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Transcriptional profiling of *Zea mays* genotypes with different drought tolerances – new perspectives for gene expression markers selection

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Abstract

Drought is considered to be one of the greatest limiting factors in agriculture. Therefore, the ability of plants to adapt to drought conditions is crucial to sustain worldwide crop production. The aim of this work was to identify gene expression markers for drought tolerance in *Zea mays* with the potential to assist breeding. Selected maize genotypes were characterized with respect to drought tolerance by measurements of the relative water content after drought stress conditions. Tolerant genotypes were screened for up-regulated genes in drought conditions detected by previous microarray hybridizations. Five cDNAs coding for drought inducible genes in maize were identified and further characterized by semi-quantitative reverse transcription polymerase chain reactions. The expression levels of two of them (MZ00023411 and MZ00037881) allow a significant discrimination between tolerant and susceptible genotypes. In tolerant genotypes (MK01, MK01xRF7, 002x250) the expression level of these genes was at least twice higher as compared to susceptible genotypes (XL12, 005, 005x301) throughout all conditions tested. Almost for all tolerant genotypes were registered significant differences ($P \leq 0.05$), versus susceptible ones, in expression of these genes. Specific primers for these two genes allow discrimination of drought tolerant and susceptible maize genotypes even after 2h of dehydration stress based on expression level and are suitable as potential gene expression markers, associated with drought tolerance.

Keywords: maize, drought, tolerance, DHNs, GEM

Introduction

Maize became the number one production crop in the world. This was possible due to the high productivity per acre and the wide spectrum of commercial uses of this crop (Lawrence et al, 2008). Drought is considered one of the greatest limiting factors in agriculture and the ability of plants to adapt to such conditions is crucial to sustain worldwide crop production (Chaves et al, 2003). The development of maize varieties with enhanced drought tolerance and the development of new breeding technologies continue to be important objectives.

The appearance of molecular markers had revolutionized the selection and breeding processes of crop plants. All molecular markers generated by classical methods (AFLP, RFLP, RAPD, SCAR, etc) are based on DNA sequences of whatever kind and allow the detection of genetic variation of an organism, tissue, cell. In last decade appeared a new concept of markers - expression markers, derived from mRNA of transcribed genes. They are used to establish an expression fingerprint characteristic for a certain cell, tissue, organism, but changes continuously, depending on the developmental stage and environment. In contrast to DNA markers, that characterize certain regions of the genome, the dynamic expression markers define the potential of a cell, tissue, organism in a given environment (Weising et al, 2005). With the appearance and development of microarray techniques a new era of gene expression markers (GEMs) began. Transcript abundance data from cRNA hybridizations is used to identify genetic markers: polymorphic transcript-derived markers (TDMs), nucleotide polymorphism of the particular gene (SFPs - single feature polymorphisms), polymorphism resulting in extreme variation of gene expression – GEMs (Gene Expression Markers) (Potokina et al, 2009). At the molecular level hundreds of genes have been identified in crop plants, which are induced under stress conditions (Xiong et al, 2002; Zhu et al, 2002; Shinozaki et al, 2003; Suprunova et al, 2004; Liu X et al, 2003; Marilyn et al, 2006; Yu and Setter 2003). These data open possibilities for screening and identification of new GEMs associated with drought tolerance.

A combined product of traditional genetics and molecular biology that allows for the selection of genes that control traits of interest is marker-assisted selection (MAS). Combined with traditional selection techniques, MAS has become a valuable tool in selecting organisms for traits of interest. MAS in

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a breading context involves scoring indirectly for the presence or absence of a desired phenotype or phenotypic component based on the sequences, banding patterns or expression level of molecular markers. Markers can increase screening efficiency in breeding programs especially in the cases when the ability to screen for traits are extremely difficult, expensive or time consuming to score phenotypicaly (i.e. environmentally sensitive traits such as pests tolerance, abiotic stresses tolerance, etc). Also they allow selection of individual plants with desirable character(s). Markers associated with tolerance for a variety of environmental stresses rank as important targets for MAS and cereal breading because these complex traits are often difficult to screen using classical selection techniques (Edwards et al, 2007). For these reasons detection of new molecular markers associated with valuable traits in crops will have a considerable economic impact for breeding programs through the creation of various plant hybrids and lines with valuable features characterized by an increased production.

The overall objective of this study was to evaluate some up-regulated genes in drought conditions, detected in previous microarray investigations, as potential GEMs associated with drought tolerance.

Materials and Methods

Plant material and stress conditions

A set of 31 maize genotypes from germplasm collections of Institute of Genetics an Plant Physiology, Moldova and University of Hohenheim, Germany, common for amelioration programs in Moldova and Germany where used. Seedlings were grown in the glasshouse with a 16 hours illumination period at 25/20˚C (day/night) and watered with tap water. Ten day old seedlings were selected for each genotype for dehydration treatments, physiological and molecular analyses, in three biological repetitions. Dehydration treatments were done as described by Rampino et al, (2006). Well watered ten day-old seedlings, of each genotype, were collected and used as control. For drought stress treatment the seedlings were placed on dry filter paper for 2, 4, 6, 8, and 24h at room temperature under constant light. At the end of stress whole seedlings were frozen in liquid nitrogen and stored at -70˚C until use for molecular analyses.

Determination of drought tolerance level trough germination in osmotic solution

We performed the screening for drought tolerance by a classical method – determination of germination index in osmotic solutions (Kojushko, 1988). As osmotic solution a sucrose solution of 13% was used that create an osmotic pressure of 12atm. For each genotype 25 seeds were placed in a Petri dish on filter paper and 25ml sucrose solution in experimental dishes (three repetitions) and 25ml distilled water in control ones (two repetitions) was added. The Petri dishes were placed in a thermostat for 5 days at 30ºC. According to mathematical procedures were determined the germination index, standard deviation and intervals size:

$$P = \bar{a} \diagup \bar{b} * 100\%$$

$\bar{a}$ - average number of seeds germinated in sucrose solution

$\bar{b}$ - average number of seeds germinated in distilled water

$$P \pm tSp$$

P - germination index
t - Student coefficient (for 0.05, t=1.98)
Sp – standard deviation

$$Sp = \pm\sqrt{\frac{P(100-P)}{n}}$$

n – number of seeds tacked for germinated

Determination of intervals size:

Analyzed genotypes are divided in groups, number of which should be ≥ 2 and ≤ 5. Interval's size were calculated according to formula:

$$k = \frac{X\max - X\min}{r}$$

X_{max} – maximum % of germinated seeds
X_{min} – minimum % of germinated seeds
r – number of groups in which will be divided analyzed genotypes.

All analyzed genotypes were divided in 2 groups: tolerant and susceptible to drought.

RWC (Relative Water Content)

For the measurement of the relative water content expanded maize leaves, of approximate same size, were excised and fresh weight (FW) was recorded from control and stressed plants as described by Rampino et al (2006) with some modifications. Five leaves per each genotype were soaked in distil water for 4h at room temperature and constant light, and the turgid weight (TW) was determined. Total dry weight (DW) was recorded after drying the leaves for 24h at 90ºC. RWC was calculated according to the formula: RWC (%) = [(FW-DW)/(TW-DW)]*100 (Barrs et al, 1962).

Primer design

The primers were designed from the five EST sequences used for oligo construction from Maize Oligonucleotide Array Project database (http://www.maizearray.org/index.shtml), corresponding to up-regulated DHNs and drought related genes of maize drought tolerant genotype. Primers designed from

exons 2 and of the maize actin gene were used as internal control for data normalization. The intron spanning design of these primers allows also for the detection of gDNA contamination of RNA samples. All primers were constructed using Primer3 software (http://frodo.wi.mit.edu/) and were synthesized commercially (Metabion, Germany). Genes ID and annotation are presented in Table 1.

sqRT-PCR

For semi-quantitative RT-PCR total RNA was isolated using peqGOLD TriFast reagent (PEQLAB, Germany) according to manufacture instructions. Based on photometrical quantification and denaturing gel-electrophoresis, 5 µg of total RNA (treated with DNase1) was transcribed into cDNA using Revert Aid H Minus M-MuLV kit (Fermentas) and dT20 as primer. For PCR 0.5 µl of cDNA sample of the reverse transcription reaction were used in a final reaction of 25 µl containing (20mM Tris-HCl pH 8.4, 50mM KCl, 1.5 mM $MgCl_2$, 0.05mM dNTPs, 5pM of each forward and reverse primers and 0.5 U of Taq polymerase). For amplifications Biometria TProfessional Thermocycler were used and the cycling parameters were as follows: 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. At different numbers of cycles, between 20 and 30 cycles, 5 µl aliquots were taken from the reaction in order to detect the exponential phase of PCR product accumulation before the plateau of amplification. PCR products were separated in 2% agarose gels with pre-added ethidium bromide and visualized in gel-doc station. GeneTools 4.01 software was used to analyze band intensities. For data normalization control amplification with primers corresponding to maize actin gene were run in parallel with every analyzed gene. All reactions were run in triplicates using three individual cDNA samples per genotype.

Sequence and analysis of PCR products

PCR products were purified using PCR clean-up kit (Fermentas). Four individual PCR products obtained after amplification with specific primers on cDNA from drought tolerant and susceptible genotypes (two of each type) were sequenced (GATC Biotech). The obtained sequences were analyzed against public databases using BLASTN and BLASTX programs (http://ncbi.nlm.nih.gov) and tools at (http://magi.plantgenomics.iastate.edu) to Blast the sequences against maize BACs in order to determine their positions on maize chromosomes (Fu et al, 2005)

Statistics

Statistical analysis of the difference in genes expression level was performed with one-tailed Student's t-test. The confidence level for the differences in genes expression of tolerant and susceptible genotypes was calculated as follow: the values for every tolerant genotype at each stress point (2h, 4h, 6h, 8h) were compared independently with values for every susceptible genotypes at the same stress points and the difference was considered statistically confident if the highest value of Tail Probability (P) obtained from all four was ≤0.05.

Table 1 - ID and annotation of the genes used for specific primer construction in sqRT-PCR. Up expression levels of selected genes in drought conditions in comparison with normal conditions for drought tolerant genotype (MK01) and susceptible (DH1).

Gene ID	Annotation from Maize Oligonucleotide Array Project database	primer sequences (5' to 3')	up expression level - DH1	up expression level - MK01
MZ00041440	dehydrin {Zea mays;}	CCCGTACGTACCAAAGCCTA ACAAGAGCTCGGGTGAAGAA	3	2,1
MZ00025109	Dehydrin DHN1 (M3) (RAB-17 protein) {Zea mays}	GCGAGAAGAAAGGCATTATG GGAAACTGTCCCTGTCCCTG	1,9	1,7
MZ00043105	putative dehydrin (having alternative splicing products) {Oryza sativa (japonica cultivar-group)}	CCGGTGATCTTGTCCTTGAT AAGAGAGCGAGAAGCAGTCG	1,9	1,4
MZ00023411	22 kDa drought-inducible protein {Saccharum hybrid cultivar}	CTCGTCCTTCTTGTGGTGGT GCTAGCCAGCCATCCTACTG	3	1,6
MZ00037881	water-stress protein {Zea mays}	AGGGCCTACACCAAAGTCCT GCAATTTGGGTACCAGCAGT	1,5	1,2
Actin ex2+3		GAGGTCACGCCCCGCAAGAT TCAACCCCAAGGCCAACAGAG		

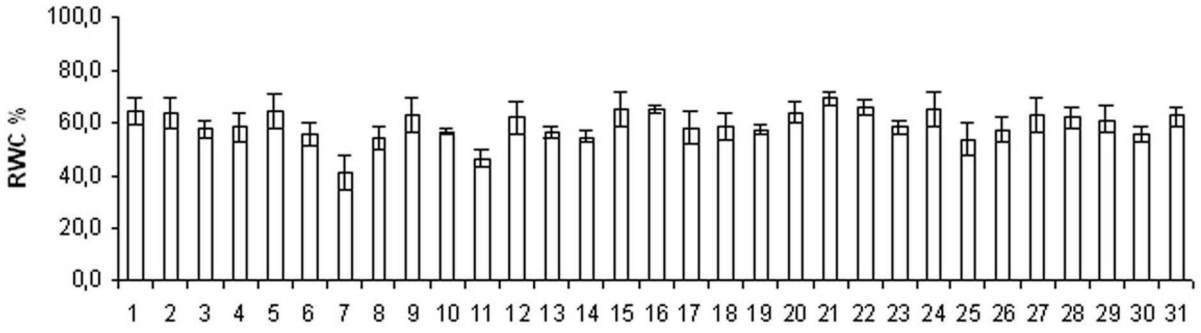

Figure 1 - RWC test for various maize genotypes: 1- RF7; 2- MK01; 3- M1; 4- W47; 5- CH9; 6- DH1; 7- XL12; 8- Mf; 9- 092; 10- 002; 11- 005; 12- Rf7xM1; 13- M1xRF7; 14- RF7xDH1; 15- RF7xMf; 16- W47xRF7; 17- 092xRF7; 18- MK01xRF7; 19- M1xMf; 20- XL12xM1; 21- DH1x 092; 22- DH1xMK01; 23- XL12xMf; 24- 301x250; 25- 005x301; 26- 301x005; 27- 250x005; 28- 301x002; 29- 002x301; 30- 250x002; 31- 002x250. Ten days old seedlings were exposed to 24h dehydration stress. Five independent measurements were performed for each genotype and the mean value ±SE is displayed.

Results

Physiological analysis of drought tolerance

In order to characterize the drought tolerance of the group of maize genotypes RWC tests were performed, by exposing the plants to 24h dehydration stress. In control seedlings, RWC values were high (93-99%), but in plants subjected to stress these values decreased considerably (41-70%, data not shown). We classified the analyzed genotypes by assuming all maize genotypes with RWC values above the average (59%) as drought tolerant and those with RWCs below the average as stress susceptible (Figure 1). Based on the RWC tests, we selected the 17 most divergent genotypes and determined the germination index in osmotic solution to further specify the drought tolerance level. This test showed greater differences between tolerant and susceptible genotypes. According to mathematical calculations of this method we considered those genotypes with germination percentage greater than 60% as tolerant and those genotypes with germination percentage lower than 60% as sensitive (Figure 2). Based on these data, we selected three drought tolerant maize genotypes (MK01, MK01xRF7, 002x250) with a germination index ranging from 77 to 95% and three susceptible genotypes (XL12, 005, 005x301) with germination index ranging from 24 to 43% for the expression analysis of selected genes.

Selection of up-regulated genes from microarray data

In previous experiments, microarray analysis of 5 maize genotypes from Institute of Genetics of Moldova germplasm collection (high drought tolerant MK01, DH1xMK01; low drought tolerant XL12, XL12xDH1; susceptible to drought DH1), were performed to determine the group of differentially expressed genes with ≥ 1.5fold expression difference in drought conditions (Badicean, 2008). We used a reference design for this microarrays experiment which utilized a direct comparison between samples (drought and control) and four biological replications for each treatment. Hybridizations were conducted according to the protocols from Maize Oligonucleotide Array Project (http://www.maizearray.org/index.shtml) using a Tecan HS4800Pro Hybridization Station. We chose the two most contrasting genotypes: MK01 (tolerant) and DH1 (susceptible) and focused on up-regulated genes. Based on existing annotation from the maize Oligonucleotide Array Project database we selected only those genes that are implicated in molecular response to drought: DHNs, water-stress proteins, HS proteins, etc, and have different or contrasting levels of expression between tolerant and susceptible maize genotypes. Based on these criteria five genes were selected: two dehydrins and one water related protein (ws1) of *Zea mays*, one gene with homology to a dehydrin of Oryza sativa and one gene with homology to a drought-inducible protein of Saccharum hybrid. The expression level of these genes detected with Microarray is presented in Table 1.

Molecular analysis of DHNs and drought related genes expression under dehydration stress

To further test the expression pattern of DHNs and drought related genes, selected by previous microarray experiments, under dehydration stress, we used the total RNA from control and stressed maize seedlings for sqRT-PCR analyses. Three drought tolerant (MK01, MK01x RF7, 002x250) and three susceptible (XL12, 005, 005x301) maize genotypes were used. The results reported in Figure 3 show the difference in expression of the analyzed genes depending on stress duration and genotype. From five selected genes, three of them (MZ00043105, MZ00023411, MZ00037881) were also expressed, at relative low levels, under well watered conditions, for some tolerant and susceptible genotypes. For tolerant and susceptible maize genotypes the expression profiles of analyzed genes are different. Following the kinetics of particular gene expression in all studied genotypes we revealed an increase of expression level with

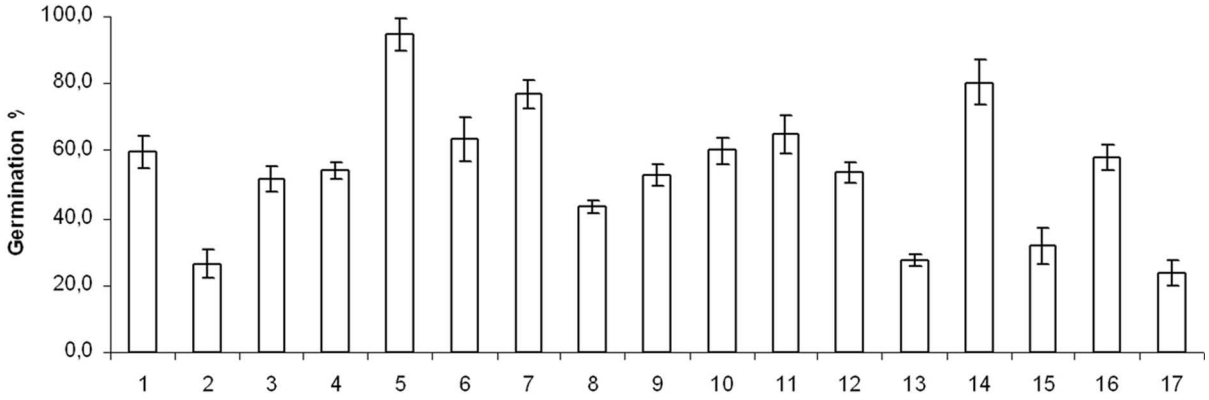

Figure 2 - Germination in osmotic solution test. Seeds were germinated in sucrose 13% solution for 5 days at 30ºC. Maize genotypes: 1.DH1, 2.XL12, 3.M1, 4.W47, 5.MK01, 6.CH9, 7.MK01xRF7, 8.250x002, 9.092, 10.002, 11.Rf7xM1, 12.RF7xMf, 13.005, 14.002x250, 15.Mf, 16.W47xRF7, 17.005x301.

stress duration for the majority of the genotypes.

The expression of MZ00041440 was not detected under control conditions for tolerant and susceptible maize genotypes. It was expressed at relative high values in both types of genotypes under dehydration. No significant differences were detected between expressions of analyzed genes for drought tolerant and susceptible maize genotypes. MZ00025109 expression increased gradually with the severity of stress, with higher values in drought tolerant maize genotypes. Significant differences in gene expression were obtained only for one tolerant genotype (MK01xRF7) after 6h and 8h of stress. MZ00043105 followed approximately the same expression pattern. Only for drought tolerant hybrid 002x250 significant difference in gene expression after 2h of stress were detected.

The most interesting results where obtained for genes MZ00023411 and MZ00037881, encoding *Zea mays* abscisic stress ripening protein and *Zea mays* water-stress protein (ws1) respectively. The expression pattern of these two genes showed the best discrimination between tolerant and susceptible maize genotypes. Their expression was detected for almost all genotypes (with exception of two susceptible ones – XL12 and 005, in case of MZ00037881) in control conditions at high RWC values. In tolerant genotypes (MK01, MK01xRF7, 002x250) the expression level of these DHNs was at least twice higher as compared to susceptible genotypes throughout all conditions tested. For all tolerant genotypes almost at each stress point (2h, 4h, 6h, 8h) significant differences in expression of these two genes were detected (Figure 3). Almost for all tolerant genotypes were registered significant differences ($P \leq 0.05$), versus susceptible ones, in expression of these two genes.

Characterization of cDNA sequences encoding analyzed genes

After performing RT-PCR with specific primers for investigated genes, four individual PCR products corresponding to drought tolerant and susceptible maize genotypes subjected to dehydration for different periods of time (two samples for every genotype) were selected.

In order to confirm their identity these PCR products were sequenced. BLASTN analysis revealed that the obtained sequences correspond to maize DHNs or drought related genes. Two analyzed genes (MZ00043105, MZ00023411) based on Maize Oligonucleotide Array Project database (http://www.maizearray.org/index.shtml) were annotated as putative dehydrin (*Oryza sativa*) and 22 kDa drought-inducible protein (Saccharum hybrid cultivar) respectively. After BLASTN analysis of these two obtained sequences we found 100% similarity with *Zea mays* genes encoding dehydrin 13 and abscisic stress ripening protein, respectively. The characteristics of obtained sequences are presented in Table 2.

In order to confirm that the RT-PCR targets were the same genes as the microarray ones we performed BLASTX analysis. Significant similarities were found only for two cDNA, corresponding to MZ00043105 and MZ00025109. Finally we used MAGI (Maize Assembled Genomic Island) tools to Blast our sequences against maize BACs in order to determine their positions on maize chromosomes (Fu et al, 2005). As a result we obtained that analyzed sequences, corresponding to genes MZ00041440 and MZ00043105, are located on chromosome 1; MZ00025109 – chromosome 6; MZ00023411 – chromosome 10; MZ00037881- chromosome 2 (Table 2).

Discussion

Creation of new drought tolerant crop varieties is one of the main goals of breeders in order to attenuate the negative effect of changing environment, the water recourses scarcity in certain regions and to prevent yield losses. Classical breeding techniques for crop inbreeding are very laborious and time consuming: monitoring of thousands of individual plants and 5-10 years for elite lines to be identified (Col-

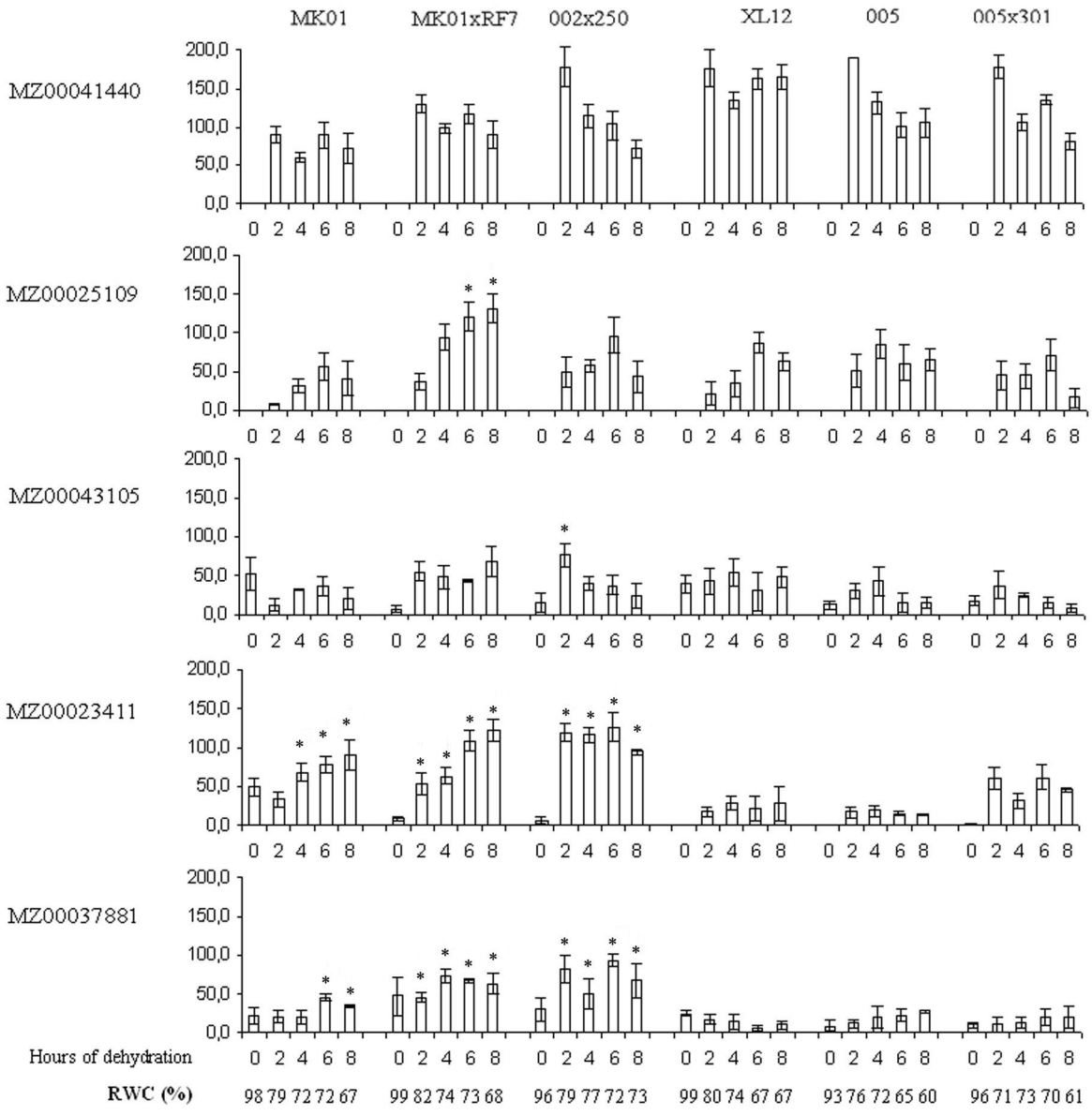

Figure 3 - Relative expression levels of studied DHNs in control and stressed seedlings of maize contrasted by drought resistance: tolerant genotypes - MK01, MK01x RF7, 002x250; susceptible genotypes - XL12, 005, 005x301. Expression levels were analyzed by sqRT-PCR using specific primers for selected genes. Band intensities were analyzed (GegeTools 4.01 software) and normalized against actin and are expressed in %. Analyses were performed in 3 independent biological repetitions and the reported values are the mean and standard deviation. Significance level: *, $P<0.05$

lard et al, 2008). For these reasons more emphasis is putted on biotechnology, marker technology and MAS because these new approaches have the potential to overcome the obstacles of classical breeding. Molecular markers have enormous potential to improve the efficiency and precision of conventional plant breeding. That is why identification of new molecular markers associated with drought tolerance of crops remains an important task.

The aim of this work was to analyze up-expressed genes in drought conditions detected in previous microarray investigations, as potential GEMs associated with drought tolerance. We focused on five DHNs and drought related genes that were up-expressed in drought tolerant maize genotypes according to microarray analyses. In order to evaluate the drought

Table 2 - Characteristics of obtained sequences encoding maize DHNs and drought related genes.

cDNA	PCR product length (bp)	GeneBank accession	Best identity (%)	E value	Chromosome location
MZ00041440	230	NM_001111857.1	Zea mays dehydrin (dhn2), mRNA gb:L35913.1 MZELIPASE (99%)	2e-109	1
MZ00025109	143	X15994.1	Zea mays RAB-17 gene; (99%)	8e-66	6
MZ00043105	154	EU971108.1	Zea mays clone 357636 dehydrin 13 mRNA (100%)	4e-74	1
MZ00023411	105	EU960308.1	Zea mays clone 223410 abscisic stress ripening protein 2 mRNA (100%)	9e-44	10
MZ00037881	40	NM_001111629.1	Zea mays water-stress protein (ws1), mRNA gb:AF533364.1 (100%)	1e-10	2

tolerance level of various maize genotypes with different genetic background, we applied two classical screening tests: RWC and germination in osmotic solution. High RWC values of maize plants in control conditions (99-93%) in comparison with lower values under dehydration stress (60-68%) indicates that the dehydration stress of seedlings at room temperature can successfully be used to induce drought stress in maize. The same method was also successfully used for wheat and barley seedlings to simulate drought stress in laboratory conditions (Rampino et al, 2006; Suprunova et al, 2004). Germination in osmotic solutions (sucrose, manitol and polyethylen-glicol) also simulates a drought stress reactions. Seed germination performance in such conditions, on one hand, reflects hereditary germination capacity in low humidity conditions, on the other hand, it reflects the presence of suction force, which determines the capacity to rapidly assimilate the necessary quantity of water. Because was demonstrated a good correlation between drought tolerances in seedlings and mature plants, this classical method is often used for screenings of different crops before sowing (Kojushko, 1988; Bajji et al, 2002; Murillo-Amador et al, 2002; Yang et al, 2007; Zhu et al, 2006). That is why we decided to perform this test on selected maize genotypes, based on RWC test. In comparison with RWC test this type of screening showed a better discrimination of the analyzed maize genotypes with respect to drought tolerance allowing us to select most contrasted genotypes for molecular analyses.

Drought is very complex abiotic stress that influences the expression of a large number of genes. Dehydrin genes family (DHNs) belongs to LEA (Late Embryonic Abundant) genes group and is one of the most analyzed drought-inducible gene families. DHN proteins are normally synthesized in maturating seeds during their desiccation, and also in vegetative tissues of plants treated with abscisic acid or exposed to environmental stress factors that result in cellular dehydration. The dehydrins are considered as stress proteins involved in plant protective reactions against dehydration (Allagulova et al, 2004). The expression of great majority of members from this family is differentially regulated under different types of biotic and abiotic stress conditions (Shinozaki et al, 2007; Wang et al, 2007). From our previous microarray analyzes we selected five DHNs and drought related genes with differential expression level in drought tolerant and susceptible maize genotypes. We have identified and characterized five cDNA corresponding to different DHNs and drought related genes, expressed at high levels in stressed plants, confirming the role of these genes in molecular mechanisms activated by the plant to tolerate and survive water deficit conditions. Different expression profiles, depending of genotype and stress duration, were detected for every analyzed gene suggesting their individual functions in the complex response to drought. This fact also has been reported in wheat and barley (Rampino et al, 2006; Suprunova et al, 2004). In case of wheat, Rampino et al (2006) studied the expression patterns of five DHNs under dehydration stress. They revealed that DHNs genes are induced in tolerant genotypes when RWC values are still high in comparison with induction of the same genes at lower RWC values in susceptible genotypes, suggesting that induction of DHNs at high RWC values allow the plant to prepare better for the stress and determine its tolerance. We revealed the same induction pattern of drought inducible genes for our analyzed maize genotypes.

Transcripts analyses techniques (Microarray,RT,

qRT, sqRT-PCR) have demonstrated significant power for genome-wide analyses of gene expression and identification of transcript-derived markers (TDMs), SFPs, GEMs (Potokina et al, 2009; Marilyn et al, 2006). One condition for using a gene as a GEM for environmental stress is its specific and differential expression. Brosché et al (1999) cloned and fully sequenced the ribosomal protein S26 cDNA from pea (*Pisum sativum* L). The gene PsRPS26 was shown to be differentially regulated by ozone and UV-B radiation in opposite ways. Ozone gave rise to increased mRNA levels, whereas UV-B led to a decrease in S26 transcript abundance. Thus, the expression of PsRPS26 can be used as a GEM to differentiate between these two environmental stresses. In our experiments we detected an expression difference of two maize drought inducible genes that allow discriminating between tolerant and susceptible genotypes under stress conditions. Specific primers for these genes (MZ00023411 and MZ00037881) allow discrimination of drought tolerant and susceptible maize genotypes even after 2h of dehydration stress, based on expression level and are suitable as GEMs, associated with drought tolerance.

The feasibility of using a gene expression as an alternative approach for early detection of potato nitrate deficiency was examined by Li et al (2009). In their experimental system, GEMs detected a reduction of nitrate supply prior to measurable reductions in plant growth or in N status measured using common chemical or optical methods. Messmer et al (2009), by the joint analyses of the well-watered and water stressed flowering maize plants, identified several clusters of QTLs for different traits located on chromosome 1. These regions are clear targets for future marker-assisted breeding.

With our detected GEMs it is possible to select drought tolerant individual maize plants at the stage of first full expended leaves that make them feasible for use in MAS. Forward analysis on larger population is needed in order to validate these markers. Our results in the identification of GEMs associated with drought tolerance, based on Microarray data, may contribute to better understanding of the role of analyzed genes in molecular response to drought and favor creation of new drought tolerant maize lines and hybrids via their use in MAS.

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