results of separation of PCR products using gel electrophoresis, confirmed the presence of *ro/B* gene in the genome of hairy root cells.

### **Conclusion:**

The results showed that the type of explant and strains of *Agrobacterium rhizogenes* affect the production of hairy roots in alfalfa plants. The bacterial strain A4 had more efficiency in inducing hairy roots, especially in leaf explants. In the conditions of using hypocotyl explants, there was no significant difference between A13 and A4 strains regarding the percentage of hairy root induction. Bacterial strain 318 had the lowest transformation efficiency among the used

*Agrobacterium rhizogenes* strains. According to the results, inoculation of leaf explants with A4 strain of *Agrobacterium rhizogenes* is recommended to produce hairy roots in alfalfa.

**Keywords:** *Bacterial strain, Explant, Hypocotyl, Plasmid, Transformation*