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The effect of manganese and salicylic acid on gene expression of Menthone reductase and Menthol content in *Mentha piperita*

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A B S T R A C T

The effect of manganese and salicylic acid on gene expression of Menthone Reductase (MR) and menthol content in *Mentha piperita* were investigated. The research was conducted by a factorial experiment design in a randomized complete block with three replications. The experimental factors include the treatments by manganese (500 µM) and salicylic acid (1 mM) and time (1, 3 and 5 days) after spraying. The results of ANOVA indicated that the manganese and salicylic acid treatments had significant effect on gene expression of Menthone Reductase and menthol over all three times after spraying. The lowest gene expression of MR and menthol casused by manganese at 5 days after spraying was significantly different from control. The maximum gene expression of MR and menthol content was achieved by salicylic acid at 5 days after spraying. The analysis of the interaction of manganese and salicylic acid showed that salicylic acid reduced the manganese toxicity to some extent in the expression levels of MR and the menthol content. The results of this experiment showed that a direct correlation exists between the MR expression and the amount of menthol in peppermint, so that over time by increasing the MR expression, the amount of menthol was also increased by salicylic acid while the expression of MR and the amount of menthol was decreased by manganese. However, it was observed that salicylic acid acts as a powerful hormone and stabilizes the effect of manganese on MR gene expression and menthol content in peppermint plant.

Key Words

Gene expression, Menthol Reductase, Menthol, *Mentha piperita*.

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Optimization of transient expression by Agrobacterium in almond

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A B S T R A C T

Since permanent transfer of genes and transgenic plant production, particularly in perennial plants such as almonds are time and cost consuming, optimization of various factors affecting the transgenic plants is critical. Transient genetic expression using *Agrobacterium* (Agroinfiltration) in plant is a useful tool to study gene function indifferent gene expression systems. Since optimization of factors that could affect the transgenic methods is necessary for gene transfer programs, Because This study was aimed at developing an efficient system for transient expression via Agrobacterium in almond leaves for rapid prediction of systems performance. Here the lichenasereporter gene was usedto assessthe parametersinfluencing thelevel oftransient expression. The main advantagesof using thisgene includeeasy operation, high sensitivity and biosafety. Three different constructs carrying lichenase gene and differentregulatoryelementswereinfiltrated intoleaves ofmamaei and shahrood 12cultivars,by two Agrobacteriumstrain, LBA4404 andGV3501,using syringewithoutneedle.After threedays,the amount of active proteinwas measured. Statistical analysis showedthat the highestamount ofprotein was obtainedin theleavesof mamaei cultivar transformedby theexpression systemcausing accumulation ofproteins in theendoplasmic reticulum by LBA4404 strain. So forrapidproduction ofrecombinant protein in Almond, theagroinfiltration method by using the related expression system, LBA4404 strain and mamaei cultivar, isrecommended.

Key Words

Transient expression, Agroinfiltration, Agrobacterium, Expression system, Almond.

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Different expression pattern of NPR1 and some of the pathogenesis-related genes in response to salicylic acid and methyl jasmonate treatment in rice

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A B S T R A C T

Salicylic acid (SA) and Jasmonic acid (JA) signaling pathways are mutually antagonistic and sometimes synergistic. This regulatory cross talk may have evolved to allow plants to fine-tune the induction of their defenses in response to different plant pathogens. *NPR1* plays a significant role in regulating the interaction of salicylic acid and methyl jasmonate pathway. In fact, *NPR1* activation depends on the levels of these two hormones in plant. Therefore, antagonistic and synergistic effects of salicylic acid and methyl jasmonate spraying were investigated on the expression of the genes *NPR1*, *Thionin*, *PDF1.2* and *PR1* using Real time PCR technique in two rice cultivars Khazar (resistant to blast) and Hashemi (susceptible to blast) at 0, 6, 12, 24 and 48h after spraying. Results showed that the studied genes had different expression patterns in different genotypes and times after spraying, in response to salicylic acid and methyl jasmonate, indicating an association between regulatory pathways of these hormones and induction of the defense related genes. The expression of the studied genes was increased by SA and MJ treatment in khazar, while no significant differences were observed among different times after SA and MJ treatment for *PR1* in Hashemi. The expression of the other genes was also low in this cultivar. In general, it could be concluded that antagonistic and synergistic effects of SA and MJ lead to changes in the expression of the genes studied at different times after spraying and in the formation of the immune system in resistance genotype.

Key Words

Methyl jasmonate, Pathogenesis related genes, Rice and Salicylic acid.

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Bioinformatical analysis of Isolated ESTs from *Aeluropus littoralis* under salinity stress

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A B S T R A C T

Biotechnology needs the genetic resources to establish the sustainable tolerance to salinity stress in GMO plants. *Aleluropus littoralis* is known as a good model plant for understanding the genetic and molecular mechanism of salt resistance in monocots. This plant has a high genetical homology with rice. In this study, 150 ESTs were isolated from *A. littoralis*. Firstly, repeated sequences were eliminated and then the possible function and similarity of the remained ESTs were compared with other ESTs of different plants using international genetic databases. Results of this study showed that, 71 of the selected ESTs were similar to the ESTs in the databases and their function have been determined. Moreover, 14 ESTs were similar to the ESTs in the databases but their functions have not been determined yet. Seven ESTs did not show any similarity. Alignment analysis showed that the isolated ESTs were categorized in 19 different groups. 16 ESTs acted as a transporter, 12 ribosomal proteins and the other ESTs were related to channel transfer regulators, RNA editing and cell division. By the relatively high level of homology with rice genes, the genetic studies on *Aleuropus* could help in determining the differences in salt-tolerance between glycophyte and halophyte grass

Key Words

Aeluropus littoralis, Databank, Bioinformatics, Salinity stress and EST.

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Molecular detection of native isolates of *Bacillus thuringiensis* from soils samples with different vegetations

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A B S T R A C T

Native isolates of *Bacillus thuringiensis* were obtained from soil samples of various ecosystems in Mazandaran province and *Cry1*, the insecticidal protein-encoding gene, was detected in the isolates. About 491 bacilliform isolates were obtained from 128 soil samples through sodium acetate inhibition procedure. Two hundred ninety one spore-forming, 85 spore and cap-forming and 113 spore, cap and crystal forming bacteria representing 59.67, 17.31 and 23.01% 0f the isolated strains were identified by phase contrast microscopy. Molecular identification of *Cry1* and its 14 related genes were carried out by 14 pairs of gene-specific primers. Fifteen isolates contained the *Cry1* gene in the expected size. *Cry1Ac* and *Cry1I* were detected in all of the isolates but *Cry1Aa*, *Cry1F*, *Cry1G* and *Cry1K* were not entirely found. *Cry1D*, *Cry1E* and *Cry1J* were amplified in some of the isolates in different sizes: this could be attributed to the fact that they might have contained one or more new *Cry* genes. Detecting the effective genes of native Bt strains and their application in genetic engineering can be a useful component in future of insect pest management system.

Key Words

Crystal protein, *Cry1* gene, *Bacillus thuringiensis.*

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Study of *cry1Ab* transgene locus structure in a transgenic rice (*Oryza sativa L.*, cultivar Tarom Molaii)

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ABSTRACT

The level and stability of transgene expression can be influenced by factors such as structure of expression cassette, integration pattern and locus structure. The structure of transgene locus is important in its expression mechanisms. The aim of this experiment was to characterize the *cry1Ab* transgene locus structure in a transgenic rice (*Oryza sativa*, cultivar Tarom Molaii). This transgenic rice, co-transformed with two different plasmids (pCIB4421 and pChitIHygII), had a simple integration pattern and stable expression over several generations. In order to characterize the *cry1Ab* transgene locus stracture, the exact insertion site was identified using specific primers corresponding to 3´ and 5´ ends of *amp*R gene, 3´ end of *cry1Ab* gene and 5´ end of PEPC promoter on pCIB4421 plasmid. Then, DNA sequences of the flanking insertion sites were amplified using two methods of one-side PCR including TAIL-PCR and Splinkerette PCR. Sequencing of the PCR products and bioinformatics analysis revealed that pCIB4421 plasmid break for insertion was 148 bp after 3´ end of *ampR* gene. A fragment corresponding to 1499bp downstream sequences and 311 bp upstream of insertion site were isolated. BLAST search was performed against the pCIB4421, the pChitIHygII and *Oryza sativa* genome. Downstream sequences of the insertion site was scrambled fragments of delivered DNA and rice genome, however the upstream of the insertion site was non-continuous fragments of the delivered DNA.

Key Words

Biosafety, *cry1Ab* transgene, Flanking sequence of transgene, Transgenic rice.