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The involvement of MSD1 metalloprotein in the mechanism of resistance to salinity by interaction with SOS3

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A B S T R A C T

Among the various stresses, salinity is a great threat to plants and the study of the mechanism of salt tolerance in plants is important. In one of the salt tolerance pathways in the model plant Arabidopsis, Salt Overly Sensitive 3 (SOS3) protein, which is a sensor of calcium ions and the initiator of a salt tolerance pathway, activates various processes in order to tolerate salinity by the formation of an SOS3-SOS2 complex. On the other hand, superoxide radicals that are produced in response to various stresses such as salinity are harmful to the cell and must be eliminated. How to remove superoxide radicals and protect cells from oxidative damage in salt-tolerant plants is not clear. In this study, the SOS3 protein interactions in Arabidopsis cDNA library were investigated using the Yeast Two Hybrid System (Y2HS), and double transformation of *Saccharomyces cerevisiae* AH109 strain with pGBT9-SOS3 and pGADT10-AtcDNA. DNA extraction was performed on four selected yeast colonies on SD-AHWL and pGADT10-AtcDNA vectors were amplified in *E. coli*, sequenced and compared to the Arabidopsis data bank. One of the most important interactions was found on Manganese Superoxide Dismutase 1 (MSD1). MSD1 neutralizes superoxide radicals to protect plants from oxidative damage caused by stresses. The interaction and the recruitment of MSD1 by SOS3 in the salinity resistance pathway should save the plant from oxidative trauma. Afterward, the interaction of SOS3-MSD1 was confirmed by isolation of complete MSD1 cDNA and cloning in yeast two hybrid vectors. In parallel, using a GST-Pull Down assay, it was shown that the SOS3 protein produced in bacterium from pGEX2TK-SOS3 vector directly interacted with the radio-labeled MSD1 produced from pGBKT7-MSD1. This is the first report of the interaction of salinity tolerance pathway (SOS3) with the elimination pathway of harmful superoxide ions (MSD1) that shows interaction between these molecular mechanisms.

Key Words

Yeast two hybrid system, SOS3, salinity, Manganese superoxide dismutase, GST-Pull Down

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Optimizing tissue culture and GUS gene transformation to shallots using *Agrobacterium*

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A B S T R A C T

Shallot (*Allium stipitatum*) is an edible vegetable that has important pharmacological properties. Therefore, optimization of an efficient *in vitro* regeneration and transformation system for shallot breeding through genetic engineering would be useful. Embryo, root and bulb explants of shallot were cultured *in vitro* on MS basal medium supplemented with different concentrations of growth regulators (NAA, 2,4-D and BA) for callus induction and plantlet regeneration. Explants were transformed using *Agrobacterium tumefaciens* strain LBA4404 and pBI121 plasmid carrying the *gus* reporter gene. The results indicated that bulb had a higher efficiency of callus induction and plantlet regeneration (100%) compared to embryo and root explants. The highest percentage of regeneration (100 %) was observed on MS medium supplemented with 5 $mg.l^{-1}$ BA and 1 $mg.l^{-1}$ NAA for bulb explants. Polymerase chain reaction (PCR) analysis of *gus*-positive transformants confirmed genetic transformation of the cultures. Furthermore, the lack of *Agrobacterium*-related infection was confirmed using *vir*G-specific markers. In this study, the efficiency of transformation was 10 and 6.6% in embryo and bulb, respectively.

Key Words

Agrobacterium, GUS gene, Gene transformation, Shallot, Tissue culture

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Assessment of hairy roots induction of the medicinal plant Alecost (*Tanacetum balsamita* L.) using *Agrobacterium rhizogenes*

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A B S T R A C T

Alecost (*Tanacetum balsamita* L.), belongs to the Asteraseae family, is a pharmacologically important species rich in important secondary metabolites including flavones, sesquiterpene lactones, phenylpropane compounds and derivatives, tannins and essential oils. Alecost has been used both fresh or dried as flavouring or food additive. Additionally, it has medicinal properties and is applied in aromatic products. In addition to traditional farming, in vitro hairy root culture has been found to be suitable for the production of secondary metabolites. Therefore, in order to establish a protocol for hairy root culture of alecost, root induction by co-cultivation with *Agrobacterium rhizogenes* (strain A4) was assessed in this study. Different explants (cotyledon, young leaf, stem and root) showed different responses to hairy root induction by *Agrobacterium rhizogenes*. Moreover, the frequencies of hairy root induction for different types of explants were considerably different. The induced roots were shown to be transformed by PCR using primers specific for *rolB*. This is the first report of hairy root induction in alecost and the results may be useful in genetic manipulation of *Tanacetum balsamita* and use of hairy root culture to produce high-value secondary metabolites.

Key Words

Agrobacterium rhizogenes, Hairy root, Medicinal plant, Secondary metabolites, *Tanacetum balsamita*

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Proteome analysis of *Xanthomonas citri* subsp. *citri* protein extract with elicitor activity on *Arabidopsis thaliana*

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A B S T R A C T

Extensive research is currently going on in different research centers of the world to find new elicitors from microorganisms and their related receptors from different plants. Comprehensive knowledge about these elicitors and their cognate receptors could be used for development of transgenic plants displaying resistant against plant pathogens. As a crude protein extract of *Xanthomonas citri* subsp. *citri* (Xcc) has strong elicitor activity on *Arabidopsis*, in this study protein extracts of this bacterium were prepared and fractionated and a semi-purified sample displaying elicitor activity was analyzed by LC-MS/MS. About 60 different proteins were detected in the analyzed sample. Among the detected proteins, only three were similar to the elicitor activity observed in a protein extract of *Xcc* bacterium: cspA (cold shock protein A), csrA (carbon storage regulator protein A) and an unknown conserved hypothetical protein from the DUF1456 protein family. This protein is soluble in water and it has no bacterium membrane-targeting sequence as determined by PSORTB and SOSUI analysis, suggesing with high probability that it is a cytoplasmic protein.

Key Words

Arabidopsis, Elicitor, Ethylene, Proteome, *Xanthomonas*

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Isolation, cloning and bioinformatic study of a resistance gene against an Asian strain of citrus greening librobacter in grapefruit plant

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A B S T R A C T

Citrus greening disease is the most important bacterial diseases of citrus and is present in the southern region of Iran. Due to the nature of the pathogen, disease control is difficult. cDNA-AFLP analysis shows that the expression pattern of NBS-LRR (Nucleotide Binding Site- Leucine-Rich Repeats) genes was upregulated in grapefruit plant interacting with *Candidatus* Leiberibacter aciaticus. In this study we have isolated the *NBS552* gene from grapefruit by PCR and clone it by ligation of this product to pGEM-T vector transformed *E. coli* bacteria. Sequencing analysis revealed an open reading frame for a gene of 171 bp that encodes a protein of 57 amino acids. Results of bioinformatic analysis showed that this gene contains regions that are conserved in different plant species. The NBS552 protein is associated with membranes and its extracellular secretion is low. The aim of this study was isolation and cloning of *NBS552* genes from the grapefruit genome and characterization of the genes in view of their potential for practical use in plants.

Key Words

Grapefruit,C itrus Greening, Gene *NBS552*, Resistance

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A method for Evaluation of RNA Silencing Suppression Activity in Plants Using two Proteins of *Grapevine Fanleaf Virus*

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ABSTRACT

Suppressor proteins of RNA Silencing have been well described in most genera of plant viruses. The methodology for identifying gene silencing suppressor activity of a protein in a plant system has proven to be an important tool. *Grapevine fanleaf virus* (GFLV) is one of the most common viral diseases in grapevines worldwide and can cause up to 80% crop losses. GFLV is a bipartite member of the *Nepovirus* subgroup A in the family *Secoviridae* and has a single-stranded positive-sense RNA genome. A viral suppressor of RNA silencing (VSR) in Nepoviruses has not yet been described and our knowledge of symptom determinant genes in nepeoviruses is very limited. In this study, a transgenic GFP gene was silenced in transgenic *Nicotiana benthamiana* line 16c, and then the ability of GFLV polymerase and movement proteins to suppress gene silencing was evaluated. The efficiency of these two proteins was compared with positive and negative controls. The results showed that neither protein had the ability to suppress gene silencing. However, these proteins are involved in symptom production and systemic infection. This study demonstrates a model method for evaluation of suppressor activity of RNA Silencing.

Key Words

GFLV, RNA Silencing, Suppressor of Gene Silencing

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The status study of Biotechnology in East Azerbaijan Province

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A B S T R A C T

Biotechnology is one of the most important scientific strategies in today's world and involves application of various techniques in direct or indirect usage of living organisms or their byproducts in natural or modified forms for different fields. Significant advances of biotechnology in recent decades, especially after the acquisition of new methods of genetic engineering for gene isolation, their modification and transferring from one species to another have made biotechnology one of the most productive present and future technologies. Iran with a wide variety of climate and the variety of living organisms is the most suitable region for access to technologies derived from biological sources. It is evident that management and planning in biotechnology, as in all fields, requires a good understanding of the researchers in that field, In this study we attempted to identify researchers, institutes, universities and companies operating in various fields of biotechnology along with their research areas so as to identify strengths and weaknesses of biotechnology to help making targeted decision in the province and the country. The results of this study showed that more than 40% of the provincial biotechnological activities are related to agriculture, about 25% are related to animal science and 35% to other sectors. The main activities in the agricultural biotechnology are related to study of different growth conditions of plants. Study on medicinal plants, plant tissue culture and production of transformed plants are other activities in this section. Factors affecting the growth of livestock, animal health and livestock products are main activities related to animal biotechnology as well as cloning of useful genes and control of pathogenic microorganisms in microbial biotechnology. Review, treatment, and diagnosis of genetic diseases through biotechnology in the medical field and, finally, production of pharmaceutical products and the use of biotechnology in the food industry are also activities which are carried out by researchers in various fields of biotechnology in East Azerbaijan Province. Based on these results, activities related to environmental biotechnology and especially industrial biotechnology are under-represented in East Azerbaijan Province and serious attention should be paid to this section.

Key Words

Biotechnology, Development, East Azerbaijan Province

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Study of Root Induction in Several Medicinal Plant Species using *A. rhizogenes rol* Genes

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A B S T R A C T

Agrobacterium rhizogenese can induce hairy root phenotype in many dicotyledonous plants via the transfer of *rol* genes into the plant genome. The characteristics of induced roots such as a rapid growth, high stability of genetic material, ease of conservation and their ability to grow in a hormone-free medium have led to their use in the production of secondary metabolites. In the present study, we have investigated the effect of *rol* genes, particularly the *rolB* gene, on root induction of three medicinal plants *valeriana officinalis*, Echinacea angustifolia and *Foeniculum vulgare*. Leaf explants and wounded parts of stems were inoculated with bacterial suspension and the presence of bacterial DNA as well as its expression in induced roots was demonstrated using PCR and RT-PCR techniques with *rolB* specific primers. The results showed that maximum rooting occurred with *Valeriana officinalis* leaf explants cultured in MS medium and the wounded parts of stems did not show rooting at inoculated areas.

Key Words

Agrobacterium rhizogenese, hairy root, *rol* genes, RT-PCR