



Comparative analysis of the drought-responsive transcriptome in soybean lines contrasting for canopy wilting

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ABSTRACT

Drought stress causes significant yield losses in major oil seed crops, such as soybean [*Glycine max* (L.) Merr]. Few soybean lines have been identified as canopy-wilting tolerant; however, the molecular mechanism conferring tolerance is not fully understood. To understand the biological process, a whole genome transcriptome analysis was performed for leaf tissues of two contrasting soybean lines: drought-susceptible (DS) Pana and drought-tolerant (DT) PI 567690. A pairwise comparison of the DS and DT lines under drought and control conditions detected 1914 and 670 genes with a greater than two-fold change in expression under drought conditions. Pairwise treatment comparison and gene enrichment analysis on the DT line showed the down-regulation of genes associated with protein binding, hydrolase activity, carbohydrate/lipid metabolism, xyloglucan endo-transglycosylases associated with cell-wall, apoplast, and chlorophyll a/b binding proteins. On the other hand, genes that were associated with the biotic stress response, ion binding and transport, the oxido-reductive process and electron carrier activity were up-regulated. Gene enrichment analysis detected UDP glucuronosyl transferase activity-encoding genes to be differentially expressed in PI 567690 under drought stress conditions. We found valuable SNPs variation in aquaporin genes of the DT line that are conserved in known slower canopy-wilting lines, this should facilitate marker-assisted selection in soybeans with improved drought tolerance.

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1. Introduction

Soybeans are a major oil seed crop worldwide and an indispensable dietary source of oil, protein, macronutrients, and minerals [1]. Drought stress has devastating effects on soybean yields [2], and this situation is further exacerbated because most US soybean cultivars have a very narrow genetic base [3]. Recently soybean breeding programs have identified some exotic drought-tolerant lines with good yield potential from around the world [4]. Evaluating soybeans lines for drought tolerance is problematic and complex, as it is an unpredictable event under field conditions [5]. Canopy wilting is a visual symptom of soybean response to water deficit [6] and has been used to identify drought-tolerant lines under field conditions [3,5]. This trait is polygenic in nature and

could be affected by various attributes [7], delaying the appearance of wilting symptoms. [8]. Ries et al. [9] reported distinct combinations of key physiological traits, such as radiation-use efficiency and water-use efficiency, which result in soil moisture conservation among slow-wilting lines. The molecular mechanisms that are involved in drought/canopy-wilting tolerance remain unclear. Several studies have associated silver nitrate sensitive aquaporins with the canopy-wilting trait in soybean [10,11], but none have identified specific aquaporins that are involved in this trait. Aquaporins are small integral membrane proteins that belong to a large family of water channel proteins and help in selective water transport across cellular membranes [12]. In addition to water transport, aquaporins are involved in the uptake of nutrients and carbon and nitrogen fixation [13].

Sinclair et al. [14] proposed that soybean genotypes with slower canopy wilting and the ability to sustain nitrogen fixation under drought (NFUD) yielded better under water-limited environments. Extensive research on the effects of soil drying and nitrogen fixation in soybean [15] has shown that nitrogen fixation in soybean

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Table 1

Genes in leaves that were differentially expressed between control and drought treatments in DS and DT.

Genotype	Condition	Rep	Expressed genes (FPKM)	Average	Non-expressed genes (FPKM)	Average
Pana (DS)	Control	1	38518	41268	15657	12907
Pana	Control	2	42081		12093	
Pana	Control	3	43205		10970	
Pana	Drought	1	43817	44071	10357	10103
Pana	Drought	2	44117		10057	
Pana	Drought	3	44279		9896	
PI567690 (DT)	Control	1	43971	43997	10978	10436
PI567690	Control	2	44640		9535	
PI567690	Control	3	43380		10795	
PI567690	Drought	1	42627	43311	11547	10863
PI567690	Drought	2	43445		10729	
PI567690	Drought	3	43862		10313	

declines under drought [16,17], which ultimately affects seed yield [14,18]. Drought/canopy-wilting tolerance and nitrogen fixation efficiency under drought are correlated with nodule size and weight in soybeans [19,20]. Most of the key canopy-wilting-tolerant soybean PI lines have a high nodule size and weight that enhance the nitrogen fixing ability under drying soil conditions [19,21,22]. Libault et al. [23] reported eight fruit-weight-like genes that were associated with nodule organogenesis and that determine the nodule number and size in soybeans. However, the expression pattern and influence of these genes on the maintenance of nodules under drought stress have not been reported so far. Recently, canopy-wilting QTLs [8] and nodule-related traits [24] were mapped in the recombinant inbred line (RIL) that was developed between KS4895 and Jackson.

Recent advancements in sequencing technology and gene expression platforms have facilitated the discovery of genes that are associated with drought/dehydration stress in *Arabidopsis* [25], rice [26], and soybean [27]. Studies based on soybean Affymetrix gene chips provide only a limited picture of the whole genome transcriptome; however, next-generation sequencing technologies allow deeper and more robust transcriptome analyses [28]. RNA-sequencing approaches provide precise information on differential gene expression in different organisms [29,30,31]. Transcriptome studies in soybean have been performed at the tissue level [28], in response to bacteria [23], and in the apical meristem tissues [32]. Transcriptome studies (with Affymetrix/RNA sequencing technology) aimed at understanding abiotic stresses in rice utilized contrasting lines for drought stress [26,33], but most of the studies in soybean used a single genotype [27,34]. Transcriptome studies with contrasting lines will give comparative and detailed information on differentially expressed genes (DEGs) that could help identify useful genes that are involved in drought tolerance. A recent transcriptome study with contrasting soybean lines (Benning × PI416937) enabled the derivation of a deeper understanding of the drought response in soybean [35]. In addition, assessing genomic differences among soybean lines with the trait of interest (canopy wilting) will enable us to understand the molecular mechanism [36] and identify genes for marker-assisted selection. Furthermore, integrating advances in -omics technologies (DNA and expression transcriptome) will enable us to hasten our understanding of complex traits and drought stress tolerance mechanisms in soybean [37,38]. In this study, a drought-tolerant and slow-wilting genotype, PI 567690, and a contrasting drought-susceptible genotype, Pana, were used to elucidate the possible molecular mechanisms underlying these complex traits. The goal of this study was to identify the candidate genes that are involved in drought/canopy-wilting tolerance under drought conditions that could be potentially used in marker-assisted selection and in the development of drought-tolerant soybean lines.

2. Materials and methods

2.1. Plant materials and drought stress treatment

This experiment was conducted in 2012 under greenhouse conditions at the Division of Plant Sciences, University of Missouri, Columbia, Missouri, in a randomized, complete block design experiment with three replications. A mixture of soil and sand (2:1) was used in 26.5 L pots (the top and bottom diameters were 30 cm and 27 cm, respectively, and the height was 37 cm). The two genotypes, cultivar Pana, selection from Jack × Asgrow A3285, and a plant introduction (PI) 567690 from China, were selected as contrasting canopy-wilting lines based on a drought study that was conducted under greenhouse [39] and field [22] conditions. Further details on growing conditions in greenhouse and phenotypic variation in the physiological and biochemical traits of these two lines under drought stress conditions can be obtained from Mutava et al. [39]. At the V5 stage [40], withdrawing water for 15 days imposed drought stress. After the stress treatment, the plants were in the R2 stage [40]. The relative water content based on the leaves that were collected from the fourth trifoliate leaf on the main stem at midday (11:00–13.30 h) was 48–59% under severe stress; these samples were collected in a 25 ml Falcon tube and flash frozen in liquid nitrogen for transcriptome analysis.

2.2. RNA isolation and qRT-PCR analysis

One hundred milligrams of leaf tissue was used for RNA extraction using the RNeasy Plant mini-kit (Qiagen, 214CA, USA) with on-column DNA digestion performed using the RNase-Free DNase Set (Qiagen, CA, USA) according to the manufacturer's protocol. Total RNA (2 µg) from each sample was reverse-transcribed to cDNA in a 20 µL reaction volume using the RNA to cDNA Ecopry™ Premix (double-primed) cDNA Synthesis Kit (Clontech, CA, USA) as per the manufacturer's protocol. Quantitative RT-PCR (qRT-PCR) was performed using the cDNA product corresponding to 25 ng of total RNA in a 10 µL reaction volume using the Maxima SYBR Green/ROX qPCR Master Mix (2×) (Thermo, USA) on a detection system (ABI 7900HT [Life Technologies, Grand Island, New York, USA]). Seventeen genes were selected from comparative drought transcriptome data to validate the RNA sequencing expression data from leaf samples (3 biological and 2 technical replicates) using qRT-PCR analysis. The PCR conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. To normalize the gene expression, Ubiquitin (Glyma20g27950) was selected as a housekeeping gene [41] because it showed no expression change between genotypes and stress treatment. All of the gene-specific primers were designed using the Primer3 web inter-

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