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Evaluation of total carbohydrate and soluble sugars in transgenic potato resistant to potato tuber moth

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A B S T R A C T

Based on the regulatory frameworks in most countries, careful safety assessment based on comparison methods must be performed before the adoption and commercialization of GM crops and products. One of the safety assessments of GM plants is the comparison of key nutrients and metabolites between transgenic and non-transgenic lines. This study was designed to identify undesirable potential changes resulting from genetic manipulation (for example, as a result of the entry of foreign genes into the genome and new metabolite production) in transgenic potato resistant to potato tuber moth (*Phetorima operculella*). This process is known as substantial equivalence. This study attempted to examine total and soluble sugars such as sucrose, fructose and glucose in transgenic potato which were produced in Agricultural Biotechnology Research Institute of Iran. For this purpose, four transgenic lines (B2, B8, B11, B12) that have shown high levels of potato tuber moth resistance in bioassay tests were used. First, molecular analyses were performed on plant to be ensured of the presence of transgene, and the result of PCR showed that *cry*1Ab gene was present in all transgenic samples. The transgenic and control plants were transferred to greenhouse to produce the tubers. The harvested tubers were treated under light and dark conditions and then used for more analyses together with leaf samples. Evaluation of total sugar showed no significant differences between transgenic and control plants. Moreover, evaluation of soluble sugar showed that the contents of sucrose, fructose and glucose were not significantly different between transgenic and control plants. We conclude that according to evaluated components and regulatory rules of Codex, these transgenic potatoes are safe to use.

Key Words

*cry*1Ab gene, soluble sugar, substantial equivalence, total sugar, transgenic potato

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Evaluation of strain compatibility effects on Agrobacterium-mediated transient GUS expression in melon cultivars

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A B S T R A C T

Transient expression of a candidate effector gene in host plants following Agroinfiltration is a useful method to investigate the role of the gene in pathogenecity. In this study, the effects of two *Agrobacterium tumefaciens* strains on four standard melon lines and two Iranian melon cultivars were investigated to establish transient expression in melon. Compatibility of the LBA4404 and GV3101 strains was defined by injection of the bacterial suspensions into the leaves of cultivars. Viability of the injected *Agrobacterium* strain cells in leaf tissues was evaluated 24 hours after injection by prokaryotic GUS reporter gene assay. The LBA4404 strain harboring the pCAMBIA3301 vector containing an intron-GUS reporter gene was used to confirm eukaryotic GUS expression in the plant cells. The LBA4404 strain was transformed by the pBI121 and pCAMBIA3301 expression vectors and transient transformation was confirmed by colony PCR technique using the PSh3-F/R primers. The efficiency of transient transformation of the melon leaves was evaluated 48 hours after Agroinjection of the LBA4404 strain containing pCAMBIA3301 by the histochemical GUS assay. The leaves that were injected by LBA4404 harboring pBI121 showed GUS activity. All the melon cultivars transiently expressed the GUS reporter gene 24 hours after Agroinjection. These findings could be used in the future studies to evaluate function of candidate effector genes in interaction with standard melon lines.

Key Words

Agroinjection , GUS assay, Melon, Transient expression

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Study on Expression of some Transcription Factors Associated with Resistance to Black Stem Disease in Sunflower

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A B S T R A C T

Black stem is one of the most important fungal diseases of sunflower caused by *Phoma macdonaldii*. In this study, expression level of some transcription factors (TF) including HD-Zip, AP2 domain, MYB- related, WRKY family and MYB family were studied using quantitative RT-PCR in sunflower genotypes, including ENSAT-B5 (susceptible to all 3 studied isolates; MA6, MP8, MP10), AS613 (resistant against MP8 and MP10 and susceptible to MA6), and M5-54-1 (a mutant genotype, resistant against MA6 and MP10 and susceptible to MP8) following infection by MA6, MP8 and MP10 isolates of *P. macdonaldii*. Among studied TFs, the expression level of two TFs, HD-Zip and MYB-related, were significantly different in genotype-isolate combinations but the expression level of three other TFs including AP2 domain, WRKY family and MYB family, were not significantly different in several genotype-isolate combinations. The expression of HD-Zip and MYB-related were suppressed in infected genotypes. Results revealed that increased repression of HD-Zip and decreased repression of MYB-related are affective in induction of resistance to MP8 and MP10 isolates in AS613 genotype. In this study, the induction of resistance in mutant genotype against MA6 and MP10 isolates was accompanied with decreased suppression of HD-Zip and MYB-related genes.

Key Words

Sunflower, black stem, transcription factors, gene expression

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Transformation and expression of recombinant insulin monomer in Arabidopsis plant

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A B S T R A C T

The prevalence of diabetes is predicted to rise significantly in the coming decades. Also, the incidence of this disease is increasing in Iran every year. Aspart is an insulin analog. Aspart insulin has a more rapid peak than Lispro and can be used in insulin pumps, insulin pens and injection methods. In this research, Two *Agrobacterium tumefaciens* strains, EHA101 and GV 3101, containing pJawohl3 carrying the Aspart insulin gene were used to transform *Arabidopsis thaliana* Col-0 ecotype plants by floral dip infiltration method. Seeds of floral dip infiltrated plants were sown on the pots. Then, seedlings were treated by BASTA herbicide. Expression of the Aspart insulin gene was detected in transgenic plants by RT-PCR and western blot. Evaluation of the infiltrated plants revealed that the EHA101 strain of Agrobacterium was more efficient than GV3101 strain in gene transformation.

Key Words

Agrobacterium, *Arabidopsis thaliana*, Aspart insulin, Transformation

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Indentifying, Cloning, and Determining the Sequence of MT-ND2 Gene in Khorasan's Native Chickens

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A B S T R A C T

Discovering the sequences of mitochondrion has made it possible to study the presence of controlling genes in this organelle. Mitochondrion is responsible for producing 90 percent of the energy that the cell needs. Some differences in the broiler chicken growing function and the resulting phenotype and food efficiency may be related to differences in mitochondrion function. The aim of this study was to clone and to analyze the ND2 gene among Khorasan's native chickens in order to investigate possible mutations. To this end, genomic DNA was extracted from blood sample taken from this population. Then, using ND2 specific primers, PCR was conducted in order to multiply this gene. The PCR product was cloned into pTZ57R/T linear vector and sequenced. Comparison between the sequenced fragments and the registered gene revealed four mutations. A similarity of 99% was observed between them and the complete mitochondrial genome sequence of *Gallus gallus* with the accession numbers of X52392.1 (reference), GU261709.1, GU262712.1, AP006746.1, KF826490.1, HQ857210.1, and AY23557.1. Comparison between proteins showed that the resulting sequence was similar to protein sequences related to mitochondrial ND2 gene of the poultry in the data bank. The maximum similarity (100% similarity) was with the ND2 gene in *Gallus gallus* with the accession number of BAC57576.1. The minimum similarity (88% similarity) was with the ND2 gene in *Chrysolophus amherstiae* with the accession number of AAF65702.1 and *Tetra stessewerzowi* with the accession number of ABH01111.1. Also, the amino acid sequences of Khorasan's native chicken had 99% similarity with reference sequence and other sequences with the accession numbers of YP272073.1, BAD11115.1, NP_006916.1, ADB06584.1, and ADW41566.1. After translating the obtained nucleotide sequence to the amino acid sequence, it was compared with the reference proteins sequence. The result of this comparison showed a difference in one amino acid. The amino acid Leucine 130 was changed to Methionine.

Key Words

Chickens- Mitochondrial Genes- Cloning- MT-ND2

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Genetic diversity of *Barbus grypus* in the Karkheh River in Khuzestan Province and cultured fish studied using a microsatellite marker

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A B S T R A C T

Shirbot (*barbus grypus*) belongs to the *Cyprinidae* family and is widely present in the west and Southwest's water resources of Iran, especially the Karkhe River in Khuzestan Province. The aim of this study was to compare levels of genetic polymorphism between Karkhe River and cultured *Barbus grypus* populations using seven microsatellite loci. Genetic diversity was investigated by studying 60 samples collected from two regions. According to the results, the F_{st} value was 0.033, which indicates low genetic diversity between the populations. Most of the loci showed deviation from Hardy-Weinberg equilibrium. Also a relatively high level of gene flow was found among the population. Genetic variations in Karkheh: mean number of alleles per locus, N_a=10.286, mean effective number of alleles, N_e=6.789, observed heterozygosity, H_o=0.691 and expected heterozygosity, H_e=0.840 and cultured fish N_a=13.286, N_e=9.141, H_o=0.800 and H_e=0.883 were not statistically different. Also, analysis of molecular variance showed that there is low genetic variation among populations and most of the observed variation is within the populations.

Key Words

Genetic Diversity, Hardy-Weinberg Equilibrium, Microsatellite, Shirbot (*Barbus grypus*)