

The two dimensional electrophoresis irfan sadiq biology essay

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With the increase in world population there has been increasing demand of food. However crop production is not increasing at the same rate as population because of environmental stresses, which negatively affect plant growth and productivity. To overcome negative effects of stresses plants adapt themselves according to the conditions by changing gene expression at transcriptional, posttranscriptional or posttranslational level. Detailed studies of these changes can help in development of stress resistant crop varieties. Wheat is an important staple food in many parts of the world and with the increase in world population there is desire to increase its production to meet the increasing global demands. Decreased availability of water resources have resulted in drought stress which have resulted in decreased production of wheat. There is desire to identify genes and proteins which play important roles in drought stress tolerance in wheat. This information could be used to develop better crop varieties which can cope with these unfavorable conditions. Use of wild relatives of domesticated crops could play an important role in identification of stress responsive genes and proteins. Wild emmer wheat (*T. diccoides*), which is believed to be ancestor of domesticated durum and bread wheats (*T. durum* and *T. aestivum*) could provide important information in this case. Next-generation sequencing (NGS) technologies have revolutionized biology and are applied to address critical issues in plant biology. NGS is becoming increasingly popular for transcriptome studies due to the power of vast read depth and read pair technology. Deep sequence coverage is important for gene discovery and gene expression analysis. There are very few studies conducted to study gene expression analysis using NGS in wheat. The

present study will help to dissect signaling mechanisms in wild emmer and durum wheat in response to drought stress. Proteomic study will help to see the posttranscriptional regulation of differentially expressed genes at protein level. The information obtained by this study will help deep insight into regulatory mechanisms in response to drought stress which will benefit plant breeders to generate new varieties which are tolerant to drought stress.

Objectives

To study drought responsive transcriptome expression profile by Next Generation Sequencing
To study drought responsive proteomic profile of emmer and durum wheat

Experimental Methodology

Plant material and stress treatment

Triticum turgidum ssp. *dicocoides* genotype TR39477, along with modern durum wheat variety *T. turgidum* ssp. Kızıltan are selected for this study based on the previous findings of Ergen and Budak, 2009. Drought stress treatment will be applied as previously described by Budak et al. (2013). Seeds of both genotypes will be surface sterilized in 1 % sodium hypochlorite and will be pre-germinated in petri dishes. Germinated seedlings of similar growth will be transferred to pots that contain 3: 2 clay: sand mixture supplemented with 200 ppm N, 2. 5 ppm Fe, 100 ppm P, 20 ppm S and 2 ppm Zn. Plants will be grown under controlled conditions: 10-12 h photoperiod, $25 \pm 3^{\circ}\text{C}$ temperature. Drought stress treatment will be applied after four weeks of normal growth by withholding water for nine days, while

control plants will be continuously irrigated. After 9-day-drought treatment leaf and root samples will be collected and stored at -80°C

RNA Extraction

Total RNA will be isolated from all tissue samples using Trizol reagent (Invitrogen) according to manufacturer's instructions. The quality and concentration of isolated RNAs will be measured using a Nanodrop ND-100 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The integrity of the isolated RNA will be assessed by running on 2% agarose gel. Contaminants including polysaccharides, polyphenols will be removed from freshly extracted RNA by LiCl precipitation (An et al., 2011).

Construction of cDNA library and Illumina deep-sequencing

The cDNA libraries will be constructed by following Xu et al. (2012). Briefly cDNA libraries will be prepared by following the Illumina mRNA-Seq assay for paired-end transcriptome (Illumina Inc., San Diego, CA). mRNA will be enriched from 20 µg total RNA using polyA selection and then cleaved into 200-700 nt fragments by incubation with RNA Fragmentation Reagent. The fragmented mRNA will be converted into double-stranded cDNA by priming with random hexamer primers, purified with a QiaQuick PCR extraction kit (QIAGEN Inc., Valencia, CA, USA), and then washed with EB buffer for end repairing and single nucleotide adenine addition. Finally, sequencing adaptors will be ligated onto the fragments, and the required fragments will be purified by agarose gel electrophoresis and enriched by PCR amplification to construct the cDNA library. Each cDNA library will be loaded as an independent lane on a Genome Analyzer II (Illumina, San Diego, CA) which

will be used to obtain more detailed information about gene expression. The data sets will be made available at NCBI SRA database.

De novo assembly and sequence clustering

The raw data will be cleaned by filtering out adaptor only reads, reads containing more than 5% unknown nucleotides, and low quality reads. De novo assembly of the clean reads will be performed to generate non-redundant unigenes using SOAPdenovo and the Trinity methods. Sequence directions of the resulting unigenes will be determined by performing BLASTX searches against protein databases, with the priority order of NR (non-redundant protein sequences in NCBI), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes database (KEGG), and COG.

Calculation of RPKM values and the detection of differentially-expressed transcripts

The expression levels of unigenes will be calculated as the number of clean reads mapped to its sequence. The number of clean reads mapped to each annotated unigene will be calculated and then normalized to RPKM (reads per Kb per million reads) with ERANGE3.1 software and adjusted by a normalized factor

qPCR Validation of differentially expressed genes

RT-qPCR analysis will be conducted of some selective genes to validate differential expression observed by RNA-seq data in response to drought stress. Three independent biological replicates will be used for control and treated samples.

Functional categorization of unigenes

The Gene Ontology terms assigned to the differentially expressed transcripts will be obtained from InterProScan 5 Release Candidate 2 (<http://code.google.com/p/interproscan/>)

Proteomics

Protein extraction and Quantification

Soluble proteins will be extracted from leaves and roots according to Sadiq et al. (2011). Tissue samples will be grind in liquid nitrogen and homogenized with 1 mL of extraction buffer (5M urea, 2 M thiourea, 40 mM Tris-HCl, 2%, CHAPS, 50 mM DTT). The homogenates will be centrifuged for 15 min at 15, 000 x g. Supernatants will be precipitated using TCA (15%, v/v) containing 0. 007% β -mercaptoethanol in acetone at -20°C for 2 h and then at 4°C for a minimum of 2 h. Samples will be centrifuged at 4°C for 15 min at 14, 000 x g, supernatants will be discarded and pellets will be washed twice with ice cold acetone containing 0. 007% β -mercaptoethanol. Pellets will be dissolved in a rehydration buffer (5 M urea, 2 M thiourea, 4%, CHAPS, 40 mM DTT). Protein quantification will be performed using a Bradford-based assay kit assay (BioRad Hercules, CA), using bovine serum albumin as a standard.

2-D Electrophoresis

Isoelectric focusing (IEF) of total proteins will performed using immobilized pH gradient (IPG) strips of varying pH and lengths. The protein sample will be mixed with a rehydration buffer, 0. 5% IPG buffer (v/v) of respective pH range and 0. 002% bromophenol blue and loaded onto the IPG strips by passive rehydration. For analytical gels (silver stained), 100 μ g of the protein

sample will be loaded. For the preparative gels (Coomassie brilliant blue (CBB) staining) 1 mg of the sample will be loaded. IEF will be carried out according to Sadiq et al. (2011). Before running the second dimension SDS-PAGE, IPG strips will be equilibrated twice in an equilibration buffer (6 M urea, 30% glycerol (v/v), 50 mM Tris-HCl, 2% SDS for 15 min. The first equilibration will be carried out using 1. 2% DTT (w/v) in an equilibration buffer, while in the second equilibration; DTT will be replaced by 1. 5% iodoacetamide (w/v). SDS-PAGE will be performed using 12. 5% polyacrylamide gels at 15°C. After completion of the electrophoresis, gels will be fixed and stained. The analytical gels will be stained for image analysis with silver nitrate as described by Oakley et al. (1980), while for the MS analysis; the preparative gels will be stained with CBB according to the manufacturer's instructions. Three independent biological replicates, each with three technical replicates will be run for the analytical gels.

Image Analysis

The silver stained 2DE analytical gels will be scanned at 300 dpi resolution and will be saved as TIF images for image analysis. Two-dimensional image analysis will be performed using the Proteomweaver software (Definiens AG, Munich, Germany) to detect differentially expressed protein spots on 2DE gels.

Trypsin digestion

Protein spots showing differential expression will be excised from preparative gels and will be reduced, alkylated, and in-gel digested overnight with bovine trypsin (Roche Diagnostics Corp.) as previously described (Shevchenko et al.,

1996). In-gel digested protein spots will be used for the analysis. After trapping and desalting the peptides on an enrichment column using 1 % acetonitrile, 0.5 % formic acid solution for 5 min, peptides will be separated on a Zorbax 300 SB C18, 75µm x 150 mm column (Agilent) using an acetonitrile/0.1 % formic acid gradient from 5 to 40 % acetonitril for 40 min. MS spectra will be acquired by Esquire 3000 plus according to manufacturer's instrument settings for nanoLC-ESI-MS/MS analyses. In search parameters ion charge for ions from ESI-MS/MS data acquisition will be set to "+1, +2 or +3" according to the instrument's and method's common charge state distribution. Peak lists will be searched via Mascot Daemon 2.2.2, first in a custom (trypsin and common keratins) contaminant database derived from the publicly available cRAP repository. Unmatched signals will then be searched in NCBI (National Center for Biotechnology Information, Bethesda, USA). Functional classification of identified proteins will be performed with the Clusters of Orthologous Groups of proteins (COG) tool of NCBI (<http://www.ncbi.nlm.nih.gov/COG/>).