

## Cloning and Transformation of Bacteriophage T7RNA Polymerase in Tobacco

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### ABSTRACT

**M**olecular farming refers to the production of pharmaceutical proteins and industrial enzymes in plants by genetic engineering. T7RNA Polymerase is one of the most important enzymes which is used in various fields of biotechnology and molecular biology to produce RNA and study the structure and function of RNA *in vitro*. The *in vitro* synthesized RNA is employed in hybridization, microarray, anti-sense RNA and RNAi experiments. This enzyme has also applications in expression systems depending on the T7 promoter and terminator. T7RNA polymerase is a bacteriophage enzyme which acts mainly in the cytoplasm. This study aimed to clone and transform eukaryotic cells with the T7RNA Polymerase gene for production of T7 polymerase in tobacco plants. Specific primers with proper restriction enzyme sites were designed corresponding to the ends of the T7RNA polymerase gene and the gene was isolated and cloned into the plant expression vector, pCAMBIA1304. Successful of gene insertion was confirmed by PCR, restriction enzyme digestion and DNA sequencing. The recombinant construct was transformed into *Agrobacterium tumefaciens* LBA4404 strain for tobacco transformation. The transgenic tobacco plants were regenerated on selective media containing hygromycin and cefotaxime antibiotics. The presence and expression of the T7RNA polymerase gene in the transgenic plants was confirmed by PCR and RT-PCR techniques.

### Key Words

T7RNA polymerase, Cloning, Transformation, Tobacco, Molecular farming

## Evaluation the *Beauveria bassiana* fungus efficiency on biological control of *Rhizoctonia* damping-off disease in cotton plants

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### ABSTRACT

**R**hizoctonia rot disease is one of the most important diseases of crop plants and has a worldwide distribution. Despite the relative effects of certain chemicals to control the disease, it seems that biological control is one of the appropriate methods of control. *Beauveria bassiana* is an endophytic fungus that can colonize a wide range of plants in a systemic manner and enhance plant resistance. In this study the ability of three strains of *B. bassiana* fungus for control of cotton damping-off were investigated. Results showed that the isolates used, in addition to their endophytic activities, can significantly reduce the disease severity in plants. Based on the result of greenhouse studies and measurement of peroxidase activity and total plant protein, the KJ24 isolate at a concentration of  $10^7$  spores/ml was found to be the most suitable treatment to control the disease. It appears that this ability is due to the stimulation of plant growth. Moreover, concentrations of  $10^7$  spores/ml of isolates TS7 and TS12, were also able to significantly reduce the severity of the disease. Based on the observed increase of total protein in plants treated with these isolates, the reduction of disease could be due to the induction of plant resistance by these isolates.

### Key Words

Biocontrol, *Beauveria bassiana*, cotton, peroxidase enzyme, *Rhizoctonia solani*

## Imported infected cucurbit seeds provoked the establishment and spread of central Europe isolates of *Zucchini yellow mosaic virus* in Varamin (Sothern Tehran, Iran)

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### ABSTRACT

**Z***ucchini yellow mosaic virus* (ZYMV) is one of the most important viruses infecting cucurbits and the epidemiology of the virus is of considerable practical significance. The coat protein (CP) of Potyviruses is a phylogenetically informative sequence, but there is no ZYMV-CP sequence available from Varamin, an important cucurbit producing region. Therefore, the aim of this study was isolating the ZYMV-CP gene in Varamin isolates and studying its phylogenetic relationship(s) with previously reported ZYMV isolates. To this end, melon leaf samples were collected from Varamin, Pishva and Pakdasht (Southern Tehran) during the spring and summer of 2014-2015. ZYMV was detected in 34% of the collected samples. Sequencing data showed ZYMV-CP in the Varamin isolate was 840 nucleotides in length. Phylogenetic trees based on the entire ZYMV-CP gene showed all the ZYMV isolates could be placed in three main groups A, B and C in which group A was further subdivided into 8 subgroups, A1-A8. Iranian isolates of ZYMV are placed in A1, A6 (including the Varamin isolate) and A8 subgroups. Subgroups A7 and A8 were detected for the first time in this study. However, in the N-terminal region of the CP, the most variable region, central Europe isolates harbor a landmark conserved motif of N<sub>16</sub>N<sub>17</sub>A<sub>27</sub>M<sub>37</sub>. This motif was detected in the ZYMV-Varamin isolate but not in other Iranian isolates, suggesting that this isolate might have been imported from central Europe by means of infected cucurbits seeds.

### Key Words

Melon, phylogeny, ELISA, taxonomy, aphid transmission *Tanacetum balsamita*

## **Callus induction and regeneration of bread wheat lines from coleoptile explants**

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### **A B S T R A C T**

**O**ptimization of wheat tissue culture is essential for any gene transfer process and for generation of cell suspension lines. To optimize tissue culture conditions for four bread wheat lines (C-D-4, C-D-6, C-D-8, and C-D-9), several factorial experiments based on completely randomized design with 3 replications were conducted. Callus induction potential and regeneration of lines under different hormone treatments were evaluated. For callus induction, ML1G1 medium containing three levels of 2, 4-D (2, 2.4 and 3 mg/l) and for regeneration, MS and N6 medium including different levels of NAA, BAP and Kin was used. With respect to the effect of hormones on the different wheat lines, the results showed that the highest callus induction rate was achieved with C-D-9(82%) and the highest rate of callus induction (60%) was recorded in 2.4 mg/l 2, 4-D for all lines. With respect to embryogenic callus production, ML1C2 media containing 250mg/l AgNO<sub>3</sub> produced the highest embryogenic callus percentage in C-D-9 and C-D-8 lines. The highest regeneration rate (29.62%) for C-D-9 lines was obtained in N6 (6) medium containing 1 mg/l IAA + 1 mg/l BA.

### **Key Words**

2,4-D, coleoptile segment, tissue culture and wheat

## Characterization and sequence of a hydrogen/sodium anti-porter gene in the plasma membrane of the plant *Kochia scoparia*

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### ABSTRACT

**S**odium efflux is one way to reducing of cytosolic sodium in plants. This prevents toxic effects of sodium on cytosol cellular processes. Sodium/hydrogen transporters in the plasma membrane (SOS<sub>1</sub>) are one of the best known proteins in this process. In this study *Kochia scoparia*, a halophyte dicotyledonous plant was used as a source to gene isolation. Using designed primers based on conserved regions of other plants, a gene coding sequence of approximate 3600 nucleotides and 1200 amino acids was identified and sequenced. Analysis with BLAST verified that maximum nucleotide homology of the sequence with other SOS<sub>1</sub> proteins was 84%, and in amino acid homology 92%. Additional *in silico* analysis was performed to characterize the putative protein and its relation with other SOS<sub>1</sub> proteins, which contain a hydrophilic alpha helix region as passenger fragment through plasma membrane and in contrast to this, a sequence with less alpha helix and hydrophilic properties, is as protein intracellular fragment. These results support the role of sodium efflux for resistance response to salinity in *Kochia* using plasma membrane transporters.

### Key Words

Gene isolation, *Kochia scoparia*, Plasma membrane Transporters, Salinity

## Evaluation of three reference genes for real-time PCR normalization in wheat roots under salt stress

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### ABSTRACT

Gene expression studies by real-time PCR constitute a powerful tool to analyze the mechanisms underlying plant abiotic-stress tolerance. One of the crucial steps of this technique is the selection and validation of reference genes to normalize target gene expression under different stress conditions. In an attempt to find the best reference genes for wheat root salinity research, we evaluated the stability of the three most commonly used reference genes (*GAPDH*, *Actin* and *Ta.22845*) using the NormFinder, BestKeeper statistical algorithms and the comparative  $\Delta\text{CT}$  method. The results indicated that *Actin* was the most suitable reference gene for gene expression normalization under salinity treatment in wheat root.

### Key Words

Wheat, Salinity stress, Housekeeping genes, Internal control, Gene expression.

## Optimization of *Agrobacterium tumefaciens*-mediated transformation of Barley and Production of Fertile Transgenic Plants

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### ABSTRACT

In recent years, gene transfer to cereal crops has become an important tool for improving agronomic traits. Barley is one of the most important cereals and like other crops its modification by genetic transformation is an important method for increasing tolerance to biotic and abiotic stresses. Optimization of the various factors that can influence transformation and regeneration of the target plant is the first step in the gene transfer. In this research, some of the factors influencing transformation of barley with *Agrobacterium* such as the *Agrobacterium* strain, bacterial concentration, co-cultivation period and *Agrobacterium* infection medium were investigated. To confirm the presence of possible transferred genes in the transgenic plants, a PCR test was done and expression of the transferred gene was carried out using a GFP gene expression test. Regeneration of transgenic explants was carried out successfully and fertile transgenic plants were obtained. The results showed that in the transformation of immature embryo explants of barley, the LBA4404 and AGL1 *Agrobacterium* strains were the most effective strain and a concentration of OD<sub>600</sub>=1 was the optimal bacterial concentration. Also, the usage of LB as the inoculation medium and a 2-days co-cultivation period of explants with *Agrobacterium* resulted in maximum transformation of barley.

### Key Words

*Agrobacterium*, Barley, GFP, Hygromycin, Transformation.

## Effect of Pirimicarb and Thiamethoxam on detoxification enzyme activity in the black bean aphid, *Aphis fabae* Scopoli (Hem: Aphididae)

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### ABSTRACT

In many organisms, detoxification enzymes play important roles in detoxification of xenobiotics and are considered as biomarkers of toxic effects of exposure to a variety of these agents. In the current study, the effects of thiamethoxam and pirimicarb in sublethal concentrations were evaluated on *Aphis fabae* Scopoli (Hemiptera: Aphididae) detoxification enzymes. The median lethal concentrations for the female aphids exposed to thiamethoxam and pirimicarb were 113.85 (68.94-625.16) and 2.94 (1.71-3.99) mg (ai)/L, respectively. The activity of monooxygenases was measured as detoxification enzymes. The effects of both insecticides on *A. fabae* caused significant increase in monooxygenase ( $P < 0.0001$ ). Glutathione-s-transferase (GST) was likewise induced in *A. fabae* by increasing the sublethal concentrations of pirimicarb ( $df=5, 12, F= 18.17, P= 0.0001$ ). Finally, thiamethoxam in higher concentrations (48.07, 75.06 and 113.85 mg (ai)/L) induced this enzyme ( $df=5, 12, F= 9.15, P= 0.0009$ ). The results illustrate that two important aphicides, thiamethoxam and pirimicarb in sublethal concentrations can be detected by detoxification enzymes as biochemical markers. Consequently, prediction of poisonous effects on the aphid populations in the field will be possible.

### Key Words

*Aphis fabae*, pirimicarb, thiamethoxam, detoxification enzymes, biomarkers



## Effect of salicylic acid and phenylalanine on expression of key genes involved in the sesamin biosynthesis pathway in sesame

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### ABSTRACT

**S**esame (*Sesamum indicum* L.) is not only a source of manganese and copper, but is also rich in calcium, magnesium, iron, phosphorus, vitamin B1, zinc and dietary fiber. Sesame contains a group of fibers called Lignans, including Sesamin and Sesamolol, which are especially useful as they decrease cholesterol in the human body. Due to its use in medical treatments, especially in cancer cases, recent decades have seen numerous efforts aimed at a better understanding of Sesamin production and the genes underlying its biosynthesis, with the aim of enhancing expression of genes involved in this pathway. In this study, the effects of salicylic acid and phenylalanine on the expression level of two key genes, *CYP81Q1* and *C3H*, involved in the Sesamin biosynthesis pathway were evaluated using sesame cell suspension culture and qRT-PCR. For this purpose, sesame cell suspensions were treated using 0.1, 0.5 and 1 mg/l salicylic acid and 0.1 mg/l phenylalanine. Sampling was carried out 24, 48 and 72 hours after treatment. Gene expression analysis was performed by the qRT-PCR technique. The results showed that expression of genes involved in Sesamin biosynthesis increased in all the samples treated with salicylic acid and phenylalanine. The highest increase in expression level of *CYP81Q1* occurred in 1 mg/l of salicylic acid 72 h and 0.1 mg/l of phenylalanine 72 h after treatment. The highest increase in expression level of the *C3H* gene occurred in 0.1 mg/l of salicylic acid 24 h and 0.1 mg/l of phenylalanine 72 h after treatment.

### Key Words

Sesame, Sesamin, salicylic acid, phenylalanine, qRT-PCR

## ***In vitro* regeneration of red nightshade from different explants and evaluation of gene transfer using a biolistic gun**

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### **A B S T R A C T**

**R**ed nightshade (*Solanum alatum* Moench.) is one of the important medicinal plants from the *Solanaceae* family. Tissue culture technology is a very useful application of biotechnology which can be used for plant preservation, rapid multiplication and in genetic engineering for molecular breeding or improving secondary metabolite production. In this study, plant regeneration from three different explant sources derived from young seedlings, including hypocotyl, coleoptile and leaf segments, was evaluated. In this research, efficient protocols for regeneration of red nightshade from all three explants are reported. The best shoot regeneration response (100%) was obtained at 0.5 mg/l BAP concentration for leaf explants. Regenerated plants adapted to greenhouse conditions with a high efficiency (100%) and produced normal flowers and seeds. Also, leaf explant was found to be the most efficient explants for plantlet regeneration, with a mean number of 29.93 shoots per explant. Finally, the potential of leaf explants for gene transfer and expression by means of a gene gun (at two rupture disk pressure of 1100 and 1350 psi), was evaluated using transient GUS expression, and leaf explants were found to be suitable for this purpose.

### **Key Words**

Red nightshade, Micro-propagation, *in vitro* regeneration, *Solanum alatum*

## **Genetic analysis of the cytochrome b sequence in Khazak native chickens of Sistan**

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### **A B S T R A C T**

**F**or conservation of indigenous poultry genetic resources and their use as genetic material in poultry breeding programs, it is necessary to obtain accurate genetic knowledge about these animals. The aim of this study was to investigate the genetic and phylogenetic characteristics of Khazak native chicken of Sistan using the nucleotide sequence of mitochondrial cytochrome b. For this purpose, blood samples were collected from 20 Khazak native chickens of Sistan. After DNA extraction, an 864 bp fragment of cytochrome b of mitochondrial genome was amplified by primers. The amplified fragments were purified and sequenced. A total of five different haplotypes were determined based on three single nucleotide polymorphism sequences. The final sequences from each haplotype with 789 bp in length were obtained, including 26.37% of adenine, 13.31% of guanine, 36.50% of cytosine, and 23.82% of thymine. The results indicated that the Khazak native chicken of Sistan has the lowest genetic distance from Japanese and Chinese native chickens.

### **Key Words**

Phylogeny, Cytochrome b, Khazak native chicken of Sistan, Genome Mitochondrial

## Identification, isolation and sequence analysis of a $\beta$ -Conglycinin seed-specific promoter

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### ABSTRACT

Promoter sequences are one of the key factors in directed gene expression. Different types of promoters can be used according to the type of tissue or expression levels desired.  $\beta$ -Conglycinin is one of the major storage proteins in soybean seed embryos and is produced in the seed development stage. The synthesis of storage proteins is primarily controlled at the transcriptional level and seed-specific expression has been shown to be conferred upon the promoter regions of many storage protein genes. In this research, a seed specific promoter ( $\beta$ -Conglycinin) of Iranian *Glycin max* was isolated from genomic DNA. Then, the promoter was cloned into pTZ57R/T and sequenced. Sequence analysis showed that the cloned promoter contained all of the typical conserved motifs such as TATA box, CAAT box as well as other previously identified seed-specific motifs such as SKn-1, RY repeats, G box. Finally, the  $\beta$ -Conglycinin promoter was cloned into pBI121 binary vector for further research.

### Key Words

Promoter, Soybean,  $\beta$ -Conglycinin, Motif, Signal peptide

## **The application of plant viral vectors in biotechnology**

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### **A B S T R A C T**

**O**ver the past 38 years, there has been a re-orientation of virology studies toward the beneficial use of viruses, and several plant viruses have been employed as expression vectors. The use of plant virus vectors for production of recombinant proteins has advantages such as speed of expression, high yield, reduced cost and duration of studies, and an extremely high throughput of recombinant proteins. Thus with such vectors large scale production of recombinant proteins required for pharmaceutical and industrial applications is accessible. Plant viruses can also be used for improved vaccine efficacy. Such vaccines can be made by ligand conjugation of a non-immunogenic peptide to the outer surface of the virus coat protein. Finally, plant virus vectors can be employed as library construction tools for discovery of gene function in genomics studies. Here, we review the use of plant viruses in expression of recombinant proteins.

### **Key Words**

Gene Functions, Plant Viruses, Recombinant Proteins

## **Applications of plant viruses in bionanotechnology**

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### **A B S T R A C T**

**T**he emphasis on viruses as disease-causing agents has skewed scientists' perception of other aspects of viruses. In recent years, there has been a re-orientation of virus studies toward the beneficial use of viruses, independent of their disease-causing phenotypes. For example, plant viral capsids have been utilized as biotemplates, drug vectors and nanodevices in many practical applications. By studying the morphology, genetics, spatial folding, replication and assembly strategy of viral capsids, we can manipulate the viral genome and viral proteins for in various fields. At present one of the most important uses of plant viruses is in nanomedicine, where they can be employed in diagnosis and therapy. Their low toxicity for humans and their specific effects are the main advantages of using plant viral nanoparticles. This review discusses applications of plant viruses in bionanotechnology.

### **Key Words**

Bionanotechnology, biotemplate, capsid, nanomedicine, plant viruses, viral nanoparticles

## **The simultaneous progress of conventional breeding and biotechnology for sugar beet improvement**

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### **ABSTRACT**

**S**ugar beet is one of the most important agricultural products which currently provides about 20% of the sugar around the world. With classical breeding methods, good traits can be introduced in sugar beet breeding programs but it can take years to find the perfect cross. Genetic engineering (precision breeding) is the most advanced plant breeding method that is currently used. In the last two decades, this method has been applied in sugar beet breeding for resistance to pests and disease, abiotic stress, and also by-product production. Genetic engineering has not only elevated the efficiency of breeding programs efficiency but also in some cases, such as introducing resistance in sugar beet to herbicide application, significantly reduced costs. The present study will describe recent progress in the application of genetic engineering in sugar beet improvement.

### **Key Words**

Breeding, genetic engineering, sugar beet