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Comparative transcriptomes between *viviparous1* and wildtype maize developing endosperms in response to water deficit

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ABSTRACT

Maize kernel development is particularly sensitive to water stress at the early post-pollination phase. Endosperm plays an essential role in support of embryo development and represents the bulk of carbohydrate storage in the kernel. In the present study, we compared transcriptomes of developing maize endosperms between an ABA-insensitive mutant, *viviparous1* (*vp1*), and wild type (wt) under water deficit. A total of 122 and 164 transcripts were significantly affected by water deficit in *vp1* and wt, respectively. Transcript profiles indicated that the Vp1 transcription factor contributed to regulation in response to water stress at early stages of maize endosperm development. Genes involved in transcriptional regulation and signal transduction were particularly dependent on presence of a functional Vp1 allele, as 83% the stress-affected genes in these categories were up-regulated by water stress in the wt, but only 34% were up-regulated in the mutant. This suggests that the loss of ABA sensitivity altered signaling networks in response to water deficit such that the mutant was unable to up-regulate the expression of many genes that normally play regulatory roles. Thus, the Vp1 gene plays a role in regulating transcript expression in maize endosperm development in response to water deficit, and this regulation is likely modulated via ABA signaling pathway.

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1. Introduction

Maize kernel development at the early post-pollination phase is particularly sensitive to water stress. Previous studies in maize have indicated that reproductive abortion by water stress may involve, in part, ABA response (Setter et al., 2001). ABA accumulates dramatically in both endosperm and placenta during water stress and returns to normal after rewatering (Setter et al., 2001; Wang et al., 2002; Yu and Setter, 2003). Plant response to ABA levels is largely controlled by the alteration of gene expression, and complex networks of ABA-regulated genes have been identified in plants (for reviews see Rock, 2000; Finkelstein et al., 2002). Using cDNA microarrays, we previously monitored global gene expression of developing maize endosperm and placenta/pedicel tissues under water deficit and rewatering episodes and identified 135 genes regulated by the stress (Yu and Setter, 2003).

The viviparous1 (Vp1) gene in maize is a transcription factor that provides sensitivity to abscisic acid (ABA) in developing maize kernels. In maize, Vp1 is specifically expressed in endosperm and embryo during kernel development. In well-watered plants its expression is low at early stages post-fertilization, rising to highest levels during rapid storage material accumulation (McCarty et al., 1991). The presence of ABA normally inhibits the expression of genes required for germination (such as α-amylase), while it stimulates expression of genes associated with kernel maturation such as anthocyanin-synthesis enzymes and LEA proteins (McCarty et al., 1991; Hoecker et al., 1999). Null alleles at *Vp1* result in a loss of sensitivity to ABA, leading to vivipary (precocious germination), and loss of desiccation tolerance (McCarty et al., 1989). During late embryogenesis when kernel water content declines, several genes co-regulated by ABA and Vp1 are thought to contribute to tolerance of low water potential. In addition to LEA proteins, these include heat shock proteins (Sun et al., 2001) and oxidant tolerance genes (Guan and Scandalios, 1998). The availability of a mutant for kernel-specific ABA-signaling provides a system for investigating gene regulation in kernels while stomatal closure and other ABA responses in vegetative plant parts are unaffected.

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In *Arabidopsis*, ABI3 encodes a transcription factor homologous to maize Vp1 (Koornneef et al., 1984; McCarty et al., 1991; Giraudat et al., 1992). Studies have indicated that in *Arabidopsis* and several other species, ABI3 and its homologs are involved in ABA-related expression in vegetative as well as seed tissues (Rhode et al., 2000), and it is involved in responses to water deficit in vegetative tissues (Shiota and Kamada, 2000; Zou et al., 2004). Moreover, Suzuki et al. (2003) created transgenic *Arabidopsis* expressing maize 35S:Vp1 in an abi3 null mutant background and identified 354 genes that were co-regulated by Vp1 and ABA. Most recently, Suzuki et al. (2014) characterized functions of COAR and B3 domains of maize VP1 by ectopic gene expression in *Arabidopsis*. They found that the COAR domain function of VP1 mutants that lacked B3 DNA binding activity was sufficient for complementation of the desiccation intolerant seed phenotype of abi3, and the B3 domain was dispensable for most VP1 induced gene expression and ectopic phenotypes.

In our previous study (Yu et al., 2014), we used the maize VP1 gene as an effector in the maize effector–reporter assay to see if it is involved in activating transcriptional regulation in addition to ZmZnF2, a transcription factor induced in maize kernels by water deficit. We found that GUS reporter expression from a promoter containing the ABA responsive element ABRC1 was dramatically increased 10 fold when both ZmZnF2 and VP1 were used in the assay in the presence of exogenous ABA. This suggests that the VP1 gene plays a critical role in regulating the ABA-mediated gene expression when combined with other transcription factors such as ZmZnF2.

The endosperm of maize has been extensively studied because of its contribution to embryo development, its effect on kernel appearance, and its role as the main carbohydrate storage tissue of the kernel. Transcriptomes of maize endosperm have been investigated and compared to the rice genome (Lai et al., 2004). Most recently, temporal patterns of gene expression in developing maize endosperm has been characterized using transcriptome sequencing (Li et al., 2014). Such high throughput platforms have allowed identification of gene expression patterns genome-wide.

To advance our understanding of maize kernel response to water deficit, we used the maize *vp1* mutant and compared the transcriptome of developing endosperms in this line with that of endosperms containing the wildtype (wt) allele, Vp1, under water stress using cDNA microarray analysis. Our objective was to determine the extent of involvement of the ABA response factor Vp1 in regulating global gene expression during the cell division phase of endosperm development under water stress.

2. Materials and methods

2.1. Plant material and stress treatments

To generate the wt and *vp1* mutant kernels, maternal plants were grown from F1 hybrid seeds produced by crossing inbreds W22 and ACR5855, where both of the inbred parents contributed mutant *vp1-R* alleles (Paek et al., 1998). Paternal plants were W22 homozygous for either *Vp1* (wt) or *vp1-R*. Plants were grown in a greenhouse with supplemental lighting and hourly irrigation as described by Setter et al. (2001). Ear-shoots were bagged before silk emergence. Just before pollination, silks were cut about 4 cm above the ear tip and split into two lateral bundles such that silks arising from florets on the left longitudinal half-cylinder of the ear were covered with one bag, while those on the right were covered with a second bag. Controlled pollination was performed such that florets on lateral halves of an ear were fertilized with pollen from either homozygous *vp1* pollen, thus creating *vp1*/*vp1*/*vp1* mutant endosperms (yellow), or *Vp1* pollen, thus creating *Vp1*/*vp1*/*vp1* endosperms (red–purple) which were phenotypically wild type. Treatments (control and stress) were randomly assigned to paired equivalent plants in each batch. Plants were subjected to water deficit treatment beginning at 5 days after pollination (DAP). At 5 DAP, plants were fully irrigated, allowed to drain for 1 h on the same day, and the pots containing plants and soil were weighed on scales connected to an automatic irrigation system. Irrigation was withheld until plants depleted water to a set-point of 50% of initial weight of plant + pot + soil. At this set-point, plants had reached the wilting point (e.g., leaf rolling) but leaves did not die. The automatic irrigation system added water when water level was below the set-point. The set-point was maintained for 5 days until sampling at 10 DAP. According to our previous study (Ober and Setter, 1990), leaf and kernel water potentials of stressed plants declined at 5DAP and continued to decline throughout the treatment period.

2.2. Abscisic acid and transpiration measurements

Abscisic acid was measured according to Setter et al. (2001). Briefly, maize kernels from stressed and control plants were dissected, weighed, and placed immediately in cold 80% methanol on ice. Tissues were macerated to extract ABA and stored at −20 °C. The ABA extracted from the same stressed or control plant was fractionated by C_{18} reverse-phase chromatography, and the ABA fractions were assayed by enzyme linked immunosorbant assay (Setter et al., 2001). Transpiration rate was measured using the amount of water loss (kg) per plant per day (Setter and Parra, 2010).

2.3. RNA extraction and gel blot analysis

10–24 kernels were sampled from the apical region of the ear (the upper 33% with respect to ear length). Immediately after excising each kernel, the endosperm was dissected free of embryo, nucellus and pericarp and frozen immediately in liquid nitrogen until RNA extraction. Total RNA was extracted from the same stressed or control plant using a kit that employs guanidine isothiocyanate and a silica-gel based membrane (Qiagen, CA) according to the manufacture's procedure. RNA was purified by oligo (dT) cellulose column (Life Technologies) and the purified mRNA was used for labeling with aminoallyl dUTP via first strand cDNA synthesis followed by coupling of the aminoallyl groups to either Cyanine 3 or Cyanine 5 fluorescent molecules, according to the protocol of Yang et al. (2002). For gel blot, 10 mg total RNA was loaded on the denatured gel for electrophoresis and blotted to Hybond N membrane according to Sambrook et al. (1989). The membrane was then hybridized with ^{32}P labeled cDNA probes. Ribosome RNAs stained by ethidium bromide were used as gel loading control.

2.4. Microarray processing and data analysis

Slides of the maize unigene microarray, Unigene 01-02 were obtained from the microarray laboratory of the Maize Gene Discovery project as described in the website (http://archive.maizegdb.org/microarray.php#md). This array contained about 8000 expressed sequence tags (ESTs). Our focus was on the developmental time frame of early post-pollination when the placenta and pedicel were still actively growing and differentiating, and when the endosperm was in its phase of most rapid cell division. Given that the target tissue in this study was developing endosperm, the Unigene 01-02 array was an ideal resource because it contained cDNAs from several tissue- and developmental-stage-specific libraries, including cDNAs from an immature endosperm library. Microarray slides were hybridized to labeled cDNA from endosperms of stressed and control plants according to the protocol recommended (Fernandes et al., 2002). After washing, the