DROUGHT RESPONSIVE GENE MECHANISMS DURING THE INITIAL GROWTH PHASE IN MAIZE (*ZEA MAYS* L.)

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Abstract. Climate change, global warming and increasing drought are critical issues for the world and particularly for developing countries. Drought severely affects maize and causes heavy yield losses. Improving maize genetically against drought stress is an integral strategy to overcome this problem. Identifying drought responsive genes and their expression profiling is helpful for this purpose. Drought-responsive genes from phylogenetically related crops were hunted for the maize genome. Candidate maize genes with unknown functions were analyzed. Out of 9 selected candidate genes, two genes *GSNOR* and *CDPK7* were found differentially regulating under drought stress. They displayed down regulation in roots, whereas, in leaves both genes were up-regulated under drought conditions. Apparently, under stress conditions signaling of Ca^{+2} and NO initiated in roots, then moved to stem and leaves shifting its load to aerial plant parts to overcome water deficit conditions. Results suggest that *GSNOR* and *CDPK7* have potential to enhance maize plants' ability to resist water deficit conditions. *GSNOR* and *CDPK7* enable maize plants to encounter drought stress through nitrosylation and phosphorylation of their target proteins. Thus, they modify downstream gene expression, protein functions and activity. Identified genes may prove useful in improving maize plants to face drought stress conditions.
Keywords: *drought responsive genes, phosphorylation, gene expression profiling, S-nitrosoglutathione reductase (GSNOR), calcium dependent protein kinases (CDPK7)*

Introduction

The developing countries of South East Asia face the challenge of water scarcity (Greve et al., 2018). Unfortunately, Pakistan also confronted by agricultural and domestic water shortage. Pakistan is one of the countries which are facing water scarcity and the situation is expected to get worse in the forthcoming years (Khoso et al., 2015). Scarcity of water inflicts a quite significant and heavy economic loss to the agricultural crops and climatic changes taking place globally are predicted to make the problem worse (Battisti and Naylor, 2009; Marris, 2008). Drought and salinity are the two factors that cause about 50% reduction in yields of main crops throughout the world (Majeed and Muhammad, 2019). The drought problem is quite ubiquitous, alarming and it may have a catastrophic effect on economy of a country relying on agriculture produce (Ali et al., 2017).

In case of maize, water deficient conditions influence the crop physiology thus inflicting heavy production losses. Due to water deficiency at initial reproductive growth stages, defoliation takes place in maize crop which ultimately reduces the grain yield (Jaleel et al., 2009; Monneveux et al., 2006). Water deficiency has negative impacts during anthesis, pollination, anthesis-silking stage and formation of the grains in corn (Aslam et al., 2013). An unfortunate fact found by the plant breeders was that

tolerance against drought in maize is quite complex feature, thus it becomes difficult for them to make a selection, screening and breeding for the suitable features (Luo et al., 2019). One of the possible solutions to cope with drought conditions is development of drought tolerant cultivars (Nuccio et al., 2015). However, lack of sufficient genetic resources available in maize is the reason for slow progress towards development of drought tolerant cultivars (Mugo et al., 2005). Several studies have employed the forward genetic approaches like proteome analysis, quantitative real-time PCR and field evaluation for identifying differential expression of candidate genes under drought stress in maize (Wang et al., 2019). A large number of genes are conserved across the plant species which have similar structure and function. These conserved genes are searched in other phylogenetically related plant species (Alexandrov et al., 2009). Identification of genes which are responsive against water deficit conditions can be a useful tool to understand biological systems adapted by the plants against drought stress (Langridge and Reynolds, 2015). Several molecular markers have been developed and genes identified on the basis of differential expression of candidate genes and these findings are being applied to maize improvement programmes focused on drought tolerance (Mao et al., 2015). Identification of genes which are responsive against water deficit conditions can be a useful tool to understand biological systems adapted by the plants against drought stress (Langridge and Reynolds, 2015).

Several genes are reported to play their role under drought stress conditions. For instance, Liang et al. (2011) stated that *RAB18* showed its response against water deficit conditions in the presence of ABA as stimulus via ABA-dependent pathway. SNF1-related protein kinase 2 proteins (*SnRKs*) play major function in phosphorylation process of AREB1 through ABA dependent pathway (Yoshida et al., 2010). Similarly, *NRGA1* from Arabidopsis acts as pyruvate carrier for mitochondria and it negatively controls guard cell signaling, induced by ABA (Li et al., 2014). When *nrga* loss-of-function mutants were tested, plants showed high sensitivity in the presence of ABA in perspective of stomatal movements, demonstrated an intensified tolerance against drought stress. In contrast to this, over-expressing lines for *NRGA1* depicted contrasting stomatal responses, decreased tolerance against drought and activation for anion channels. Another gene *AtCIPK1* depicted its key importance in stress induced pathways. *AtCIPK1* combine both ABA-dependent as well as ABA-independent features for signaling against abiotic stresses (D'Angelo et al., 2006). It was found that *AtCIPK23* initiates channel for potassium ions, controls transpiration process in leaves and absorption of potassium ions in Arabidopsis while interacting along with *CBL9* and *CBL1* (Cheong et al., 2007). Another stress responsive gene *CIPK* found in *Pisum sativum* showed up-regulation in coordination with *CBL* and gave relative response against biotic and abiotic stresses, SA and calcium (Mahajan et al., 2006). For various environmental signaling, calcium ions play a central part as secondary messenger intracellularly. Calcineurin B-like proteins, as well as their targeted proteins depicted mediatory role in signaling network against various environmental stresses faced by the plants (Chen et al., 2011). Nakashima et al. (2007) implied that a drought responsive gene in rice *NAC6* showed its expression in biotic and abiotic stress conditions. If *OsNAC6* is used in combination with stress inducible promoters, this technique can be beneficial in molecular breeding against stress tolerance. Transgenic tomato plants were developed by inserting yeast *TPS1* gene. These transgenic plants showed enhanced chlorophyll as well as starch contents and as a result their tolerance against drought, oxidative stress and salt stress was increased (Cortina and Culianez-Macia, 2005).

GSNOR plays pivotal role under various stresses. The gene plays vital part in maintaining homeostatic balance of nitric oxide and thus regulating the process of nitrosylation. S-nitrosoglutathione reductase (GSNOR), catabolizes and splits GSNO into oxidized glutathione (GSSG) and ammonia. As a result, *GSNOR* regulates and maintains GSNO levels in various biotic as well as abiotic stresses (Leterrier et al., 2011). Moreover, GSNO is able to perform transnitrosylation, during which NO functional group is shifted from S-nitroshthiol to any cysteine thiol group of a particular target protein. GSNO is studied for S-nitrosylation of various proteins as GSNO is associated with responses of plants which are exposed to various types of stresses (Chaki et al., 2009).

Nitrosylation and phosphorylation are analogous in cellular biology, as both change the expression of their target genes and alter protein activity as well as function. As GSNOR regulates the process of nitrosylation, similarly CDPKs perform phosphorylation reactions. Both *GSNOR* and *CDPKs* regulate the process of nitrosylation and phosphorylation respectively, thus affecting downstream events taking place in the cytosol, for the purpose of drought management. Several biochemical reactions in plants are closely linked with phosphorylation of proteins (Luo et al., 2018). Calcium ions play a significant role in transmitting messages for several signal transduction routes among such signal transduction pathways (Asano et al., 2012). In response to a particular environmental stimulus (e.g., abiotic stress) concentration of Ca^{2+} is enhanced temporarily and this elevation is identified by many Ca^{2+} sensors or Ca-binding proteins (Rudd and Franklin-Tong, 2001). The elevation of Ca^{2+} introduces modified protein phosphorylation as well as changes in the gene expression patterns as downstream effects (Tuteja and Mahajan, 2007).

Identification and characterization of drought responsive genes is one important step in this direction. The main objective of this study was to identify novel drought responsive genes in maize and conducting their expression profiling. As we know genes are conserved among all species, therefore it was hypothesized that drought responsive genes, reported in crops which are phylogenetically related to maize, would be present in maize genome as well. Therefore, such genes were hunted in maize genome and their gene expression profiling was carried out under drought stress conditions. The main objective of this research was to identify drought responsive genes in maize genome and to perform their expression profiling.

Materials and methods

Plant materials and growth conditions

The research was carried out in PMAS Arid Agriculture University Rawalpindi, Pakistan. The seedlings of maize genotype Soan-3 were grown in glasshouse (32 ± 4 °C) to examine their drought tolerance at early development stage. Soan-3 cultivar was taken as Reference Genome. The selected genotype was grown in potting media with the composition of sand, soil and farm yard manure (FYM) at 1:2:1 ratio, respectively. Size of all pots was 250 ml.

Drought stress treatment

The maize seedlings were grown in pots and watered regularly up to 14 days. Two-weeks old maize seedlings were maintained under similar growth conditions and subjected

to drought stress by withholding water for two days. After withholding water for two days i.e., for 48 h, then the time points for six drought stress treatments were counted. The drought stress treatments were given at 0 h, 6 h, 12 h, 24 h, 30 h and finally 10 h after providing recovery irrigation. These six time points of collecting samples were based on observations of gradual increase in symptoms of drought stress on maize seedlings. BBCH growth stage of the maize seedlings was V3. After two weeks of seed germination, it was V3 stage when drought stress treatments were given and samples were collected. Four plants represented one treatment of drought stress for each time course.

Selection of drought responsive genes

The drought responsive genes were searched in crops which are phylogenetically related to maize. Then their orthologues were searched in maize genome with an assumption that their homologous sequences may be present in maize genome as well. Maize genome sequence databases (Mittal et al., 2017) and available online tools were used (Jiao et al., 2017). The decision of selecting identical sequences was taken on protein homology as proteins are functional entities rather than nucleotides. Thus, several amino acids' sequences were used through Basic Local Alignment Search Tool (BLAST) (Pearson, 2013). Among all the searched genes only those were chosen as our candidate genes which depicted more than 70% homology of amino acid sequences and their role against drought stress was not reported in maize (*Table 1*). As a result of this search, nine genes were shortlisted with their orthologues present in maize genome.

Table 1. *Short-listed genes chosen for the expression profiling*

S. No.	Gene name	Plant	References	Best hit in maize genome	Protein		
					Coverage	Seq. ID	C.W score
1	*RAB18*	Arabidopsis	Muller et al., 2012	GRMZM5G83647_T05	93	85	83.5
2	*SnRK2*	Arabidopsis	Zheng et al., 2010	GRMZM2G155593_T01	84	86	80.9
3	*GSNOR*	Arabidopsis	Kwon et al., 2012	GRMZM5G824600_T03	99	90.4	89.4
4	*BADH15*	Sorghum	Wood et al., 1996	GRMZM2G135470_T01	100	81	82.4
5	*AtCBL1*	Arabidopsis	Kudla et al., 1999	GRMZM2G107575_T01	100	77	77
6	*OsCDPK7*	Rice	Saijo et al., 2000	GRMZM2G314396_T01	100	89	89.6
7	*OsNAC6*	Rice	Nakashima et al., 2007	GRMZM2G014653_T01	81.5	80	83
8	*AtNRGA1*	Arabidopsis	Li et al., 2014	GRMZM2G003642_T01	90	77.5	70
9	*OsTPS1*	Rice	Li et al., 2011	GRMZM2G068943_T01	94	84	88

Total RNA isolation

Collected leaves samples were immediately frozen in liquid nitrogen (LN_2). To extract RNA TRIzol method was used (Sambrook and Russel, 2001). After extraction it was resolved on TAE agarose gel of 1.2% concentration at 60 V for 45 min. Then it was stained with Ethidium bromide and observed under UV light. It depicted a good quality of RNA with two distinct bands of *28S* and *18S* ribosomal RNA. The purity and concentration of RNA was calculated by mass spectrophotometry. Later, RNA samples were equilibrated by adding nucleases free water accordingly.

Synthesis of 1st strand of cDNA

The mRNA preps were used for the synthesis of first strand of cDNA by using cDNA synthesis kit. To verify successful synthesis of cDNA, PCR was performed using primers

of *ZmActin,* followed by gel electrophoresis. Moreover, the uniformity of bands for all the six drought stress treatments confirmed the synthesis of cDNA with equal concentration.

Primer designing

Primers were designed using available online tools justbio.com and primer3 (*Table 2*). Only exon regions of sequences were used to design primers. Every primer pair was specifically designed so that it should uniquely amplify the conserved region of the gene under investigation.

Table 2. *Detail of the primers designed for the putative short-listed drought responsive genes*

S. No.	Gene name	Sequence	L	GC%	Tm	PS	References
1	RAB18-F	GATTCCTCCTCCTCCTCGTC	20	60	60	453	Ayarpadikannan et al., 2014
2	RAB18-R	AGCAAATTCAATCCCCTCCT	20	45	58		
3	SnRK2-F	CTGATTTGTGGCGTCAGCTA	20	50	56	721	Kulik et al., 2011
4	SnRK2-R	GGACTTGCTTCACCTGCTTC	20	55	58		
5	GSNOR-F	AAGTTTTGCAAGAGCGGAAA	20	40	52	309	Xu et al., 2013
6	GSNOR-R	TTCCAATGCACTCAAAGCTG	20	45	54		
7	BADH15-F	TAGGCCTACCACCAGGTGTC	20	60	60	635	Wood et al., 1996
8	BADH15-R	TGCAAGTTCCACAGCTTCAC	20	50	56		
9	AtCBL1-F-	GGCCTGATCAACAAGGAAGA	20	50	56	226	Kudla et al., 1999
10	AtCBL1-R	CAATAAACCCTGTGCCATCC	20	50	56		
11	OsCDPK7-F	CAAGAACGTCGTCGCTATCA	20	50	56	428	Wang et al., 2008
12	OsCDPK7--R	AAAATGGAGGCACACCACTC	20	50	56		
13	OsNAC6-F	TGGTGATGCACTACCTCTGC	20	55	58	391	Nakashima et al., 2007
14	OsNAC6-R	ACCCAGTCATCCAACCTGAG	20	55	58		
15	AtNRGA-F	CATGCACTGGACTCATTTGG	20	50	56	168	Li et al., 2014
16	AtNRGA-R	CTTCCAGTTGTGAAGCAGCA	20	50	56		
17	OsTPS1-F	TGTGAGGATGAGCGCACTAC	20	55	58	416	Kim et al., 2005
18	OsTPS1-R	ATTGCAGCACCCTTGGTAAC	20	50	56		

PCR for gene expression profiling

PCR was performed using primer pairs specific for each candidate gene. PCR conditions were optimized. Initial denaturation temperature was 94 °C while the annealing temperatures were kept a few degrees lesser than the melting temperature (T_m) of primers. Extension was performed at 72 °C for 30 s. The final extension was performed at 72 °C. The reaction products were separated by gel electrophoresis at 70 V for 45 min.

Tissue specific gene expression

For Tissue-Specific gene expression similar procedure was repeated. Same size of pots i.e., 250 ml was used, water was withheld for two days i.e., 48 h and then six drought stress treatments were given as 0 h, 6 h, 12 h, 24 h, 30 h and then 10 h after recovery irrigation. In this experiment, samples of stem and roots were collected from maize seedlings to investigate the gene expression in roots and stem other than leaves. Procedure of RNA isolation from samples, RNA equilibration, cDNA synthesis, primer designing and PCR was repeated for gene expression profiling in stem and roots. For quantifying the intensity of expressed genes, image J software was used.

Results

Gene expression profiling

In genes expression study, two genes namely *GSNOR* of Arabidopsis (*FDH1*) with its maize orthologue GRMZM5G824600 and *CDPK7* from rice with its maize orthologue GRMZM2G314396 were identified. However, seven genes *RAB18, SnRK2, BADH15, AtCBL1, OsNAC6, AtNRGA1, OsTPS1* could not show their expression.

GRMZM5G824600 – A maize orthologue for GSNOR

The maize orthologue GRMZM5G824600 found as a result of BLAST on Maize GDB with its source gene *AtGSNOR* from *Arabidopsis thaliana*, expressed during the gene expression profiling. The gene showed a steady up regulation with increasing levels of drought stress (*Fig. 1a*). When two weeks old maize seedlings were subjected to water deficit conditions, *AtGSNOR* gene started showing its presence. At 0 h, which was a control experiment, expression of the gene was at its minimum level and could be hardly detected. At 6 h the gene expression was observed easily. The drought stress was at its peak with successive increase of drought stress duration at 12 h, 24 h and finally at 30 h. The gene showed a gradual increase with highest intensity at 30 h. However, this gene showed a reverse trend in its expression intensity after 10 h of recovery irrigation.

PCR for GRMZM2G314396 – A maize orthologue for OsCDPK7

The gene selected from rice Os*CDPK7* expressed itself as the maize orthologue, GRMZM2G314396 and showed its presence in maize genome. The gene displayed a certain expression pattern during the step wise exposure of the maize seedlings to drought stress conditions. The expression profiling provided evidence that it is functioning as drought responsive gene by showing up regulation from 0 h till 12 h of drought stress in maize genome. However, after 12 h of drought stress it showed a gradient of down regulation (*Fig. 2a*).
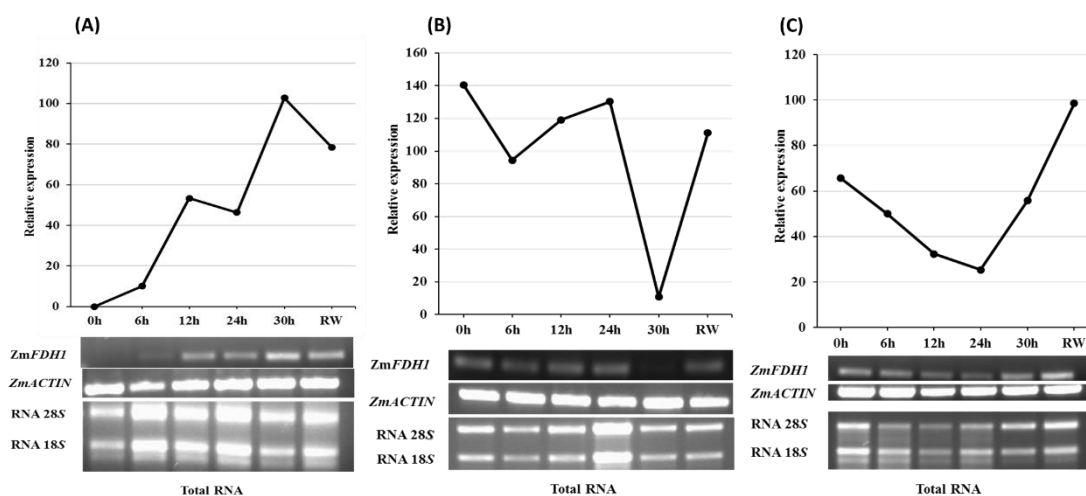

Figure 1. *Expression pattern of GSNOR with size 309 bp upon exposure to drought stress. ZmActin is maize Actin gene loading control. Total RNA obtained shows ribosomal RNA bands of 28S and 18S. The line graphs depict the gene expression pattern. (A) Gene expression profiling in leaves showing upregulation followed by down regulation at recovery irrigation. (B) Expression profiling obtained in roots. (C) Gene expression pattern in stem*
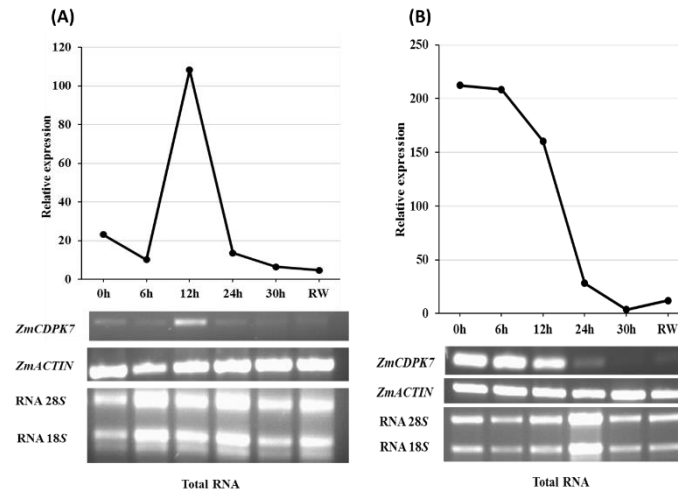
Figure 2. *Expression profiling of CDPK7 with size 428 bp at various levels of drought stress. ZmActin is used as loading control. Total RNA obtained shows ribosomal RNA bands of 28S and 18S. The line graphs display the gene expression patterns. (A) Gene expression profiling in leaves showing upregulation of the gene till 12 h followed by down regulation. (B) Expression profiling obtained in roots displaying an explicit pattern of down regulation*

GSNOR expression in tissues (roots and stem)

In addition to leaves, gene expression was analyzed in roots and stem. On performing PCR for finding gene expression pattern of GSNOR in roots, it was observed that GSNOR displayed an overall declining pattern. Especially at 30 h of drought stress, expression of the gene was at its minimum level (*Fig. 1b*). The expression pattern in stem showed that the gene displayed a general trend of down regulation in first five treatments of drought stress, i.e., starting from 0 h till 30 h of exposure to water deficit conditions. GSNOR gene depicted an overall declining trend while on providing recovery irrigation it showed a sign of rejuvenation (*Fig. 1c*).

CDPK7 expression in tissues (roots and stem)

In roots an obvious down regulated pattern for *CDPK7* was observed (*Fig. 2b*). Initially its expression was found higher at 0 h and then it displayed a steady decline with subsequent increased levels of stress while at 30 h the gene expression almost vanished. However, after 10 h of recovery irrigation when *CDPK7* expression was checked it showed a sign of little reappearance. While expression of *CDPK7* could not be detected in stem.

Discussion

GSNOR/FDH1 gene is induced by ABA and therefore it is included among the genes which follow ABA dependent pathway (Wang et al., 2015). ABA plays a very important role under abiotic conditions and more specifically under water deficit situations (Roychoudhury et al., 2013). Upon exposure to drought conditions, the expression of a drought responsive gene induced by ABA is quite logical. ABA accumulation in leaves makes the guard cells flaccid and results in closure of the stomata thus preventing loss of water through transpiration (Yamaguchi-Shinozaki and

Shinozaki, 2006). Accumulation of ABA initiates Nitric Oxide (NO) signaling. When plants are exposed to various environmental stresses, NO plays its role as secondary messenger in transduction of signals against these stresses (Gill et al., 2013). Along with NO, the part played by S-nitrosoglutathione (GSNO) is equally important. GSNO is greatly important because it acts as internal reservoir for NO in cells and by possessing lesser molecular weight as compared to other biological molecules it acts as a carrier of NO (Leitner et al., 2009). Thus, GSNO performs S-nitrosylation of its target proteins and modify their functions. GSNO is associated with responses of plants against stress conditions (Chaki et al., 2009). The gene *GSNOR* catalyzes GSNO and modulates its level thus maintaining homeostatic environment in plants (Staab et al., 2008). *GSNOR* mutants showed thermotolerance defects, resistance to programmed cell death and diminished fertility (Chen et al., 2009). Such kinds of pleiotropic effects provide sufficient evidence that *GSNOR* participates not only in maintaining homeostatic environment, but it also takes part in responses against biotic as well as abiotic stress conditions (Xu et al., 2013). While considering the role of *GSNOR,* it is worthy to mention that it scavenges reactive oxygen species by controlling enzymes involved in anti-oxidation activity (Cheng et al., 2018).

In our experiment *GSNOR/FDH1* depicted a steady up regulation in leaves thus proving its role under conditions of drought stress (*Fig. 1a*). Upon comparing the results displayed in *Figure 1a, b* and *c*, it can be concluded that *GSNOR* gene was initially being expressed in roots. On gradual exposure of maize seedlings to drought stress, gene expression shifted from roots and stem towards leaves in order to modulate the consequences of consistently increasing drought stress. *GSNOR* is expressed constitutively in all tissues (Shi et al., 2014). In addition, as nitric oxide plays its role in root elongation and handling osmotic stress condition through accumulation of osmolytes, therefore its presence and expression in roots is understandable and can be justified (Leitner et al., 2009). GSNO, which is the substrate of the enzyme GSNOR, is the carrier and donor of NO (Malik et al., 2011). GSNO has lesser molecular weight as compared to other biological molecules (Corpas et al., 2013), therefore it is trans located through phloem to upper parts of the plant (Espunya et al., 2012) and while passing through stem, it eventually reaches to the leaves for donating NO. Analyzing the expression of *GSNOR* in leaves and roots shows that in case of roots the gene expression is minimum at 30 h while in contrast to this, in leaves its expression is at its peak. These results clearly indicate that due to the translocation of the substrate GSNO, the need for enzyme GSNOR gradually increased in leaves instead of roots and thus its expression displayed a gradual shift between tissues. The expression pattern in roots is almost opposite to the gene expression profile in leaves (*Fig. 1a, b, c*). Thus, it can be concluded that *GSNOR* shifted its expression from roots and stem towards leaves upon step wise exposure of the maize seedlings to drought conditions. The expression of *GSNOR* is closely associated with GSNO which is the substrate of the enzyme GSNOR. We can say that the activity of GSNOR enzyme is correlated with its substrate GSNO. When and where the GSNO concentration gets higher and seems to disrupt the homeostatic balance in cytosol, the GSNOR enzyme turns active and starts decomposing its substrate, hence the expression of *GSNOR* intensifies. The literature illustrates the significance of GSNO as carrier and donor of nitric oxide. Malik et al. (2011) reported that GSNO is trans located through phloem and it acts as a carrier of nitric oxide, trans locating it to the distant targets. It is necessary for signaling through mechanism of oxidation reduction reactions. It can be deduced that when concentration

of GSNO was higher in a certain tissue the need for its catabolism into GSSG and GSH was also greater. Upon excessive accumulation of GSNO, the NADH-dependent enzyme S-nitrosoglutathione reductase (GSNOR) catabolizes and splits GSNO into oxidized glutathione (GSSG) and ammonia. As a result, *GSNOR* regulates and maintains the GSNO levels under biotic and abiotic stress conditions (Xu et al., 2013).

Due to abiotic stress conditions and more specifically under water deficit conditions, ABA concentration increases (Zhu, 2002). The rise in ABA enhances the levels of Ca^{2+} in cytosol (Kohler and Blatt, 2002). Whatever information are encoded in various stimuli, this information introduces an enhanced level of Ca^{2+} in cytosol and thus it determines the nature of response. This increase in calcium ions concentration is termed as calcium signatures (Evans et al., 2001). The elevation of Ca^{2+} ions is detected by a group of calcium sensors known as calcium responders for instance kinases which transmit the message downstream to their concerned targets (Reddy and Reddy, 2004). Similarly, Harmon et al. (2000) reported that calcium dependent protein kinases (CDPKs), sense, decode and ultimately translate the elevation of Ca^{2+} concentration into increased activity of proteins kinases and consecutive downstream signaling. When kinases e.g., CDPKs gets active they bring downstream events of phosphorylation of their target proteins and as a result enable the plant to cope against drought stress conditions. Phosphorylation of proteins is a reversible reaction which plays a significant role in stress signaling in eukaryotes. It is considered that protein phosphorylation regulates several basic cellular incidences (Xu et al., 2009). Plentiful evidences disclosed that several biochemical reactions in plants are closely linked with phosphorylation of proteins (Shen et al., 2004). On induction of *CDPKs* the phosphorylation cascade is triggered and stress responsive genes or the transcription factors are targeted (Mahajan and Tuteja, 2005). Upon exposure of plants to various types of abiotic and biotic stresses for example drought, disease and saline environment, Ca^{2+} concentration is enhanced rapidly in cytoplasm of the cells (Hashimoto and Kudla, 2011). Thus various Ca^{2+} sensor proteins, sense and translate these Ca^{2+} signals into respective responses in the cells. The spatiotemporal changes in Ca^{2+} are regulated by sensor proteins (DeFalco et al., 2010). Kinases are an example of such kind of proteins. At first Ca^{2+} increase in cytosol. As a result, Ca^{2+} attach to elongation hand of calcium dependent protein kinase and as it is also obvious from its name that its functionality depends on attaching Ca^{2+} to its EF motif, hence upon activating, calcium dependent protein kinase starts performing phosphorylation of its target proteins.

Examining the expression pattern of *CDPK7* in leaves (*Fig. 2a*), indicates that the gene upregulated up to 12 h but later on it displayed down regulation in successive treatments of drought stress. We can interpret from this obtained pattern that till 12 h of drought stress, the expression of the gene synthesized sufficient concentration of its product i.e., calcium dependent protein kinase. Therefore, in successive drought stress treatments of 24 h and 30 h, the gene *CDPK7* did not need to be expressed with same intensity and thus it got down regulated. The expression pattern depicted in *Figure 2a* and *b* suggests that *CDPK7* is involved in signaling mechanism. The signaling initiated in roots and then it gradually moved towards leaves. A comparison of *CDPK7* expression between roots and leaves shows that in roots the gene is displaying a steady declining trend (*Fig. 2b*) while in leaves the gene up regulates up to 12 h followed be down regulation (*Fig. 2a*). This comparison indicates that *CDPK7* initiated signaling from roots which gradually switched towards the aerial parts i.e., leaves. The higher expression of *CDPK7* in roots, at control conditions (i.e., 0 h) suggests its involvement

in root development and elongation of root hair cells (Ivashuta et al., 2005). With subsequent increased level of dehydration stress the expression apparently moved towards leaves. It can be deduced that the activity of kinases is required during stress conditions as they perform phosphorylation of their target proteins (Mahajan and Tuteja, 2005) and thus altering proteins activity, functioning and at times their cellular location. The overall net impact of this phosphorylation appears in the form of better survival of the plants which encounters osmotic stress conditions.

Conclusions

Drought is one of the major problems for agriculture in present as well as in near future. Developing drought tolerant varieties should be the integral part of a comprehensive plan to face this challenge. Therefore, identifying novel drought responsive genes and investigating their expression pattern was thought necessary and provided a motivation to conduct this research. Two genes namely *GSNOR* and *CDPK7* were identified and their expression pattern was analyzed. It was deduced that these identified genes regulate certain important biochemical as well as physiological phenomena thus enabling maize plants to encounter drought stress. In future, a comprehensive screening program of various maize genotypes for drought tolerance is recommended through using gene expression profiling of drought responsive genes such as *GSNOR* and *CDPK7*. Altogether it is recommended to exploit the identified genes for developing drought resilience in maize crop through selection, hybridization and gene transformation.

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