Genetic Engineering and Biosafety Journal
Vol4, Number1, Spring and summer 2015, Bi-seasonal

Reduction of applied pesticides and cancer with the cultivation of transgenic crops

Solmaz Khosravi[1] and Masoud Tohidfar[2]*

1. Agricultural research institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

2. Department of Biotechnology, Faculty of New Technologies and Energy Engineering, Shahid Beheshti University, Tehran, Iran

* Corresponding Author, Email M_Tohidfar@sbu.ac.ir

A B S T R A C T

Pests are considered as one of the most important constraints that cause production loss. Although use of pesticides is the most common solution nowadays for pest control in agriculture, this approach has many harmful effects for human health and the environment. Up to now, almost 50 lung cancers provoked by agricultural pesticides have been recognized. Furthermore, pesticide residues can remain in the soil for a long time and can represent a threat for microbial life and can be absorbed through the root system of plants and enter the human food chain. Although strategies like organic agriculture or integrated pest management have been promulgated, the correct use of pesticides is far from having been achieved. However, new technologies like genetic engineering can help overcome the problem of yield loss while still providing healthy GM food with no pesticide residue. Since the first cultivation of GM crops, 18 years have now passed and GM crops have gained significant public acceptance among farmers and consumers. In this review, disadvantages of pesticides on human health (especially cancer) and environment will be discussed.

Key Words

Cancer, environment, GM crops, Pesticides

Genetic Engineering and Biosafety Journal

Vol4, Number1, Spring and summer 2015, Bi-seasonal

Cloning and investigation of *RGT2* gene charecteristics from an Iranian strain of *Saccharomyces cerevisiae*

Azizi[1] S. and A.R. Tarinejad*[2]

1. MSc. in Agriculture Biotechnology Department, Faculty of Agriculture, Azarbaijan Shahid Madani University

2. Department of agriculture Biotechnology, Faculty of agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran.

* Corresponding Author, Email: atarinejad@yahoo.com

A B S T R A C T

The yeast *Saccharomyces cerevisiae* has 20 genes that encode Hexose Transporter proteins, including *HXT1-HXT17*, *GAL2*, *SNF3* and *RGT2*. Two of these genes (*SNF3* and *RGT2*) act as glucose sensors while the *HXT1-HXT17* genes function in direct transportation of glucose. Earlier research has shown that alcohol fermentation can be augmented by increasing the expression of these genes, resulting in increasing ethanol production. The aim of this study was the identification and isolation of the Restores Glucose Transport 2 (*RGT2*) gene from *Saccharomyces cerevisiae* genome. Specific primers were employed in PCR so as to clone *RGT2* into a vector under a suitable expression promoter for recombinant yeast. After gene amplification, ligation was achieved between the amplified fragments and pGEM-T vector and the recombinant colonies were identified by the blue-white screening method. Candidate recombinant plasmids were sequenced. The nucleotide sequence of the open reading frame was found to be 2292 bp long with a deduced amino acid of 763 residues. The estimated molecular mass and the predicted isoelectric point of the deduced polypeptide were 83.173 kDa and 5.68 respectively. The deduced protein sequence showed a high similarity to *RGT2* sequences in the NCBI database, especially with P301 strain of *Saccharomyces cerevisiae* (100 % similarity). Finally, the *RGT2* gene was cloned into the pGBKT7 expression vector which is suitable for protein expression in yeast via the restriction sites *Nco*I and *Pst*I. A phylogenic study of the *RGT2* gene and other hexose transporter families showed that this gene has the most similarity with *SNF3*. Therefore, by isolation, cloning and sequence identification and transformation of this gene into yeast, ethanol production via alcohol fermentation can be increased.

Key Words

Cloning, *RGT2*, Fermentation, *Saccharomyces cerevisiae*

Genetic Engineering and Biosafety Journal
Vol4, Number1, Spring and summer 2015, Bi-seasonal

The Effect of Nano Cobalt and Nano Chitosan on Artemisinin production and expression of SQS and DBR2 genes in *Artemisia annua*

Bita Ghasemi [1], Ramin Hosseini [2], Fatemeh Dehghan Nayeri [3]

1- MSc in Agricultural Biotechnology, 2- Associate professor, 3- Assistant Professor, Agricultural Biotechnology Department, Imam Khomeini International University, Gazvin, Iran

*Corresponding Author, Email: raminh_2001@yahoo.com

A B S T R A C T

Artemisia annua is particularly important for the production of artemisinin, a bioproduct which can be used to combat the causal agent of malaria, treat some kind of cancers and in other activities. The low artemisinin content in the plant has caused this compound to be among the more expensive medicines. Several attempts have been made to increase artemisinin production, for example by using different elicitors, but none of the approaches has been cost effective. In this study, the expression levels of two important genes in the artemisinin biosynthetic pathway, *SQS* and *DBR2* and artemisinin content were investigated in *Artemisia* cell suspension cultures. SQS and DBR2 genes have essential roles in the regulation of artemisinin pathway. For this purpose, nano-cobalt particles in concentrations of 0.25, 2.5 and 5 mg/L were used for cell culture treatment and samples were collected after 8, 24, 48 and 72 h. The highest artemisinin content was observed 24 h after 5 mg/L nanocobalt treatment. In this case, artemisinin production was 2.25 times (113.35 mg/g d.wt) higher than that of the control. Our results showed a negative and significant correlation between *SQS* and *DBR2* gene expression and artemisinin content at different levels of nano cobalt treatments. Results also showed an increase in nano cobalt concentration after 72 hour and an increase in nano chitosan after 4h hour caused a significant decrease in the expression of *SQS* and *DBR2* genes. In conclusion, it appears that the content of artemisinin was increased by high concentrations of the nano cobalt particles because of a decrease in the expression of *SQS* and *DBR2* genes.

Key Words

Artemisia annua, Artemisinin, Nano Cobalt, Nano Chitosan, HPLC, qRT-PCR,

Genetic Engineering and Biosafety Journal

Vol4, Number1, Spring and summer 2015, Bi-seasonal

Identification and classification of the WRKY transcription factors family in barley

Beniamin Yazdani[1], Rasoul Asghari-Zakaria[2], Zahra-Sadat Shobbar[3*]

1. Ph.D. Student and 2. Associate Professor, Department of Agricultural Science, University of Mohaghegh Ardabili, Ardabil, Iran
3. Assistant Professor, Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

*Corresponding Author, Email: shobbar@abrii.ac.ir

A B S T R A C T

Biotic and abiotic stresses are the most important constraints on production by crop plants, including barley. Transcription factors are involved in the regulation of biotic and abiotic stress- response genes and the WRKY transcription factor family encodes a large group of them. Therefore, identification and classification of these factors represent important steps in our quest to find smart strategies for enhancing stress tolerance in plants. In an attempt to identify WRKY transcription factors in barley, multiple searches were done in Plant TFDB and Gramineae TFDB databases. Rice WRKY-conserved sequences were used as the templates for tBLASTN searches in the nr, EST and HTGS datasets for finding new members in barley. An HMM search was used to find sequences containing WRKY conserved domains. The identified 96 HvWRKYs as well as one member of each WRKY subgroup from Arabidopsis, rice and wheat were subjected to multiple alignment using clustalx software and phylogenetic trees were reconstructed using MEGA6 software based on neighbor-joining method with a 1000 repeats bootstrap index. Sequences were divided into 3 groups based on the number of WRKY domains and the structure of zinc-finger motifs. Conclusively, there were 13 proteins with 2 WRKY conserved domain in group I, 30 proteins with 1 WRKY conserved domain and $Cx_7Cx_{23}HxC$ zinc-finger motif in group III and other proteins with 1 WRKY conserved domain and $Cx_{4-5}Cx_{22-23}HxH$ zinc-finger motif in group II. Regarding the role of group III in plant tolerance to abiotic and biotic stresses, it can be argued that the higher percentage presence of group III members in barley that are similar to rice than to other higher plants can be attributed to duplications in wild monocotyledous ancestors and natural selection for more resistant genotypes in harsh conditions.

Key Words

Abiotic stresses, Transcription factors, HMM, Phylogenetic tree, Multiple alignment.

Genetic Engineering and Biosafety Journal
Vol4, Number1, Spring and summer 2015, Bi-seasonal

Study of Hydrogen Cyanide Effects on Salt Stress Induction in *Aeluropus littoralis*

Ahmadi N. Milad [1]*, Askari H[1].,Soltani N. Masood[2]

1. Department of Biotechnology, Faculty of New Technologies and Energy Engineering, Shahid Beheshti University, Tehran, Iran.

2. Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

* Corresponding Author, Email: milad.ah2005@gmail.com

A B S T R A C T

Salt stress is a serious problem for plant growth and development. To analyze salt stress resistance and physiological behavior of plants, the halophyte *Aeluropus littoralis* was studied. Salt stress augments ethylene hormone production in plant tissue and this leads to increased hydrogen cyanide levels. In the other hand, there is a cyanide purge mechanism involving three enzymes: Cyanase, Rhodanese and β-cyanoalanine synthase. To study plant cell growth and development under salt stress conditions, an analysis of differential expression of genes involved in biosynthesis and purge of cyanide is needed. In this study, *Aeluropus littoralis* cell suspensions were subjected to different concentrations of salt and potassium cyanide in the medium. Factorial analysis of NaCl and KCN in 0, 0/0.2, 0/0.4, 60/0, 60/0.2, 60/0.4, 120/0, 120/0.2 and 120/0.4 mM concentrations were assessed. Our study demonstrated that KCN treatment significantly reduced production of dry material. The results showed that, although cyanide has negative effect on cell growth, the cyanide detoxification gene network was not activated in these conditions. In addition, the interaction between cyanide and salinity indicated that salt stress in the presence of 0.4 mM KCN increases cell growth by 40 percent because expression of the CAS gene was reduced enormously. An increase of salinity in the presence of 0.2 mM KCN, however, reduced expression of ACO, a key gene in HCN and ethylene production. As intracellular level of HCN declined, cell growth rose. Thus external treatment of cyanide increases plant dry material and plant resistance in salt stress conditions.

Key Words

Aeluropus littoralis, Ethylene,Salt stress, Hydrogen Cyanide, qRT-PCR

Genetic Engineering and Biosafety Journal
Vol4, Number1, Spring and summer 2015, Bi-seasonal

Proteomic Analysis of Spring Barley Leaves Under Short Term Cold Stress

Rana valizadeh kamran[1,2], Mahmood Toorchi[1]*, Mohammad Moghaddam[1], Hamid Mohammadi[3]

1. Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
2. Department of Biotechnology and 3. Department of Agronomy - Faculty of Agriculture Azarbaijan Shahid Madani University, km35 Tabriz-Maragheh Road

* Corresponding Author, Email: mtoorchi@tabrizu.ac.ir

ABSTRACT

Cold is one of the most significant abiotic stresses which restrict crop growth and productivity worldwide. In order to investigate how spring barley (*Hordeum vulgare* L.) seedlings adapt to short-term periods of low temperature, the present study explored proteomic changes in leaves. Cold stress at 4 °C was applied to barley seedlings for 48 hours; third leaves were harvested and compared with seedlings grown in normal conditions (25° C). The proteomic analysis was conducted by two-dimensional electrophoresis (2-DE) and the Coomassie blue staining procedure. Fifteen reproducible protein spots showing a significant difference between the control condition and cold stress were identified; 10 of the spots demonstrated an increase in expression while 5 spots showed a decrease under 4 °C cold stress for 48 hours. By applying MALDI-TOF analysis, 7 spots were identified. These responsive proteins were involved in the Calvin cycle, photosynthetic electron transport, light reaction, and signal transduction. The upregulation of proteins involved in the regulation of the chloroplast system, the integrity of chloroplasts, energy metabolism, antioxidant defense, and photosynthesis has probably acclimatized the plant to cold stress. These findings indicate that there was greater cold stress affecting photosynthesis in spring barley and it is of crucial importance to maintain the efficiency of photosynthesis under cold stress.

Key Words

Barley, Cold, Proteome, 2DE, MALDI-TOF