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THE EFFECT OF DIFFERENT STRAINS OF AGROBACTERIUM RHIZOGENES AND TYPES OF EXPLANT IN CREATION OF HAIRY ROOTS FOR HYOSCYAMUS RETICULATUS

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Abstract

The use of bacterium Agrobacterium rhizogenes for hairy roots induction in medicinal plants species of due to increased amounts of metabolites, high proliferation rate of interest is located. We investigate the optimization of hairy root cultures Hyoscyamus reticulatus plant showed that strains of bacteria and explant type effectively transformed explants on impulse. An MSU strain has the greatest impact on transformation and the second effective strains were A7 and D7. Hypocotyl and cotyledon explants of creating transgenic plants showed more effective.

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Introduction

Hyoscyamus L genus of plants with medicinal value because of tropane alkaloids are used to refer to the distant past (Sevon et al, 2001) tropane alkaloids scopolamine and hyoscyamine, especially because many medicinal properties and are used in the medical attention. Many species of this genus have been studied by various researchers. In Flora Iranica 18 species of this genus have been reported from different parts of Iran. But because of the same name some species there are 13 species of this genus are reported by other researchers. These materials are widely distributed throughout the world. Hyoscyamus reticulatus species have been reported from different parts of Iran (Khatamsaz, 1999). *Agrobacterium rhizogenes* bacteria that cause disease in plants is the hairy roots as a tool for transformation of plant genetic engineering is used. The bacteria containing the plasmid Ri is called on the root cause of the plasmid genes are entitled rol that Integrated into the genome of the host plant root causes of ill-fitting and it can be reproduced. Rol genes in addition to the construction of secondary metabolites stimulate adventitious roots to stimulate the transcription of defense genes (Georgiev, 2007). Potential of hairy root cultures for the production of secondary metabolites of interest for the first research in this area began in the early 80's. This method is more attention recently due to its special features. These features include ultra-high stability against an undifferentiated cell cultures, high growth rate compared to conventional root, no need to plant hormones and increase the amount of secondary metabolites (1).The ability of different strains of *Agrobacterium* to induction of hairy root in plants species is different (Verma, 2007) type and age and degree of differentiation plant tissues is effective in intransformed (Sevon et al, 2001). The aim of this experiment was to optimize the establishment of hairy root culture in *H.reticulatus*.

Materials and methods

1) Material Plant

Seeds of species H.reticulatus of 33 ° 38/2245 // north latitude and 48 ° 24 / 9.52 // East longitude were collected. Then the seeds cultured in pots of 15 cm with 50% garden soil and 50% perlite .Then Pots located in a greenhouse at a temperature of 26.2 ^{o}C, 16 h light and 8 hours of darkness. Green plants from age 2 to 18 weeks at intervals of four weeks to prepare explants were used. Explants of leaf, stem, and hypocotyl and cotyledon were prepared. Explants in a solution of 1.5% were put Clorox Disinfecting after 10 minutes, rinse with sterile water were 5 times. Explants of leaf, stem, hypocotyl and cotyledon leaves were prepared.

2) Bacterial strains 15834, MSU, D7, A7, A4 were obtained from the Institute of Genetics and Biotechnology. The strains in the culture medium Yeast Manitol broth (company Biomark) prepared for 24 hours at 26 ° C and the Rotary Shaker 140 rpm in the dark, cultured and after reaching OD600= 0.4-0.5 they were used for transformation.

3) Establishment of hairy root culture

The explants were wounded with a scalpel. The explants were placed in bacterial suspensions for 10 minutes. Explant were dried with sterile filter paper to remove their excessive bacteria ,then explants transferred to the MS medium (Sigma) with 5% sucrose and pH=5.5 for 48 hours at a temperature of 26 ° C in a germinator were placed in dark conditions. After that, explants were transferred to MS medium with 5% sucrose and pH=5.5 with 400 mg/lit cefotaxime (Sigma). Inoculated explants were subcultured every seven days in MS medium and antibiotic concentration was gradually reduced. After 3 to 4 weeks hairy roots appeared.

4) Approval of transgenic

In the first Genomic DNA from hairy roots was isolated using a plant DNA extraction Kit (vivantis).PCR analysis was used to investigate the presence of *rolB* gene. Primer sequences of / 5 to 3 for Fprimer ATGGATCCCAAATTGCTATATTCCCCAGGC and was Rprimer TTAGGCTTCTTTCATTCGGTTTACTGCAGC.

5) Statistical analysis

This study was conducted in a factorial design base on randomized completely design. The first factor was the strain type of the 5 with five level and type of explant was used with 4 levels of transgenic plants in each plot were calculated from 50 explants. The analysis of variance was performed using MSTAT-C. The means comparison was done by Duncan test at 5% probability level.

Results and discussion

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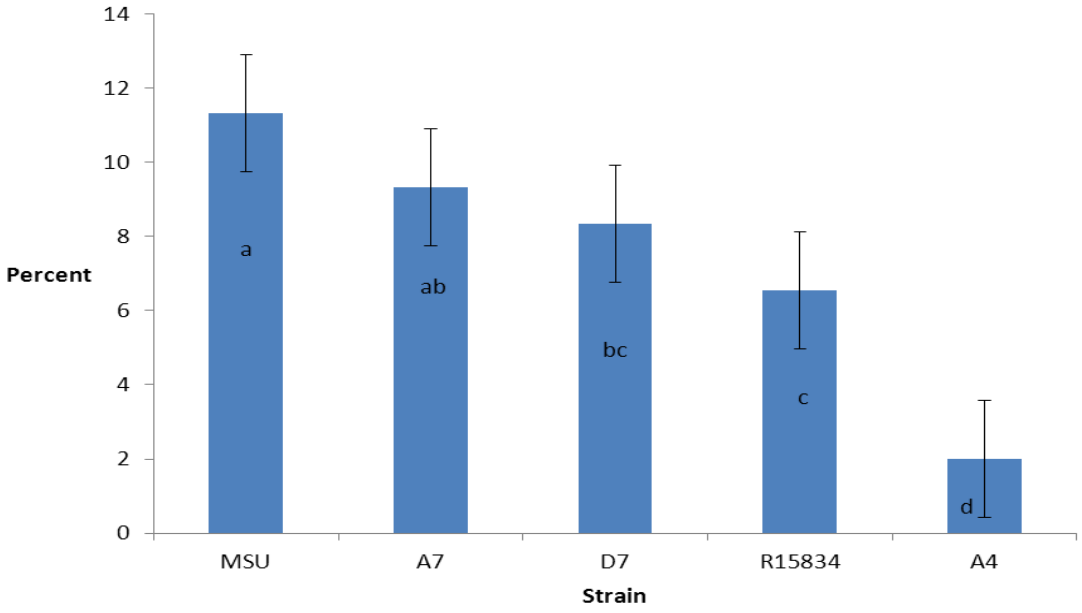
Figure 1: The effect of bacteria on the percentage of transgenic agrobacterium rhizogenes are plant explant hyoseyamus reticulatus



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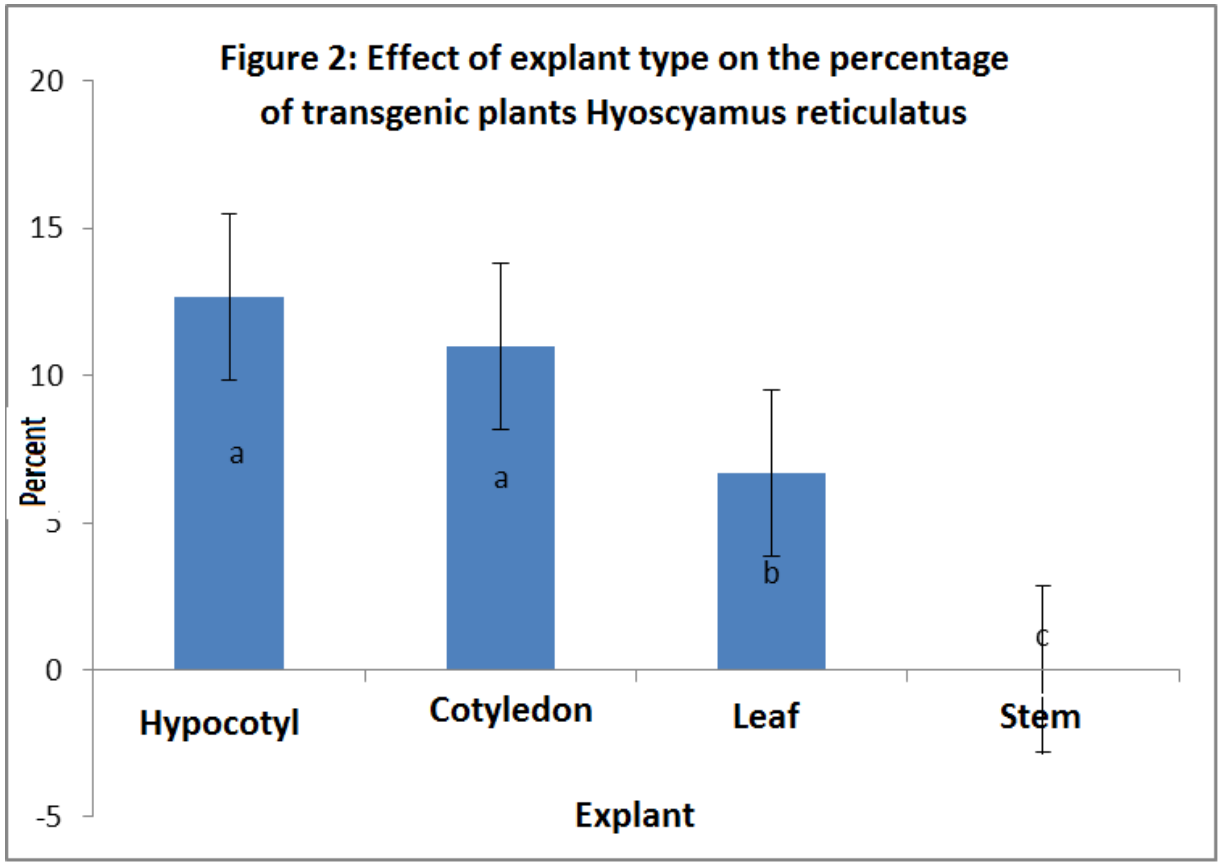

The results showed that in hairy root induction between *Agrobacterium* strains and explant type and their interactions were statistically significant differences exist. You shed the highest percentage of cultures inoculated with MSU strain obtained (11.33%) and the lowest percentage of hairy root induction was associated with the use of strain A4 (2%). No significant difference between A7 and D7 and D7 with MSU strains was observed (Figure 1). Different plant species show different degrees of sensitivity to different Agrobacterium strains and some plant species that do not show sensitivity to one strain can be transformed with other strains. Woo et al. (2004) for ginseng plant transformation strains A4, Krolicka (2001) for the transformation of plant *Ammi majus* strains A4, LBA9402, ATCC15834, Aoki (1992) A4 strains were used for transformation of plant *Hyoscyamus albus*. Also the 15,834 strain use for the transformation of peanut and *Atropa belladona* and *Ocimum basilicum*, for transformation *Psoralea*, *Solanum khasianum* clarke strain LB9402 is used (Bensaddek, 2000; Bourgaud, 1999; Jacob, 2004; Medina-Bolivar, 2007).

Effect of various agropine and nopaline strains for the transformation of plant Hyoscyamus muticu by Vanhala and his coworker showed that the agropini strain LBA9402 and nopaliny strain C58 were capable of greater hairy root induction (Sevon et al 2001).

Zehra (1998) tested alkaloids scopolamine and hyoscyamine production of root hairs and the two species *Hyoscyamus muticus* and *Hyoscyamus albus* and a somatic hybrid of the two species results showed A4 strain in transformed species and their somatic hybrid were more effective.

The explants type is important in transformation rate. there was a significant difference between explant type transformation rate. First place is for the hypocotyl 12.67 percent transformation stem explants lacking the ability to create hairy roots (Chart 2).

Explant type is effective in generating transformed plants. leaf explants *Atropa* (Bensaddek, 2000), the plant peanut cotyledon node (Medina-Bolivar, 2007), the ginseng cotyledon and leaf explants obtained from hypocotyl (Woo et al, 2004) and *Ammi majus* explants obtained from the stems and leaves (Krolica et al, 2001) were suitable for transformation. Previous studies have been conducted at the plant genus *Hyoscyamus* of leaf explants for transformation and creation of hairy roots were used (Aoki, Zehra, 1992).

In general, in studied plant type and origin of susceptible explants and suitable strains for plant transformation plant varies .Tissue age and differentiation status also affects the amount transformation rate (Sevon et al. 2001).

References:

Aoki.T,Y.Toda and K.Shimomura. 1992.Characteristic of Green Hairy Roots of *Hyoscyamus albusTransformed by Agrobacterium rhizogenes strain A4.Plant Tissue Culture Letters 9(3),214-219*

Bensaddek.L et al. 2001 .The effect of nitrate and ammonium concentrations on growth and alkaloid accumulation of Atropa belladonna hairy roots.Journal of Biotechnology 85: 35-40

Bourgaud.F. 1999.Production of flavonoids by psoralea hairy root culture.Plant cell Tissue and organ culture.56:97-104

Georgiev.M,Pavlov A,Bley T.Hairy root type plant in vitro system as source of bioactive substances .Appl Microbial Biotechnol .2007.74:1175-1185

Jacob.A,Malpathak.N.Green hairy root culture of Solanum khasianum Clarke- a new route to in vitro Solasodine production.CURRENT SCIENCE,VOL.87,NO.10,25 NOVEMBER

Khatamsaz.M.FLORA OF IRAN.No.24:Solanaceae.1998.Research Institute of Forests and Rangelands

Krolicka.A et al 2001..Establishment of hairy root culture of Ammi majus.Plant Science 160: 259-264

Medina-Bolivar.F. et al .2003.Production and secretion of resverayol in hairy root cultures of peanut.Phytochemistry 68:1992-2003

Sevon.N,et al.Transgenic Hyoscyamus muticus(Egyptain herbane).Biotechnology in Agriculture and Forestry,Vol.48.Transgenic Crops III (ed,by Y.P.S.Bajaj).Springer-Verlag Berlin Heidelberg 2001

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Veena V, Taylor CG (2007) Agrobacterium rhizogenes: Recent developments and promising . In Vitro Cell Dev Biol 43: 383-40

Verma .P. C,L. u. Rahman,A. S. Negi,D. C. Jain,. P. S. Khanuja,S. Banerjee.Agrobacterium rhizogenes-mediated transformation of Picrorhiza kurroaRoyle ex Benth.: establishment and selection of superior hairy root clone.Plant Biotechnol Rep (2007) 1:169–174

Woo.S.2004.Selection of high ginsenoside producing ginseng hairy root lines using targeted metabolic analysis.Phytochemistry.65:2751-2761

Zehar.M et al.1998.Variation in the growth and alkaloid production capability of the hairy roots of Hyoscyamus albus,H.muticus and their somatic hybrid