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BRIEF COMMUNICATION

Transcriptional profiles of immature ears and tassels in maize at early stage of water stress

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Abstract

In the reproductive organs of maize (*Zea mays* L.), the changes in transcription that occur during meiosis at early stage of water deficit were characterized. We used oligo microarray analysis, which included 57452 transcripts representing more than 30 000 genes, and combined this with reverse Northern blot analysis. After 1 d stress, 1 809 transcripts were differentially expressed (by 2-fold or greater) with 34 % of them being upregulated in the tassels, while in the ears 861 transcripts were differentially expressed with 41 % being upregulated. Of these, 33 transcripts were upregulated in both organs, including those involved in protective functions, reactive oxygen species detoxification, nitrogen metabolism and gibberellin metabolism. In contrast, the transcripts involved in polyamine and sugar metabolism were downregulated.

Additional key words: oligo microarray, water deficit, *Zea mays*.

Water-deficit stress adversely affects plant development and crop yield. Understanding maize response to water stress requires a comprehensive evaluation of stress-induced changes in gene expression. Other than quantitative trait loci approach identifyed the loci for stress tolerance in plants (Landjeva *et al*. 2008), using microarray technology, expression profiles of maize genes were analyzed under various stress conditions recently. For example, the changes of gene expression were characterized in the roots and leaves of maize seedling under drought stress (Zheng *et al*. 2004, Jia *et al*. 2006), or under salt stress (Wang *et al*. 2003). Under water stress, placenta/pedicel and endosperm differed considerably in their transcriptional responses at 9 d after pollination: 89 % genes were upregulated in placenta/pedicel and 82 % genes were downregulated in endosperm (Yu and Setter 2003). Zinselmeier *et al*. (2002) used oligonucleotide microarray containing 1 502 genes to examine genes expression at 4 d after silking and 8 d after pollination in maize ears and kernels in response to water stress. This revealed 17 genes (10 upregulated, 7 downregulated) that were affected by stress among these tissues and demonstrated that gene expression in the pedicel was more responsive than that in other tissues. Andjelkovic and Thompson (2006) characterized the similarities and dissimilarities of gene expression in developing kernels at 15 d after pollination following water and salt stress treatments.

The period from meiosis to tetrad break-up in anthers is one of the most sensitive stages; crop yield will be caused serious reduction when the maize encounters stress at this stage (Saini 1997). Meiotic-stage stress affects the subsequent development of the male gametophyte and even induces pollen sterility (Downey 1969). However, the molecular mechanism of the response to water stress at this stage is not fully understood. In order to research the molecular mechanism, the gene expression profiles of the ears and tassels at this stage were investigated at early stage of water stress in this study. The information obtained will help the development of approaches to manipulating the genes for tolerance and yield improvement of the maize crop.

Maize (*Zea mays* L. elite inbred line DH4866) plants were grown in greenhouse in pots containing soil under 16-h photoperiod (300 µmol $m^{-2} s^{-1}$, day/night temperature of 25-28/20 °C, relative humidity 55/90 ± 5 % RH). Maize plants were withheld water when the meiosis occurred at about 1/3 of the way from the bottom of tassel. Leaf tissues

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Abbreviations: ASI - anthesis-silking interval; GA - gibberellin; GS - glutamine synthetase; Sip - seed imbibition protein.
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were frozen in liquid nitrogen and thawed to extrude sap by a glass syringe, and then osmotic potential of leaf sap was determined with a cryoscopic osmometer. The day when the osmotic potential was -0.4 to -0.5 MPa at middle part of the top fully expanded leaf of the stressed plants was regarded as the first day of water stress, and the osmotic potential was -0.2 to -0.25 MPa in the leaves of the control plants. After 1 d water stress, both the immature tassels and ears (including of rachis/cob and ovaries) were collected from at least six plants per treatment for RNA extraction. Control plants at the same developmental stage were harvested and compared to stressed plants by microscopy. All materials were frozen in liquid nitrogen before RNA extraction. Other stressed plants, at least four replicates, were given adequate water for subsequent analysis of grain formation. During subsequent growth, the anthesis-silking interval (ASI) was calculated as the mean number of days to silking minus the anthesis of individuals. Pollen viability was analyzed according to the description by Imin *et al*. (2004).

Slides of *Maize Oligonucleotide Array* version *1.9* were obtained from the microarray laboratory of the Maize Oligonucleotide Array Project at the University of Arizona (Tucson, AZ, USA). The microarray used in this study contains 57452 maize 70-mer oligonucleotides representing more than 30000 identifiable unique maize genes (details at http://www.maizearray.org).

Total RNA was isolated as McCarty (1986) described. For each sample, 100 µg total RNA was transcribed into cDNAs and fluorescently labeled with Cy3 and Cy5 dyes using the SuperScript indirect cDNA labeling system (*Invitrogen*, Carlsbad, CA, USA) according to the manufacturer's instructions. Hybridizations were performed according to the protocols at the website of the *Maize Oligonucleotide Array Project*.

Data acquisition and analysis were performed on a *GenePix 4000B* scanner with *GenePix 6.0* software (*Axon Instruments*, Foster, USA). Signal values were initially normalized during the image scanning process to adjust the average ratios between two channels. Only those spots that showed fluorescent intensity levels above the 3-fold background (local) in at least one channel were input *FiRe* software for further analysis (Garcion *et al*. 2006). Signal values for each spot on the slide were calculated, using the median intensity of pixels minus median local background for each channel. In the present study, only those transcripts with a Cy5/Cy3 ratio of more than 2-fold in each independent experiment were considered to be differentially expressed. To remove redundant transcripts we used the following criteria: *1*) transcripts with their top hit in non-redundant amino acid database are the same, and *2*) they are in the identical contig using CAP3 DNA sequence analysis program (Huang and Madan 1999).

Twenty-seven cDNA clones in the subtracted cDNA library (we sequenced randomly 1000 clones) from maize leaves under water-deficit stress were selected based on the sequences that were homologous with those in *Maize Oligonucleotide Array Project* and on the microarray data whose signal values were more than 3-fold higher than local background in the ear and tassel hybridization experiments, and their inserts amplified by PCR with primers flanking the cDNA insert. The PCR products were dot-blotted twice onto *Hybond-N*$^+$ nylon membranes (*Amersham*, Little Chalfont, Buckinghamshire, UK). Maize α-tubulin cDNA was used as an internal control. Distilled water and PCR reaction solution without templates were used as negative controls. The probe preparation, hybridization, scanning and data analysis were performed as Zheng *et al*. (2004) described. A radioactive intensity more than 3-fold higher than the local background was chosen for further analysis. The transcripts with a stressed/control ratio more than 2 were considered as differential expression.

The ASI, pollen viability, ear length and grain number of the 1 d stressed plants had no significantly difference from those of control plants (data not shown). Based on these results, we concluded that there was a slight impact on maize development at early stage of water-deficit stress in this study although the meiosis was one of the most sensitive stages, which suggested that the plants were able to sense and activate different response pathways to cope with water stress.

We identified 861 (349 upregulated, 512 downregulated) and 1809 (613 upregulated, 1196 downregualted) transcripts in the ears and tassels, respectively, that showed more than 2-fold changes induced by water stress compared with those of the control groups. The transcriptional profiles of the two organs differed considerably in response to water-deficit stress. One possible reason for this difference is that the tassels undergoing meiosis are much more sensitive to water stress than the ears. This is consistent with the results of Saini (1997). A second reason is the greater stress-tolerance of the female inflorescence than male inflorescence which is a long-term adaptive response of evolution. In fields under drought the pollen sterility could be offset by an excess of pollen produced. However, the failure of growth of female spikelet could not be overcome. The differential expression of subsets of transcripts following water stress was further confirmed by reverse Northern blot ($r = 0.92$).

Thirty-three transcripts were identified that consistently showed increased expression under water deficit conditions (Table 1), including 2 well-known transcripts involved in protecting macromolecules, dehydrin DHN1 (RAB-17) and putative late embryogenesis abundant protein. A maize orthologue for a seed imbibition protein (Sip1, TM00035829) was strongly upregulated in the two organs. Since kernel maturation involves dehydration during the imposition of dormancy, perhaps Sip1 functions not only in this natural developmental process, but also in drought-induced dehydration. The upregulated expression of histone H2A (TM00042841) might indicate that the H2A was a histone variant that was uniquely expressed under the stress (Scippa *et al.* 2000). Variants of histone H2A had specialized roles through stabilization and folding of chromatin (Ausio and Abbott 2002).

Table 1. List of water-deficit stress-responsive transcripts upregulated in the ears and tassels. A ratio of stressed/control of more than 2 is considered as differential changes. The sequence information is available at the website of the Maize Oligonucleotide Array Project.

TIGR ID	Accession No.	Putative annotation	Ear	Tassel
TM00025109	P12950	dehydrin DHN1 (M3) (RAB-17 protein) (*Zea mays*)	2.57	3.05
TM00024561	AAS16887	putative late embryogenesis abundant protein (*Oryza sativa*)	2.22	2.70
TM00035829	BAC82968	putative seed imbibition protein (Sip1) protein (*Oryza sativa*)	3.91	2.18
TM00042841	P40280	histone H2A. (*Zea mays*)	2.74	2.07
TM00030371	AAP54790	putative beta-carotene hydroxylase (*Oryza sativa*)	3.90	2.46
TM00036151	T02955	probable cytochrome P450 monooxygenase (*Zea mays*)	2.58	2.22
TM00021768	E24039	chlorophyll a/b-binding protein 3A precursor (*Lycopersicon esculentum*)	2.29	2.19
TM00035077	AAB19041	type 1 light-harvesting chlorophyll a/b-binding polypeptide (*Pinus palustris*)	2.82	2.27
TM00035845	AAA34157	chlorophyll a/b-binding protein Cab-3B (*Lycopersicon esculentum*)	2.82	2.07
TM00042572	T50858	response regulator ZmRR2 (*Zea mays*)	2.50	2.02
TM00016322	P38560	glutamine synthetase root isozyme 2 (*Zea mays*)	2.13	2.73
TM00030502	BAB90150	putative GA 2-oxidase (*Oryza sativa*)	2.43	7.31
TM00043978	BAA95697	thionin like protein (*Nicotiana tabacum*)	2.97	2.30
TM00025241	AAM91471	AT3g06760/F3E22_10 (*Arabidopsis thaliana*)	2.08	2.13
TM00036295	AAL31950	CDH1-D (*Gallus gallus*)	3.26	2.33
TM00018865	BAB55503	unknown protein (*Oryza sativa*)	2.53	3.47
TM00041500	BAA84793	ESTs AU078183(C62904) (*Oryza sativa*)	2.15	4.11
TM00025243	AAN77145	fiber protein Fb2 (*Gossypium barbadense*)	2.13	2.48
TM00043630	BAB89617	hypothetical protein (*Oryza sativa*)	3.38	23.76
TM00055765	NA	NA	3.72	4.89
TM00015345	NA	NA	7.31	2.67
TM00004676	NA	NA	2.03	2.53
TM00043708	NA	NA	2.11	2.51
TM00027406	NA	NA	6.65	2.45
TM00015120	NA	NA	2.12	2.41
TM00054715	NA	NA	5.88	2.33
TM00037011	NA	NA	2.33	2.11
TM00035506	NA	NA	3.34	2.05
TM00056450	BAC07421	neurofilament triplet M protein-like protein (*Oryza sativa*)	2.41	3.57
TM00019741	E84578	probable senescence-associated protein 5 (*Arabidopsis thaliana*)	3.04	2.67
TM00045590	BAB33421	putative senescence-associated protein (*Pisum sativum*)	2.94	2.10
TM00043966	AAP06839	transformer serine/arginine-rich ribonucleoprotein (*Oryza sativa*)	2.41	3.41
TM00025117	AAS58469	ultraviolet-B-repressible protein (*Gossypium hirsutum*)	2.38	2.37

The reactive oxygen species detoxification often occurs in plants under drought stress (Fazeli et al. 2007). The β-carotene hydroxylase (TM00030371) is a key enzyme in the zeaxanthin biosynthetic pathway. It was reported that overexpression of this gene enhanced tolerance to stresses as a result of the function of zeaxanthin in preventing oxidative damage of membranes (Davison *et al*. 2002), hence its identification here as being upregulated at the mRNA level provided similar insights into its function. Cytochrome P_{450} monooxygenase (TM00036151) was upregulated in the two organs. This gene mediates the biosynthesis of lignins, terpenes, alkaloids and the variety of other secondary metabolites, which act in plant defense and oxidative detoxification (Persans *et al*. 2001). Transcripts (TM00021768, TM00035077, TM00035845) encoding components of the photosynthesis pathways were upregulated in these nonphotosynthetic organs, which had been previously reported to be stress response in maize roots (Wang *et al*. 2003). One hypothesis was that they were involved in the control of the redox state under water stress (Grossman *et al*. 2001).

Two transcripts related to nitrogen metabolism in reproductive organs were upregulated under water stress in the present study. Response regulator ZmRR2 (TM00042572) is one of two-component regulatory factors and involved in the nitrogen signal transduction in maize (Deji *et al*. 2002). Glutamine synthetase (GS) root isozyme 2 (TM00016322) belongs to GS1 family. GS is the key enzyme involved in biosynthesis of all nitrogenous compounds and essential precursors of proline. Ectopic expressing the pine cytosolic GS gene enhanced tolerance to water stress in poplar (El-Khatib *et al*. 2004), and the inhibition of the expression of *GS1* gene in tobacco led to a reduced synthesis of proline and higher sensitivity to salt stress (Brugiere *et al*. 1999). Another report showed that GS activity was not affected in maize hybrid leaves by drought (Medici *et al*. 2003). Thus further study of GS function in maize reproductive organs is warranted.

The endogenous gibberellin (GA) content could be decreased by GA 2-oxidases. Ectopic expression of GA 2-oxidase in rice resulted in inhibition of stem growth, small, dark green leaves, and impaired development of the reproductive organs (Sakamoto *et al*. 2001). In this study,

Table 2. List of water-deficit stress-responsive transcripts downregulated in the ears and tassels. A ratio of stressed/control of less than 0.5 is considered as differential changes. The sequence information is available at the website of the Maize Oligonucleotide Array Project.

TIGR ID	Accession number	Putative annotation	Ear	Tassel
TM00041253	CAB64600	S-adenosylmethionine decarboxylase 2 (*Oryza sativa*)	0.41	0.44
TM00035154	CAA83914	phosphoglycerate mutase (*Zea mays*)	0.41	0.42
TM00035502	AAM96896	glyceraldehyde-3-phosphate dehydrogenase (*Vaccinium myrtillus*)	0.41	0.24
TM00038066	T02723	glyceraldehyde-3-phosphate dehydrogenase GAPC4 (*Zea mays*)	0.31	0.21
TM00035263	PQ0179	glyceraldehyde-3-phosphate dehydrogenase (*Zea mays*)	0.37	0.21
TM00042986	AAO38821	actin (*Oryza sativa*)	0.50	0.31
TM00054816	T15035	adenosylhomocysteinase (*Petroselinum crispum*)	0.46	0.46
TM00043327	E95206	cell wall surface anchor family protein (*Streptococcus pneumoniae*)	0.47	0.29
TM00027235	B57742	cyclin Ib (*Zea mays*)	0.46	0.34
TM00048772	BAD10260	hypothetical protein (*Oryza sativa*)	0.49	0.39
TM00048623	NA	NA	0.50	0.46
TM00008860	NA	NA	0.38	0.25
TM00034170	CAD41238	OSJNBa0067K08.2 (*Oryza sativa*)	0.42	0.34
TM00015169	AAK25845	putative multispanning membrane protein (*Arabidopsis thaliana*)	0.46	0.50
TM00043042	P46265	tubulin beta chain (*Oryza sativa*)	0.49	0.48
TM00048769	AAM67255	unknown (*Arabidopsis thaliana*)	0.33	0.44

the upregulated GA 2-oxidase (TM00030502) may negatively impact the normal development of the ears and tassels. However, the crop yield of the stress plants was not significantly different from that of control plants. Therefore the roles of GA 2-oxidases in maize development and response to stress need further study. In addition, several upregulated transcripts of unknown function were identified.

Of 10 upregulated genes that were identified by Zinselmeier *et al*. (2002), 8 were different from the transcripts obtained in our experiments. The possible reasons were in different developmental stage of the ears sampled, methods of stress and different maize lines (hybrid vs. inbred). In spite of these differences, 2 genes, RAB-17 and seed imbibition protein, were upregulated in the two experiments, which suggests these two genes may play key roles in protection of the normal development of the ears under water-stress.

Sixteen transcripts were identified that consistently showed decreased expression under water deficit conditions (Table 2) including a key enzyme involved in polyamine biosynthesis S-adenosylmethionine decarboxylase 2 (TM00041253) (Tian *et al*. 2004). Four transcripts involved in the glycolytic pathways, phosphoglycerate mutase (TM00035154) and glyceraldehyde-3-phosphate dehydrogenases (TM00035502, TM00038066, TM00035263), might decrease the efficient carbon flux through glycolysis/gluconeogenesis. In addition, several downregulated transcripts of unknown function were also identified. Of 7 downregulated genes identified by Zinselmeier *et al*. (2002), none was identical to transcripts obtained in our experiments.

In conclusion, we have identified a group of transcripts from the developing immature ears and tassels of maize that had differential changes in transcript level after 1 d of water stress. The most notable upregulated transcripts were those potentially involved in protective functions, reactive oxygen species scavenging, nitrogen metabolism and gibberellin metabolism, while those transcripts involved in polyamine and sugar metabolism were downregulated.

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