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Full Length Article

Drought Stress Induces Differential DNA Methylation Shift at Symmetric and Asymmetric Cytosine Sites in the Promoter Region of *ZmEXPB2* Gene in Maize

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Abstract

β-expansin 2 (*EXPB2*) gene induces drought tolerance in different plant species including maize. Different epigenetic mechanisms like DNA methylation, histone modification and RNA interference affect the gene activities under stress conditions. DNA methylation, an important epigenetic mechanism, could be involved in the regulation of *ZmEXPB2* gene under drought stress in maize. Plants of drought sensitive variety 'Jalal' were grown till 4th leaf stage under well-watered conditions. At 5th leaf stage, plants were divided in two groups *i.e.*, well-watered (100% water holding capacity) or drought stress (0% water holding capacity for 15 days). Plants subjected to drought stress showed clear signs of stress by significant decrease in fresh weight of whole plant, 6th leaf length, stunted secondary root growth and increased primary root length. DNA methylation profile of three regions (denoted as -1.7 k, -1.3 k and -0.8 k) in the promoter of *ZmEXPB2* gene, of root DNA, were evaluated. Under well-watered conditions, heterogeneity in DNA methylation profile along the promoter sequence was observed. Regions -1.7 k and -1.3 k were methylated whereas the region -0.8 k was nonmethylated. After the comparison of DNA methylation profile of well-watered and drought stress plants, no change in -1.7 k and -0.8 k regions was observed. However, the -1.3k region had significant decrease in the DNA methylation at symmetric cytosine sites *i.e.*, cytosine-guanine (CG) dinucleotides and cytosine-adenine/cytosine/thymine-guanine (CHG where H = A, C or T) trinucleotide and significant increase at asymmetric cytosine sites (CHH) under the stress condition. In addition, significant increase in the gene expression of *ZmEXPB2* under drought was also observed. In conclusion, drought stress conditions induce DNA hypomethylation at CG, and CHG sites and DNA hypermethylation at CHH sites in the middle region of the promoter of *ZmEXPB2* gene. This shift can be associated with the up regulation of *ZmEXPB2* gene which in turn increased primary root length as a plant stress response mechanism.

Keywords: Epigenetics; Hypermethylation; Hypomethylation; Water deficit conditions; *Zea mays* L.; *ZmEXPB2*

Introduction

Plants, being sessile in nature, constantly encounter environmental changes and have developed multiple adaptive responses through regulation of different physiological and developmental mechanisms. This modulation is achieved through the regulation of activities of stress responsive genes (Farooq *et al.* 2011; Wang *et al.* 2011). In recent decades, evidences have confirmed the role of different epigenetic mechanisms, like DNA methylation, histone modification and RNA interferences, in the regulation of gene expression under stress conditions (González *et al.* 2013; Li *et al.* 2014; Kaleem *et al.* 2019). DNA methylation is addition of methyl group to either adenine or cytosine. In eukaryotes, it mainly occurs at cytosine to make it 5-methylcytosine (5-mC). The 5-methylcytosines are studied under three contexts: CG, CHG and CHH (H corresponds to A, C or T) (Zilberman *et al.* 2007; Lang *et al.* 2015). DNA methylation is evolutionarily ancient and play significant role in various important biological processes of plant growth and development like seed development, hybrid vigor, metabolite synthesis (Xing *et al.* 2015), heterosis of hybrids (Kawanabe *et al.* 2016; Lauss *et al.* 2018), fruit ripening (Zhong *et al.* 2013; Liu *et*

al. 2015; Li *et al.* 2018), synthesis of secondary metabolites (Conde *et al.* 2017), genome stability and gene regulation (Bird 2002). Involvement of DNA methylation in regulating stress responsive genes under abiotic stress especially in drought has been reported in many studies (González *et al.* 2011, 2013).

Drought is one of the most important abiotic stresses that significantly effects the plant growth and yield of crops, including maize (*Zea mays* L.), posing a serious threat to achieving the goal of food security (Hussain *et al*. 2018; Mi *et al.* 2018; Shafiq *et al*. 2019). It affects the morphological trails, photosynthesis rate and grain quality in terms of nutritional composition and quantity in terms of yield of maize (Gheysari *et al.* 2017; Hussain *et al*. 2019; Danish *et al*. 2020). Therefore, a better understanding of the plant response mechanisms against drought stress is vital.

Plant responds to drought stress by inducing changes in plant physiology through regulation in expression of many underlying drought responsive genes (Farooq *et al.* 2014, 2017). One important physiological process is cell wall elongation which influences the plant growth by regulating the cell extension and is often considered to be the earliest visible effect of stress (Gall *et al.* 2015; Ezquer *et al.* 2020). It is regulated by proteins like endo- 1,4-b-D-endoglucanase (EGase), xyloglucan endotransglucosylase (XET), expansins (EXP), and plasma membrane proton pump (PM-H+-ATPase, MHA) (Geilfus *et al.* 2011; Kaleem *et al.* 2019). Various studies have confirmed the importance of expansins in the cell wall elongation and plant response against abiotic stress (Guo *et al.* 2011; Zhou *et al.* 2015; Marowa *et al.* 2016). Among expansins, β-expansin is a large gene family, present in maize and other plants, the protein products of which play multiple roles but are mainly involved in cell enlargement through cell wall loosening by disturbing the hydrogen bonding within cell wall's cellulose fibers relying on pH as H^+-ATPase inhibition causes increase in cell wall pH (Zhao *et al.* 2012) thus contributing to growth of plant tissue (Boron *et al.* 2015). An upregulation in the expression of different expansin genes like *TaEXPB23* in *Nicotiana tabacum* and *RhEXPA4* gene in *Arabidopsis* under drought condition have been observed (Li *et al.* 2011; Lü *et al.* 2013). *EXPB2*, an important gene of β-expansin gene family, show upregulation under different abiotic stresses like salinity and drought stress in different species including maize (Guo *et al.* 2011; Zhao *et al.* 2012; Li *et al.* 2014; Kaleem *et al.* 2019). In *Glycine max*, the higher expression of *EXPB2* gene accompanied with improved root tolerance to water stress was reported in water stressed plants (Guo *et al.* 2011). The upregulation of *EXPB2* gene in *Solanum pennellii* under drought stress conditions indicated towards the strong association between *EXPB2* gene and drought stress tolerance (Egea *et al.* 2018). Interestingly, the involvement of epigenetic mechanisms at both DNA methylation and histone acetylation levels in the regulation of *EXPB2* gene in maize against salt stress have already been reported (Li *et al.* 2014; Kaleem *et al.* 2019). Li *et al.* (2014) revealed an increase in H3K9 acetylation was associated with the *ZmEXPB2* up-regulation under salt stress conditions. Similarly, *ZmEXPB2* gene up-regulation by the DNA hypomethylation in this gene induced by salt stress in maize has been reported (Kaleem *et al.* 2019). But the *ZmEXPB2* gene regulation by DNA methylation shift in maize under drought stress need to be explore. Therefore, to test the hypothesis that DNA methylation is involved in the regulation of *ZmEXPB2* gene under drought stress, this study was designed. Single base pair resolution of three regions in the promoter of *ZmEXPB2* gene through bisulfite (direct) sequencing revealed the induction of region-specific differential DNA methylation shift at symmetric and asymmetric cytosine sites in promoter of *ZmEXPB2* gene due to drought stress.

Materials and Methods

Plant material development and drought treatment

Seeds of a drought sensitive maize (*Zea mays* L.) variety "Jalal" (Basir *et al.* 2018) were acquired from Cereal Crops Research Institute (CCRI), Pirsabak, Nowshera, Pakistan. Seeds imbibition was done by soaking in distil water for an interval of four hours at a temperature of 45°C. Six pots (19.5 cm in diameter and 25 cm in height each) having 2 kg of silty loam soil (50% silt, 30% clay and 20% sand) were prepared and three imbibed seeds were then sown per pot. After germination, the seedlings were thinned to one seedling per pot. The plants were grown in 13 h daylight at a temperature around 28/22°C (day/night) in open air nursery. As water holding capacity can be determined by measuring soil moisture at filed capacity and at permanent welting point, the amount of water needed to achieve 100% water holding capacity (WHC) of the soil was measured by gravimetric method using the following formula:

$$\text{Soil moisture content} = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Dry weight}}$$

The plants were irrigated to 100% WHC till 5th leaf stage by adding 460 mL water per pot per day (Virlouvet *et al.* 2011). Each pot was provided with 15 g of NPK at 3rd leaf stage. At 5th leaf stage, drought-stress was carried out in three selected pots by withholding the irrigation and maintaining the 0% WHC for 15 days and the remaining plants were well-watered (100% WHC). The experimental design was Completely Randomize Design (CRD) consisted of 3 replicates. The whole plants along with roots were harvested. Phenotypic data from three plants per treatment was recorded for fresh plant weight, 6th leaf length and primary root length. Roots from each plant was separately sampled and stored at -80°C for molecular analysis. Two plants from each treatment were used for the molecular analysis.

DNA and RNA extraction

DNA from 200 mg root sample of each plant was separately extracted by using DNA extraction kit. RNA from 200 mg root sample from each plant was extracted through CTAB method with slight modifications (Murray and Thompson 1980). The quality of the extracted DNA and RNA was checked by running on 1% agarose gel. The quantification of DNA and RNA was done by determining the visible light-UV absorbance on the Colibri NanoDrop spectrometer (Titertek Berthold, Germany).

Bisulfite treatment and direct sequencing of the amplified PCR product

EZ DNA Methylation-Gold" kit (Zymo Research) was used to treat 350 ng of genomic DNA, from each extracted sample, with sodium bisulfite by following manufacturer's protocol. 2 μL of eluded solution was used for each PCR reaction. To analyze the overall methylation pattern of the promoter region of *ZmEXPB2*, bisulfite specific PCR primer pairs were designed to amplify three regions (denoted as -1.7 k, -1.3 k and -0.8 k) located in the promoter of *ZmEXPB2* gene (Table 1) through Methyl Primer Express v. 1.0 software (Applied Biosystems). For this purpose, 1800 bp sequence, upstream of Transcription Start Site (TSS) was used.

The PCR was carried out using 2X Topsimple DyeMIX®_multi HOT master mix with following reagent quantitates: 15 μL of final volume containing 7.5 µL of master mix, 1.5 μL of forward and reverse primer each, 2 µL of DNA template (bisulfite treated) and 2.5 μL of dH_2O. The PCR program used was as follow: an initial denaturation step (95°C for 10 min) was followed by 10 touch-down cycles (94°C for 1 min; 65°C - 55°C for 1 min (a decrease of 1°C after each cycle); 72°C for 1 min 30 s) which was followed by another 10 touch-down cycles (94°C for 1 min; 55°C -50°C for 1 min (a decrease of 0.5°C after each cycle); 72°C for 1 min 30 s) and then 20 cycles (94°C for 45 s; 50°C for 45 s; 72°C for 1 min), ending with a final elongation step (72°C for 7 min) as adopted from Khan *et al.* (2013). The amplicons were visualized on 1% agarose gel, eluded and were sequenced. The sequences were analyzed and compared through Mutation Surveyor DNA variant analysis software version 3.97 (Soft Genetics) following the previously reported parameters (Khan *et al.* 2013).

Gene expression analysis through Semi-quantitative PCR

The cDNA was produced through Hyperscript™ First strand synthesis kit (GeneAll Biotechnology) by using 500 ng of RNA of each sample. The semi-quantitative PCR was done using 2X Topsimple DyeMIX_multi HOT master mix in thermal cycler (applied Biosystem v. 1). The primers used to semi-quantitative PCR are given in Table 1. The PCR product was visualized on 1% agarose gel and was quantified by ImageJ software. ACT2 gene was amplified by using the primers (ACT2-F: 5'-CTGAGGTTCTATTCCAGCCATCC-3' and ACT2-R: 5'-CCACCACTGAGGACAACATTACC -3') as housekeeping for the purpose of normalization.

Statistical analysis

The phenotypic and molecular data was statistically analyzed by performing analysis of variance (ANOVA) by using "agricolae package (Felipe 2009) in R core" (R Core Team 2019). The model used to test the significance of variation in phenotypic traits and DNA methylation levels: $Yij = \mu\ Tj + \varepsilon ij$ where T represents treatment effect (well-watered vs drought stress conditions) and εij represents the residual effect. MS Excel was used for the calculation of standard deviation and graphical presentation of the data.

Results

Drought stress decrease plant growth

Plants showed significant decrease in fresh weight and 6th leaf length whereas a significant increase in the primary root length was also observed under drought stress (DS) conditions (Fig. 1 and Table 2). The plants under drought stress showed clear signs of wilting and stunted growth of secondary roots as compared to the plants grown under normal conditions (Fig. 1).

Overall DNA methylation pattern of promoter region of *ZmEXPB2* under well-watered conditions

Under WW conditions, fragment -1.7 k showed 77% of CG sites (7 out of 9), 73% of CHG sites (11 out of 15) and 38% of CHH sites (20 out of 52) showed certain level of methylation. Fragment -1.3 k showed 100% of CG sites (13 out of 13), 90% of CHG sites (9 out of 10) and 21% of CHH sites (8 out of 37) showed certain level of methylation. Interestingly, fragment -0.8 k showed no methylation at any of the cytosine sites present in the fragment.

Overall, 65% DNA methylation level at CG sites, 47% DNA methylation level at CHG sites and 10% DNA methylation level at CHH sites (Fig. 2) were observed in fragment -1.7 k under WW conditions. Fragment -1.3 k showed 98% DNA methylation level at CG sites, 72.5% at CHG sites and 7% at CHH sites (Fig. 2). These results confirm that the DNA methylation of the promoter region of *ZmEXPB2* gene is heterogeneous along its sequence, *i.e.*, regions -1.7 k and -1.3 k were methylated whereas the fragment -0.8 k was nonmethylated under WW conditions (Fig. 2).

Table 1: Primer sequences and specification of PCR amplicons used for DNA methylation analysis

Fragments	Primers	Sequence (5'-3')	Position of fragments in the *ZmEXPB2 gene* from 1800 bp upstream of TSS	Amplicon Size (bp)
-1.7k	F	ATATTTTTATTTAATTTGGAGGTT	302-637	338
	R	ATATTAAAACTTACTCTCCTAAACAA		
-1.3k	F	TATTTTGTTTAGGAGAGTAAGTTTTAATA	607-1031	426
	R	AAAAACACAAATAATTTTTAAATCATA		
-0.8k	F	GATTAAGGTGTTTTAAGATTTAAATAGA	1281-1571	291
	R	TAACTCACCTCACTAATCACTTATC		
ZmEXPB2-q	F	CACCACCCACCACTACTACCA	3997-4164	163
	R	AACGACTCAAAGGACCATGACAA		

Table 2: Effect of drought stress on phenotypic and molecular parameters of maize

Parameters		Well-watered	Drought stress
Phenotypic parameters	6^{th} Leaf length (cm)	35.63 ± 4.22 a	23.20 ± 1.59 b
	Fresh biomass (g $plant^{-1}$)	11.01 ± 1.92 a	6.30 ± 1.17 b
	Primary root length (cm)	28.67 ± 2.08 b	39.33 ± 1.53 a
DNA methylation percentage of CG,	CG	0.65 ± 0.01^{NS}	0.71 ± 0.007^{NS}
CHG and CHH in Fragment -1.7 k	CHG	0.48 ± 0.008^{NS}	0.51 ± 0.0078^{NS}
	CHH	0.10 ± 0.001^{NS}	0.10 ± 0.0051^{NS}
DNA methylation percentage of CG,	CG	0.98 ± 0.003 a	0.91 ± 0.0032 b
CHG and CHH in Fragment -1.3 k	CHG	0.73 ± 0.009 a	0.48 ± 0.0019 b
	CHH	0.08 ± 0.0005 b	0.21 ± 0.0005 a
Relative expression of *ZmEXPB2*		0.88 ± 0.106 b	1.90 ± 0.1414 a

Means ± standard deviation sharing same letters differ non-significantly ($P > 0.05$). NS represents non-significant results. The small alphabets represent the significant variation between well-watered and drought stressed plants at $P < 0.05$

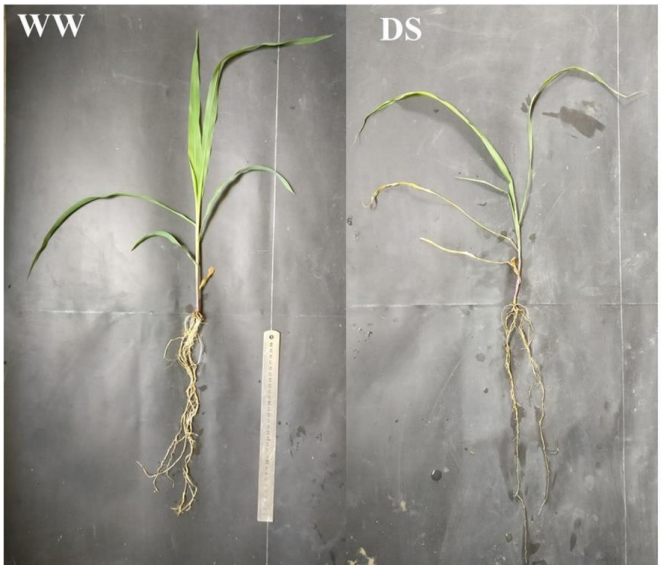

Fig. 1: Effect of drought stress on the phenotype of maize
WW and DS represent well-watered plants and drought stressed plant respectively

Drought stress heterogeneously influences the DNA methylation pattern of the different regions in the promoter of *ZmEXPB2* gene

Comparative analysis of WW and DS conditions revealed that all the three studied fragments showed their specific pattern. The fragment -0.8 k showed no DNA methylation in both normal and stress conditions. Fragments -1.7 k and -1.3 k showed DNA methylation so, the results of each of these two fragments are separately elaborated.

In fragment -1.7 k, no significant variation in overall DNA methylation profiles between WW and DS plants was observed in all three contexts (Table 2). Significant increase in DNA methylation percentage of only one out of 9 CG sites, and only one out of 13 CHG sites was observed, when site per site analysis was performed (Fig. 3). Only 05 out of 52 CHH sites showed significant shift (CHH54, CHH156 and CHH282 showed DNA hypomethylation whereas sites CHH181 and CHH282 showed DNA hypermethylation) in DNA methylation percentages due to stress conditions (Fig.

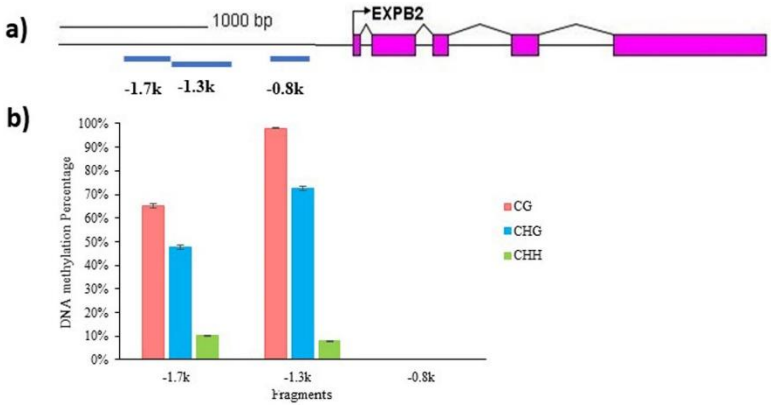

Fig. 2: DNA methylation pattern of studied regions of promoter of *ZmEXPB2* gene under normal conditions **a**) Gene structure of *ZmEXPB2* gene (based on Genebank accession GRMZM2G021621) **b**) DNA methylation pattern of regions of promoter of *ZmEXPB2* gene under well-watered conditions in three cytosine contexts
Horizontal blue lines represent the studied regions in the promoter of *ZmEXPB2*

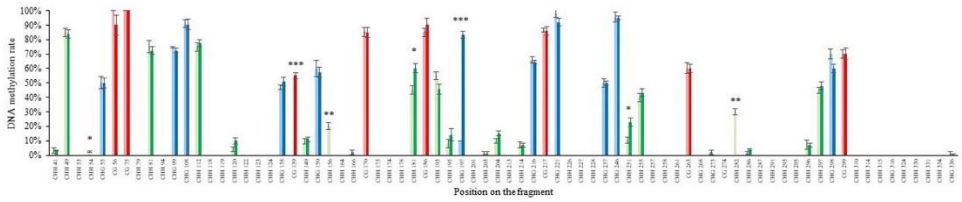

Fig. 3: Site per site DNA methylation variation in cytosine sites in -1.7 k region in promoter of *ZmEXPB2* gene due to drought stress conditions. CG, CHG and CHH contexts, are represented in red, blue and green colors respectively
Faint and strong colors represent well-watered plants and drought stressed plants respectively. The numbers with the cytosine context shown in the X-axis represent the position of that cytosine in the studied fragment. Statistical significance of methylation variation caused by drought is represented by stars with $^{**}P < 0.01$ and $^{***}P < 0.001$

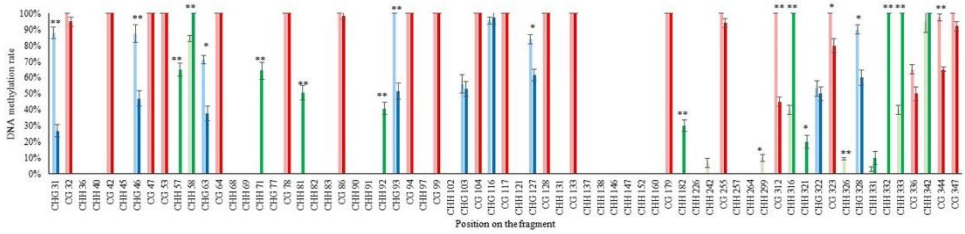

Fig. 4: Site per site DNA methylation variation in -1.3 k region in promoter of *ZmEXPB2* gene due to drought stress. CG, CHG and CHH contexts, are represented in red, blue and green colors respectively
Faint and strong colors represent well-watered plants and drought stressed plants respectively. The numbers with the cytosine context shown in the X-axis represent the position of that cytosine in the studied fragment. Statistical significance of methylation variation caused by drought is represented by stars with $^{**}P < 0.01$ and $^{***}P < 0.001$

3). These results confirm that DS does not affect overall DNA methylation profile at all the three contents (CG, CHG, CHH) in this region.

In fragment -1.3 k, the comparison of overall DNA methylation profile revealed significant shift under WD conditions in all the three contexts. DNA methylation percentage significantly decreased from 98% (in WW plants) to 90.9% (in DS plants) at CG sites, and from 72.5% (in WW plants) to 48.5% (in DS plants) at CHG sites. Interestingly, an inverse pattern of shift was observed in CHH sites where DNA methylation percentage showed significant increase from 7.8% (in WW plants) to 21.1% (in DS plants) (Table 2). The site per site analysis revealed that DNA methylation at many of the CG sites remained the same in WW and DS conditions. In contrast most of the CHG sites (7 out of 9) showed significant decrease in DNA methylation due to DS conditions. Only a small fraction of CHH sites showed DNA methylation in WW and DS conditions. Interestingly, most of these CHH sites (10 out of 15) showed pattern of DNA hypermethylation due to drought stress conditions (Fig. 4). These results confirm that DS conditions significantly affect overall DNA methylation profile by inducing a shift in DNA methylation at all the three contents (CG, CHG, CHH) in this region and this shift is context specific.

Drought stress condition causes increased gene expression of *ZmEXPB2*

Plants which experienced drought stress exhibited

significantly higher level of *ZmEXPB2* gene expression as compared to plants grown in well-watered conditions (Table 2) confirming the upregulation of *ZmEXPB2* gene under DS conditions.

Discussion

The results of this study confirm the hypothesis that DNA methylation is involved in the regulation of a stress responsive gene, *ZmEXPB2*, under drought stress conditions in maize and through this regulation, influence the plant stress response by playing its role in increasing the primary root length. Various studies have confirmed the involvement of epigenetic mechanisms like DNA methylation and histone modifications in the regulation of this gene under salt stress conditions (Li *et al.* 2014; Kaleem *et al.* 2019) but these studies particularly on DNA methylation provided very limited information as it used Methyl Sensitive Primers after bisulfite treatment which provides methylation status at only the cytosines present complimentary to primer sequence (Kaleem *et al.* 2019). Therefore, to further elaborate the involvement of DNA methylation in regulation of *ZmEXP2* gene in drought stress conditions, an in-depth analysis of DNA methylation in the promoter region of this gene was carried out.

DNA methylation was first analyzed under well-watered conditions to identify the position for initiation and termination of DNA methylation across the promoter. The data suggests that DNA methylation appeared in the distant regions of promoter, increased in the middle region and then diminished in the closer part to TSS. These results indicates towards the varying role and level of involvement of these fragments in the regulation of the *ZmEXPB2* gene and also points out that the regulatory elements present in the middle or distal parts (from TSS) in the promoter may play critical role through their involvement in the epigenetic regulation of this gene. Previous reports in both plant and mammals confirm this observed pattern that different regions in the regulatory regions or in the body of the gene may show different DNA methylation patterns and may be critical for gene regulation (Feldmann *et al*. 2013; Gent *et al.* 2013; Khan *et al.* 2013).

The comparison between well-watered and drought stressed plants also revealed heterogeneity in the pattern of shift in DNA methylation profile of the three studied fragments, further strengthening the hypothesis that different region in the promoter of *ZmEXPB2* gene have their differential roles in gene regulation. The absence of any shift in DNA methylation pattern in fragment -0.8 k and -1.7 k show that DNA methylation is not involved in regulating the cis acting elements present in these fragments. Inversely, the fragment -1.3 k was identified which showed significant shift in the DNA methylation pattern under drought conditions indicating towards the involvement of DNA methylation in the regulation of the cis acting elements of this region. Similar pattern of heterogeneity in shift in DNA methylation pattern under varying environmental conditions have been shown in different studies like *VRN1A* gene in wheat where only regions in intron 1 showed shift in DNA methylation due to vernalization (Khan *et al.* 2013) and *Asr1* gene in tomato which also showed the varying pattern of DNA methylation shift due to drought stress in different regions along the gene (González *et al.* 2011).

In order to better understand the involvement of this fragment (-1.3 k) in the gene regulation, in-depth DNA methylation analysis (site per site analysis) was performed. Interestingly, in fragment -1.3 k, symmetric and asymmetric cytosine methylation showed inverse trends in the shift in DNA methylation. Under stress conditions, symmetric cytosine methylation (both CG and CHG sites) significantly decreased whereas asymmetric cytosine methylation significantly increased. Though the mechanism of *de novo* methylation is same for the three contexts, the maintenance of DNA methylation at these three contexts require different DNA methyltransferases, it appears that these contexts may have differential roles in gene regulation. The DNA methylation at asymmetric cytosine sites is maintained by RNA-directed DNA methylation (RdDM) depandent DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) and RdDM independent CHROMOMETHYLASE 2 (CMT2) (Zhang *et al.* 2018) whereas symmetric cytosine sites *i.e*., CG and CHG sites uses METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLASE 3 (CMT3) respectively for the maintenance (Lindroth *et al.* 2001; Law and Jacobsen 2010). Recently, differential pattern of shift depending upon the context of cytosine and their distinctive roles have been reported (Wang and Baulcombe 2020). DNA hypomethylation at symmetric cytosine methylation have inverse relationship with the gene expression (Song *et al.* 2012; Roessler *et al.* 2016). This decrease in DNA methylation of symmetric cytosine methylation is in accordance with the inverse relationship between promoter methylation and increased gene expression. Similar kind of DNA hypomethylation and increase in *ZmEXPB2* gene expression under salt stress conditions have been previously reported (Kaleem *et al.* 2019). The association between increased CHH in the upstream region of the promoter and increased gene expression has also been reported in maize (Gent *et al.* 2013). Therefore, it was anticipated that hypomethyation at symmetric cytosine site and hypermethylation at asymmetric cytosine sites in the middle region may induce an upregulation of *ZmEXPB2* gene. In consistence with these expectations, a significant increase in the *ZmEXPB2* gene expression under drought stress conditions was observed in this study.

EXPB2 belongs to expansins gene family which is one of the key regulators in cell wall elongation by modifying the cross-links between cellulose microfibrils and polysaccharides (Sharova 2007). It is an important gene that is involved in the regulation of root elongation in maize and

in other species through controlling the cell wall extensibility (Kam *et al.* 2005; Guo *et al.* 2011). The significant upregulation of *ZmEXPB2* gene in the plant root under drought stress conditions indicates that it could be involved in the regulation of root length and may serve as an important candidate gene to be used in crop improvement prog. Our results of the phenotypic data confirm the correlation between the increase in gene expression of this gene and the observed increase in root length as a plant stress response which is in accordance with the previous reports (Kam *et al.* 2005; Guo *et al.* 2011). The drought stress conditions directly affect cell division and cell elongation due to deficit in external water potential (Farooq *et al.* 2009) therefore upregulation of this gene may have facilitated the cell elongation by regulating the cross-links between cellulose microfibrils and polysaccharides and induced an increase in the root length as an attempt by the plant to find more water and mitigate the stress conditions. From all these, it can be inferred that the observed increase in the primary root growth is a plant stress response against drought stress which is caused by an upregulation of *ZmEXPB2* gene that is modulated by a differential shift in DNA methylation profile in the promoter region of *ZmEXPB2* gene in maize.

Conclusion

Results of this pot experiment confirm the important role of DNA methylation in regulating the *ZmEXPB2* gene under drought stress conditions. The middle region in the promoter of *ZmEXPB2* gene was identified where symmetry dependent shift in DNA methylation across cytosine sites may cause an increase in the *ZmEXPB2* gene expression in the roots which in turn may induce the root growth under drought stressed conditions.

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Author Contributions

ZR devised the methodology, conducted the investigation and helped in drafted the original manuscript. AB and SMS curated and analyzed the data. GS, KA, MS and SR critically reviewed and edited the manuscript. ARK conceptualized and supervised the study, devised and validated the methodology, acquired funding, administered the project, provided resources and, drafted the manuscript.

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