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Production of Potato Resistant Plant to PVX using an RNA Silencing Mechanism

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ABSTRACT

Viruses are the main causes of potato yield loss. Potato X Potexvirus (PVX) is one of the most important potato viruses and the use of resistant varieties is the principal way to control it. In this study, we tried to produce potato plants resistant to this virus using an RNA silencing technique. To this end, a hairpin construct of P25 of PVX was made in pFGC5941 under control of the CaMV 35S promoter with two consecutive cloning steps. The P25 protein is a suppressor of RNA Silencing in viral host plants. The recombinant vector was transferred into *Agrobacterium tumefaciens* LBA4404 strain. Leaf and internode pieces of potato (Agria variety) were transformed by Agrobacterium. Following induction of callus, shoot and root, regenerated transgenic plants were selected and micro-propagated. Molecular analysis by PCR on DNA of plants and by RT-PCR on their RNA confirmed the presence and the expression of the transgene. Transgenic plants were propagated and then inoculated mechanically by PVX in the greenhouse. ELISA results using PVX-coat protein antibody showed resistance of two transgenic lines to PVX. Molecular confirmation of these potato lines was performed using PVX coat protein primers by RT-PCR on plant RNAs. The results of this research have led to the production of two independent lines of potato that are resistant to PVX and future work will involve analysis of resistance in field conditions and the production of their mini-tuber.

Key Words

Potato, PVX, Resistance, Transgenic Plant, RNA Silencing

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Various Tomato microRNAs could target a Mild and a Severe Strain of *Tomato Leaf Curl Virus*

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A B S T R A C T

Geminiviruses are one of the main constraints in tomato production worldwide. *Tomato leaf curl virus* (ToLCV) is a geminivirus (family *geminiviridae*) that produces various symptoms in plants including leaf curling, yellowing and stunting. MicroRNAs are endogenous small RNAs which play a key role in both plants and animal defense and development. Certain animal viruses were found to be targeted by host miRNA which prevent their establishments. In plants, on the other hand, there is no experimental evidence for such an effect on plant viruses. However, based on in silico analysis, viral plants also could be targeted by host miRNAs. Here, we investigated if different sets of tomato miRNAs could potentially bind to a mild and severe species of ToLCV using in silico analysis. We found that tomato miRNAs including mir156 bind to both strains of ToLCV. The mild strain, ToLCV-Ir, was found to be targeted by mir157, 168, 396 and 166 while the severe strain, ToLCV-Au, was targeted by other groups of miRNAs including mir159, mir319 and mir403. The possible role of the identified miRNAs in production of mild and severe symptoms by both strains is discussed. All sequences except miR156a target the coding regions of the virus. In ToLCV-Au miRNA319c binds to the V1 gene, which encodes the precoat protein and which is involved in symptom expression and virus movement. miR159c and miR403 bind to the C1 and C3 genes, respectively, that are involved in virus replication. The C1 ORF of ToLCV-Ir can be targeted by both miRNAs miR157a and miR156h. The V2 gene can be targeted by three miRNAs (miR168b, miR396b and miR166i) at different sites. This suggests the potential importance of regulation of this particular ORF by miRNAs. The possible role of the identified miRNAs in production of mild and severe symptoms by both strains is discussed.

Key Words

Bioinformatics, ToLCV, miRNA, RNA hybrid, Tomato

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Transformation of Hepatitis B Virus surface antigen (HBsAg) gene into Tobacco plants

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ABSTRACT

Hepatitis B virus (HBV) infection is one of the most widespread viral infections of humans. An effective way to treat and prevent the disease is vaccination. Since production of conventional HBV vaccines is very expensive, use of transgenic plants as an alternative bioreactor has recently become of interest to many researchers. In this study, the *HBsAg* gene has been transferred to pepper plants (*Nicotiana tabacum*) through the leaf disk technique. The recombinant plant expression vector, pCAMBIA containing *HBsAg* was cloned into *E. coli* strain JM107 and was then introduced into *Agrobacterium tumefaciens* strain LBA4404. Young tobacco leaves were used as explants and co-cultivated with *A. tumefaciens.* The transformants were regenerated on selection medium containing 1$mg.l^{-1}$ BAP, 0/1$mg.l^{-1}$ NAA, 500 $mg.l^{-1}$ cephotaxim and 15 $mg.l^{-1}$ hygromycin. After the growth of plantlets (about 15 cm), genomic DNA was extracted from putatively regenerated plants by the CTAB method. The presence of *HBsAg* gene in transgenic plants was detected using PCR analysis. Finally expression of *HBsAg* gene was tested via RT-PCR analysis.

Key Words

Agrobacterium, Edible Vaccine, Gene Transformation, *HBsAg* Gene, Transgenic Tobacco

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Characterization of Conserved Hypothetical Proteins from Proteome of *Xanthomonas citri subsp. citri,* with Ethylene Induction Activity on *Arabidopsis thaliana*

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A B S T R A C T

In previous work we showed that a purified extract of the proteome of bacterium *Xcc* that was able to induce ethylene production on *Arabidopsis*. Among the approximately 60 different putative proteins in the extract, eight were identified as conserved hypothetical proteins. The aim of this study is to use different bioinformatics tools to characterize these proteins. All of the investigated proteins ranged in size between 17.11 and 43.84 kilo Daltons with Protein NP_640497.1 being the largest. Calculated isoelectric points (pI) for proteins varied between 5.34 and 9.5. The type of protein families and domains of proteins was determined by conserved domain database (CDD-Blast) and Interpro. Among the investigated proteins, proteins NP_640912.1 had a HTH domain (Helix-turn-Helix); the central part of protein NP_640497 contained an Enoyl reductase domain; protein NP_641576.1 contained two calcium binding motifs (EF-hand, calcium binding motif); and protein NP_643454.1 contained a 4-hydroxybenzoyl-CoA thiesterase motif from the Hot dog superfamily. COACH server was used for predication of ligand binding sites of proteins and their three dimensional structure was modeled by Phyre 2 server. Results from this research can be used for better understanding of Xcc and also identification of proteins with elicitor activity from this bacterium.

Key Words

Xanthomonas, Bioinformatics, Citrus canker, Elicitor, Protein

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The effect of *rolC* gene on the medicinal plant *Catharanthus roseus*

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A B S T R A C T

Catharanthus roseus is a medicinal plant containing very important alkaloids such as Vincristine and Vinblastin that are used to treat a variety of cancers. Due to the importance of the plasticity genes such as *rolC* in the biosynthesis of alkaloids, this study we investigated possible improvement of rooting and root induction in *C. roseus* using the *rolC* gene. To this end, the seeds of this plant were superficially sterilized and cultured in *in vitro* conditions. The leaf explants of these *in vitro* plants were inoculated by *Agrobacterium tumefaciens* carrying pBI121-*rolC* plasmid (*rolC* under control of the CaMV 35S promoter). The inoculated explants were cultured in five different media with or without hormones for rooting. After nine days, the first roots appeared in leaf explants on the MS medium without hormones and containing cefotaxime antibiotic. Two lines had very rapid rooting and the roots branched quickly. Molecular analysis by PCR using *rolC* specific primers confirmed the presence of the *rolC* gene in the transgenic plants. Results showed that the *rolC* gene could be used to induce rooting in the medicinal plants and that the *rolC* gene is also more successful in induction of hairy roots than various different combinations of plant hormones.

Key Words

Catharanthus, Root, Transformation, *rolC*

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Evaluation of the effect of salinity on the germination and expression of antioxidant genes in two cultivars of tomato plant

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A B S T R A C T

Environmental stresses are considered among the most important factors limiting agricultural production. Tomato, an important agricultural product, is sensitive to high salinity levels in soil. During salinity stress, several physiological phenomena occur in plants, including oxidative damage to cellular components due to "reactive oxygen species" or ROS production. Plants produce antioxidant enzymes such as catalase, ascorbate peroxidase and superoxide dismutase to counter the destructive effects of ROS. This study was carried out to evaluate the rate of germination and the expression of *CAT1* and *APX1* genes in two tomato cultivars, caljN3 and superstrain B. To evaluate the rate and the percentage of the germination, the seeds were cultured on filter paper in a completely randomized design with three replications and the rate and percentage of germination, radicle length and plumule length were measured after 15 days. To perform the molecular study, total RNA was extracted from plants grown in control conditions and under salinity stress. The results demonstrated that by increasing the salinity, reductions in germination percentage, germination rate, radicle length and plumule length were observed in both CaljN3 and Superstrain B cultivars, although the CaljN3 cultivar showed the lowest decrease. Interestingly, upon increasing the stress level, the rates of expression of the *CAT1* and *APX1* genes in the caljN3 cultivar were nearly twice and 10 times, respectively, higher than for the Superstrain B cultivar. These results could be one of the reasons for the superior growth and salt stress tolerance of caljN3 cultivar compared to SuperstrainB cultivar.

Key Words

APX1 gene, *CAT1* gene, Germination**,** qRT-PCR, Salt stress

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Study of kappa-casein gene polymorphism association with milk production and composition in Golestan province camels

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A B S T R A C T

This study was aimed to determine the kappa-casein gene polymorphism and its relationship with some milk traits in a population of camels in the Golestan province. Blood samples were collected from100 camel dromedaries in Bandar Turkman, AqQala and Gonbad cities. DNA was extracted using the optimized salt method and then a pair of specific primers was used to produce a 488 bp fragment. Genotypes were determined by PCR-RFLP method followed by treatment with ALU1 restriction enzyme. To investigate the association of Kappa casein gene polymorphism with milk production and composition (fat, protein, lactose and solids non-fat milk), the GLM procedure of SAS software was used. Three genotype patterns including AA, AB and BB were observed with the frequencies of 0.18, 0.27 and 0.55, respectively. The effect of kappa-casein gene polymorphism on milk production and composition was not significant. Based on current results, the analysis of kappa casein gene polymorphism cannot be used for improving milk yield and composition of camels in Turkman population.

Key Words

Kappa-casein gene, Polymorphism, Camel, Milk