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Different methods of RT-LAMP for detection of potato leaf roll virus (PLRV)

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A B S T R A C T

Potato leaf roll virus is an important virus that causes economic loss in the yield and quality of potato tubers. One of the primary methods of managing infection in potato crops is using certified virus-free tuber as 'seed' for planting. Early and efficient detection of virus is essential for production of PLRV-infection free tubers. There are several techniques to detect the virus including serological test and molecular methods. LAMP is a new method to identify pathogens that is used in this study for detection of potato leaf roll virus. Potato plants with symptoms similar to PLRV were collected from Zanjan province and were subjected to a serological test. Total RNA was extracted and RT-LAMP reactions were carried out. Different methods were used to confirm performance of this reaction. Positive reaction was confirmed based on the resulting turbidity and loading product on agarose gel and using SYBR and ethidium bromide florescence dyes. The advantages of this new method include the speed (75 min), ease and safety of the method compared to other methods.

Key Words

Florescence dyes, Potato Leaf Roll Virus (PLRV), RT-LAMP Reaction, RT-PCR, Turbidity

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Mutant screening of transgenic *Arabidopsis* in genetic pathway of barley metallothionein promoter

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A B S T R A C T

In order to search for signaling factors altering the expression of barley metallothionein promoter, the mutant screening technique was used. The promoter region of metallothionein in barley (cv. Hordea) was used to drive the *gus* gene and it was transferred to *Arabidopsis thaliana*. The M_0 seeds was mutagenized using four doses (10, 20, 30, 40 Grays) of fast Neutron radiation. The chemical compound of 3-Amino-1,2,4-Triazole (3-AT) at 20 mM was chosen as the best activator using CRD statistical design. Inheritance of GUS activity was evaluated using histochemical GUS assay. 48 hours after spraying by 20 mM 3-AT the inhibition model of 3:1 GUS expression: non expression was tested. 20 potential mutant lines were selected by carrying out several screening steps. In each stage 30 seedlings of 1500 M_2 lines were checked by GUS staining. The arrangement of promising lines included 3, 5, 9 and 3 which were referred to 10, 20, 30 and 40 Gray of fast Neutron treatments respectively. These lines were analyzed using fluorometric β-glucuronidase technique and were selected as potential mutants in genetic pathways of metallothionein promoter activity for further studies.

Key Words

Barley, Fast Neutron, Mutation, Metallothionein Promoter, *gus* Gene.

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Partial purification and assessment of antifungal activity of an extracellular chitinase from an Iranian thermophile strain of *Cohnella* sp. A01

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A B S T R A C T

Chitin, poly-β-1,4-N-acetyl-D-glucosamine (GlcNAc), is the second most abundant organic polymer in nature after cellulose and it is the main part of insects cuticle and crustaceans that includes in cell walls of most fungi and some algae and nematodes. One of the enzymes that are responsible for disintegration of chitin is Chitinase. Bacteria produce Chitinase for digesting chitin, principally as a source of carbon and energy. With the objective of achieving the maximum production of Chitinase in an Iranian thermophile strain of *Cohnella sp*. A01, five different media were examined. We partially purified this enzyme from a native strain. Bacteria were transferred to a pre-culture media after an initial culture. After 24 hours, the bacteria were transferred to five different media containing colloidal chitin as the main component. Maximum enzyme production reached in a treatment that contained $(NH_4)_2SO_4$ 0.05% and Agar 0.1%. We performed Chitinase assay using the DNS (dinitrosalicylic acid) and precipitated proteins by Ammonium sulfate. After precipitation of proteins with Ammonium sulfate, enzyme activity was increased. Completely Randomized Design was used and statistical analysis was performed using SAS software. Anti-fungal effect of Chitinase partially purified in this study was tested on five plant fungal pathogens and was confirmed in four cases.

Key Words

Chitinase, Enzyme Activity, Purification of Enzyme, Anti-Fungal Effect.

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Optimization of regeneration and *Agrobacterium*-mediated transformation of citrus (*Citrus aurantifolia*)

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ABSTRACT

Mexican lime is one of the most important and economic fruit crops in the southern regions of Iran. This crop is susceptible to Citrus Tristeza Virus (CTV) and Witches Broom Disease of Lime (WBDL). Therefore, optimization of regeneration and transformation system for this plant is necessary for its improvement. In this project, the effect of different factors such as internode and epicotyl explants; two different regeneration media containing different concentrations of BAP and NAA, different Agrobacterium strains: "LBA4404" and "EHA105", each harboring a binary vector pBI were studied in a factorial experiment. The results showed that the etiolated epicotyl was the best explants for regeneration and callus production. There was no significant difference between two regeneration media. Furthermore, EHA105 was the best *Agrobacterium* strain for this purpose. Polymerase Chain Reaction (PCR) using *gus*-specific primers has been carried out on DNA extracted from all regenerated plants. Twenty-one shoots were carrying this gene but only 8 shoots out of these 21 showed the expression of this gene. Furthermore, the lack of *Agrobacterium*-related infections has been confirmed using *vir*G-specific markers.

Key Words

Agrobacterium, Transformation, Mexican Lime, *gus* Gene, Genetic Engineering.

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Application of co-transformation for *Choline oxidase* gene transfer into rice genome

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A B S T R A C T

In order to produce a marker-free transgenic rice with improved tolerance to salinity and drought stresses, expression vectors pABRII-Chl and pABRII-Cyt containing "*choline oxidase*" gene (with or without leader sequence respectively) were constructed from pChl and pCyt and pTRA132 for co-transformation. The pChl and pCyt vectors were digested with *Hin*dIII-*Bam*H and *Bam*HI-*Eco*RI enzymes. Then the resulting sequences were ligated and inserted into expression vector pTRA132, in which the *Hind*III-*Eco*RI fragment (*hph* gene) had been deleted. The constructs pABRII-Chl or pABRII-Cyt and pTRA132 (containing *hph* gene) were introduced into embryogenic calli derived from the mature seeds of a rice cv. Hashemi by biolistic transformation method. Then putative transformants were screened after 3 rounds of selection on N6 medium containing increasing concentrations of Hygromycin B from 60 to 80 mg/L. Finally, Hygromycin resistant calli were regenerated on MS medium supplemented with 50 mg/L Hygromycin B. Putative transgenic rice plants were analyzed by polymerase chain reaction PCR. Then, four of the transgenic plants were analyzed using Southern blotting. Each transgenic plant received one copy number of both *choline oxidase* and *hph* genes. Expression of the transgene was confirmed by reverse transcription PCR. The high frequency of transformation rate in this study showed that co-transformation method is a reliable method for stable transformation with the goal to make marker-free transgenic plants in subsequent steps.

Key Words

Choline oxidase, Glycine Betaine, Co-transformation, Rice, *hph* Gene, Biolistic.

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Evaluation of anion and cation contents of a genetically modified cotton expressing *chitinase* and *Bt* genes

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A B S T R A C T

One of the main goals of modern biotechnology is the production of higher yielding quality varieties to help attaining food security. In spite of numerous benefits, genetically modified crop plants have raised concerns for some of the consumers. Although some of these concerns have no scientific basis, safety evaluations of transgenic plants could reduce them. One of the studies to this end is the metabolic analysis of transgenic plants. Anions and cations (Na, K, Mg, acetat, chloride, nitrate, phosphate, sulfate, succinate and oxalate) of transgenic plants and their non transgenic counterparts were measured using Ion chromatography. After planting, sampling and extraction, Anions and cations of transgenic cotton (*chitinase* and *Bt* cotton) were measured. Significant differences in the amount of oxalate, sodium and ammonium in *cry1Ab expressing* cotton lines and oxalate and sodium in *chitinase* over expressing lines were observed.

Key Words

Anions, Cations, Safety assessment, Transgenic Cotton

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Global status of transgenic sugar beet and its advancement in Iran

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A B S T R A C T

Global status of sugar beet transformation for enhanced biotic stress tolerance is reviewed. Biosafety concerns related to deliberate environmental release and commercialization of genetically modified (GM) sugar beet are discussed. Status of production of GM sugar beet in Iran is also reviewed. A case study of enhanced insect tolerance in sugar beet is presented. A *cry1Ab* gene under the control of two different PEPC and CaMV35 promoters was transferred to sugar beet using biolistic transformation method. Insect bioassays for T_0, T_1 and F_1 generations against 3 different insect pests (Prodenia, Caradrina and Agrotis) were conducted. Results show significant enhanced tolerance among T_0, T_1 and F_1 progenies against the tested insect pests in comparison to their non-transgenic counterpart.

Key Words

Sugar Beet, *cry1Ab*, Prodenia, Caradrina, Agrotis, Genetic Engineering.