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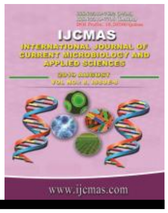

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Differential DNA Methylation under Drought Stress in Maize

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ABSTRACT

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DNA methylation plays a vital roles in plant response to drought stress. Therefore, the present study was carried out to investigate differential cytosines methylation in leaves of maize under drought stress by using AMP method. Thirty days old maize seedlings were subjected to water deficit for nine days and the leaves of drought stressed plants and control plants were collected and stored at -20 until DNA extraction and subsequence DNA analysis. We isolated and sequenced 18 differentially methylated fragments. DMF 4, DMF 10, and DMF 13 were found to be homologous to lncRNA genes, DMF3 is similar to protein dehydration-induced 19 homologue 3 and DMF 18 is homologous to nudix hydrolase 2 gene. The obtained results indicated that lncRNAs are consider as important regulators in response of maize plant to drought stress.

Introduction

Drought is one of the abiotic stresses that affect plant growth, productivity and cause significant crop losses on annual basis. Therefore, it is important to know how plants respond to drought stress by altering its biological activities. Plant faces abiotic stress by epigenetic changes that modify gene expression of the drought-responsive genes (Setter *et al.*, 2011; Boyer *et al.*, 2012; Kakumanu *et al.*, 2012; and Mao *et al.*, 2015).

Epigenetic changes, such as cytosine DNA methylation, in plants play crucial roles in gene expression and silencing of transposon. There are four different types of DNA methyltransferases that achieve the methylation of cytosines in three different forms, CG, CHG, and CHH (Chan *et al.*, 2005; Zhang *et al.*, 2006 and Suzuki and Bird, 2008). Recent studies have revealed that plant responds to abiotic stress by methylation of genomic DNA (Steward *et al.*, 2002 and Choi and Sano, 2007). Under abiotic stresses, it has been revealed that cold stress induced DNA methylation in maize (Steward et al, 2002). Drought stress induced specific cytosine hypermethylation in the pea genome (labra et al, 2002) and induced methylation alteration in leaves of maize seedlings (Tan, 2010). It was found that after drought stress in Populus, the levels of methylated cytosines significantly increased, including 2kb upstream and downstream from transcription start sites, and in repetitive sequences (Liang *et al.*, 2014).

Certain lncRNAs are induced during abiotic stress and play critical regulatory roles in plant response to environmental stress (Wang et al, 2017). It was found that drought-induced lncRNA in Arabidopsis thaliana which indicated the important role of lncRNAs in regulating plant tolerance to abiotic stress (Qin *et al.*, 2017). Although some studies on the role of lncRNAs in plants have been carried out, comprehensive studies of the response of the lncRNA to drought stress remain limited. Therefore, the present investigation was carried out to detect methylation polymorphism due to water stress in maize by using amplified methylation polymorphism method.

Materials and Methods

Growth conditions

Maize inbred line W22 was used in the current investigation, which supplied by the United States Department of Agriculture (USDA).A single seed was grown in about 7 liter plastic pots containing sand soil in July 2015. Temperatures ranged between 33 to 29 for high temperatures and between 26 to 22 for low temperatures, the average of the relative humidity was 68.47% during the period of this experiment. Maize seedlings were irrigated to field capacity until the application of drought stress treatments. Macro elements NPK fertilization, urea (46% nitrogen, monosuperphosphate (12.5% phosphate) and potassium sulfate (50% potassium) were added to each soil of plant pot at 15, 25 and 37 days after planting date. Fertilization was applied using a slow-release fertilizer consisted of 0.6% ferrous, 0.6% Manganese, 0.6%Zinc, 0.08% Copper and 0.08% Magnesium was added once a weak until the application of drought stress treatments.

Drought stress application

Drought stress was carried out on thirty days old seedlings by preventing the irrigation of plants for nine days, while control plants were irrigated regularly. Leaves of control and drought-stressed plants were collected and stored at -20°C until DNA extraction and subsequent DNA analysis. DNA extraction from leaves of maize inbred line W22 plants was carried out by using bioPLUS kit.

Determination of DNA purity and Concentration

To determine DNA purity and concentration of the genomic DNA isolated from leaves of the maize inbred line W22, the ultraviolet spectrophotometer method was used as described by Glasel (1995); Huberman (1995) and Manchester (1996).

Procedure of amplified methylation polymorphism

To detect DNA methylation, the amplified methylation polymorphism (AMP) method described by (Phutikanit *et al.*, 2010) with modification was used. Genomic DNA of leaves of inbred line W22 was digested by HpaII and MspI restriction enzymes, as recommended by New England BioLabs. Purified digested DNA samples were amplified by using a single primer contain the recognition sequence of the two isoschizomers (CCGG). PCR amplification was performed in 25 µl of a reaction mixture containing 1 unit Taq polymerase (Thermoscientific), 1X reaction buffer (containing 5 mM dNTPs, 15 mM MgCl2, stabilizers, and enhancers) and 0.5 µM of single decamer primer (Biosearch technologies, USA). The sequences of the primers used in this study are shown in table (1). The cycling program included initial denaturation of one cycle at 95 °C for 3 minutes,denaturation at 95 °C for 45 seconds, the annealing temperature of 37°C, and extension at 72 °C–1 min for a total of 35 cycles and a final extension at 72°C–7 min for 1 cycle. Amplified PCR products were separated at 6% polyacrylamide gel and stained by silver nitrate (Bassam and Gresshoff 2007).

Recovery of selected PCR products

PCR products were purified from 18differentially methylated bands as described by Sanguinetti *et al.*, (1994), reamplified with the same PCR conditions, and subjected to cloning by using the pGEM®-T Easy Vector System I.

DNA sequencing and data analysis

Positivecolonieswere sent toGenewiz(United Kingdom) for sequencing. BLAST searches against Zea mays genome databases at the NCBI (http://blast.ncbi.nlm.nih.gov) website was used to identify the resulting sequences.

Results and Discussion

Drought-stressed maize inbred line W22 plants showed the rolling leaves, a result of water deficit, as the rate of transpiration exceeds water uptake (McCue, 1990) in comparison with normal plant leaves of control plants (Figure S1). Extracted DNA samples were digested by methylation-sensitive restriction enzymes HpaII, blocked by CG methylation, and MspI, which blocked by external cytosine methylation. Both enzymes are unable to digest both cytosines methylation (full methylation). Six primers were used for amplification of the genomic DNA extracted from leaves of control and drought-stressed plants (Figure 1).

Differentially Methylated DNA Sequences Analysis

The obtained differentially methylated DNA fragments (DMFs) were sequenced (fasta sequences were mentioned at S2 file) and analyzed by NCBI BLAST (Table 2). The sequence analysis of selected differentially methylated DNA fragments revealed that five sequences, encoded as DMF3, DMF4, DMF13, DMF16, and DMF18, are homologous to intergenic regions, while DMF10 is similar to intragenic regions (Table 3). DMF3 was present only after digestion with restriction enzyme HpaII, which represents drought-induced CG methylation. DMF3 is located upstream of Protein DEHYDRATION-INDUCED 19 homolog 3 gene. Three differentially methylated DNA fragments are similar to lncRNA genes. DMF4 and DMF10 had internal and external cytosine methylation under non-stress conditions, which indicate the existence of DNA demethylation under drought stress. DMF13 had internal cytosine methylation under the control condition and was absent under drought stress. DMF10 overlapped with the second exon of loc103635457 (lncRNA gene), whileDMF4 and DMF13 are located downstream lncRNA genes. DMF18 was absent only after digestion with HpaII and MspI restriction enzymes, which represent internal and external cytosine demethylation under drought stress. DMF18 has located downstream nudix hydrolase 2 genes. The remaining twelve differentially methylated sequences are mapped to deep intragenic regions (at least 5kb away from a known gene) on the maize genome.

Table.1 Sequences of the primers used for Amp PCR

Primer	Sequence (5' to 3')
P1	TGGACCGGTG
P2	ACCCGGTCAC
P3	CACCCGGATG
P4	TCAGTCCGGG
P5	CAGTGCCGGT
P6	AGCCGGGTAA

Table.2 BLAST results of selected differential methylated fragments

DMF	Sequence location	Expect	Identities
1	Chr5:100,888,878- 100,889,347	0.0	459/470(98%)
2	Chr7:36,728,596- 36,728,988	8e-174	375/395(95%)
3	Chr10:22,664,293- 22,664,724	0.0	431/432(99%)
4	Chr6:170,351,523- 170,352,219	0.0	654/703(93%)
5	Chr1:231,772,745- 231,773,253	7e-125	428/513(83%)
6	Chr1:104,570,850- 104,571,365	0.0	513/517(99%)
7	Chr3:23,692,411- 23,692,839	0.0	425/429(99%)
8	Chr4: 4,364,531- 4,364,991	0.0	428/463(92%)
9	Chr5:181,065,261- 181,065,681	0.0	415/421(99%)
10	Chr8:81,740,729-81,741,408	0.0	672/680(99%)
11	Chr1: 293,813,390- 293,813,822	0.0	428/433(99%)
12	Chr5:43,804,361- 43,804,644	3e-138	279/284(98%)
13	Chr10: 42,514,744- 42,515,321	0.0	577/578(99%)
14	Chr5:122,150,598- 122,151,218	0.0	619/622(99%)
15	Chr1: 122,718,961- 122,719,349	5e-156	349/389(90%)
16	Chr3:118,615,902-118,616,099	3e-46	163/199(82%)
17	Chr5:152,126,082-152,126,663	0.0	544/582(93%)
18	Chr9:100,692,230-100692689	7e-165	415/463(90%)

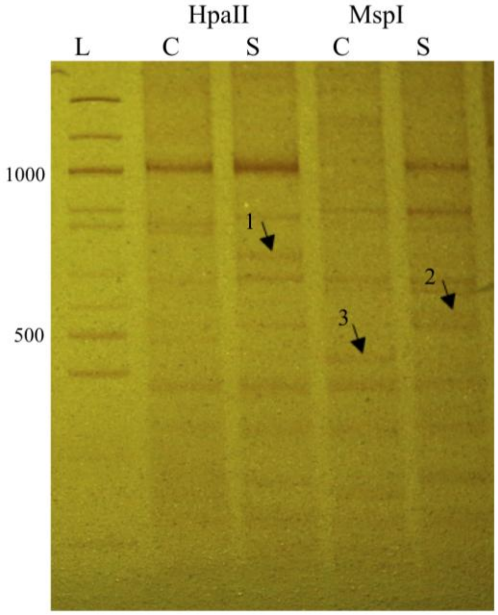
DMF: Differential methylated fragment

Table.3 Differentially methylated DNA fragments of maize inbred line W22 that show similarity to intragenic regions

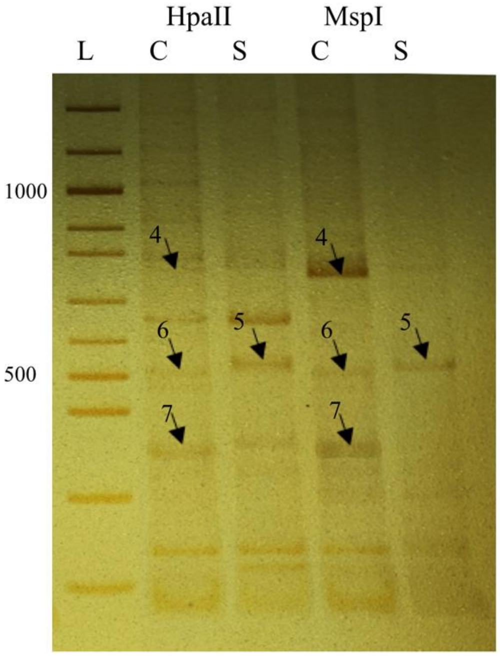
No	DMF	Status	Gene ID	Description	Position and Distance
1	3	M	LOC100284110	Protein DEHYDRATION-INDUCED 19 homolog 3	3' downstream (3474 bp)
2	4	D	LOC103630773	LncRNA	3' downstream (926 bp)
3	10	D	LOC103635457	LncRNA	Exon 2
4	13	D	LOC103641044	LncRNA	5' upstream (3889 bp)
5	16	D	LOC103652144	Uncharacterized	5' upstream (1084 bp)
6	18	D	LOC100273780	Nudix hydrolase 2	3' downstream (3403 bp)

DMF: Differential methylated fragments
M: Methylation
D: Demethylation

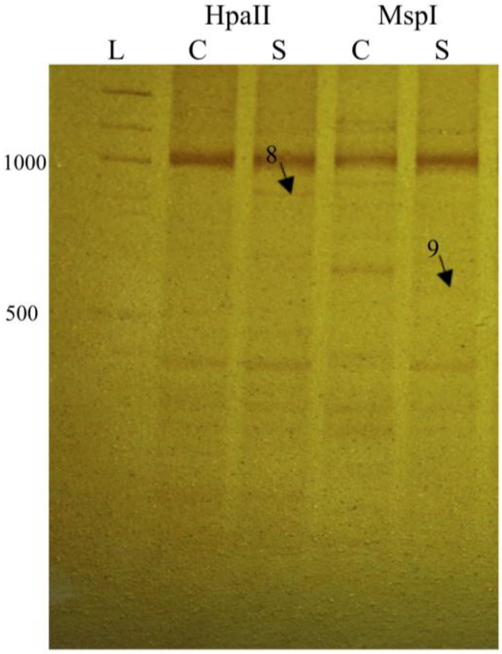
Figure.1 Representative AMP PCR profiles of control (C) and stressed (S) plants. DNA extracted from the leaves of maize inbred line W22 was digested by either HpaII or MspI and amplified by using primer 1 (A), primer 2 (B), primer 3 (C), primer 4 (D), primer 5 (E), and primer 6 (F)



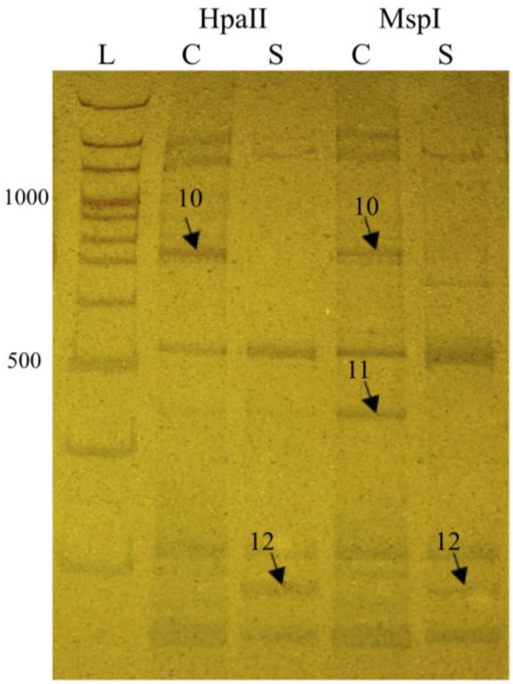
(A)


HpaII
MspI
L
C
S
C
S
1000
500
4
4
6
5
6
5
7
7

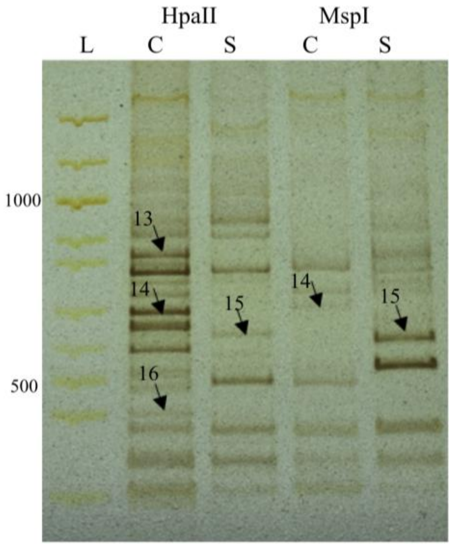
(B)


HpaII
MspI
L
C
S
C
S
1000
500
8
9

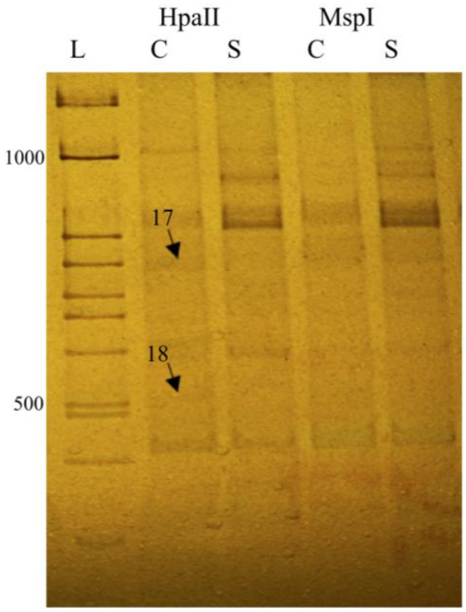
(C)


HpaII
MspI
L
C
S
C
S
1000
500
10
10
11
12
12

(D)


HpaII
MspI
L
C
S
C
S
1000
500
13
14
15
14
15
16

(E)



(F)

Supplementary Materials

Figure.S1 Represents control (left) and drought stressed (right) plants of the maize inbred line W22

S2: Fasta sequences of the eighteen differentially methylated DNA fragments

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>DMF1
        TCCGACTTCGATGCTCGAGTTTTTCAGCAAGATTGGACCGGTGGAGGACC
        CGCTTTGACTGATTCTATTGTACTTCAGCTTCGGTGATTTCCTTCTGTTT
        CTGGATTGTGACTTGACAGATTCTTGTTGTATGCCCCTTATCCTCTCCAT
        AGAAAAGGCAGAACATTTTCCTTGGTTGAGAGTTGAATCTTCCTCCGAAA
        CTCCTTCATCCTCTTCCACCTCTTGGAGTCGGTGGTCTGAACGTATTTTG
        CTGGGTTCATATGGATTGATAATGATTTTGTTGTGCCTCAATGTGACTGT
        CTTTGTTCTCGTTCTGATTTGAATTGTGTATGGTCCTGACATGCCTTGGA
        TTGAACCGTCCGCCGAAGCCCCTGGTCATCTCGGAATATCTCAAGGCCTC
        CTCTTTTCTTTGTCGAAAATCATTATTCACTCTGATATATTCATTCATCT
        TCTAGAGAAGCTTTTCCAGGGTCTGAGAGGGTTTCCTGGCGAAGTATTGT
        GGCACCGGTCCAATCTTTCTAGAAGATCTCCTACAATATTCTCAGCTGCC
        ATGGAAAATCGATGTTCTTCTTTTATTCTCTCAAGATTTTCAGGCTGTAT
        ATTAAAACTTATATTAAGAACTATGCTAACCACCTCATCAGGAACCGTTG
        TAGGTGGCGTGGGTTTTCTTGGCAATCGACTCTCATGAAAACTACGAGCT
        AAATATTCAATATGTTCCTCTTGACCAACTTTATTCTGCATTTTTTTTGA
        ACGAGGTTTAGAGCAAGCTTCAGGAAACTGAGACAGGAATTTTATTAAAA
        ATTTAAATTTTGAAGAAAGTTCAGGGTTAATAGCATCCATTTTTTTGCTTT
        GCAAGTTCCTCAGCATTCTTAACAAAAGACGTCTCTTTTGACATGTTTAA
AGTTTAAACC

>DMF2
        CCGCGTATTCCGGATGCTCGAGTTTTTCAGCAGATTGGACCGGTGAAGGT
        GAGTGCGGGGTGAAGTGGGGCGCGCTTCGGGGAGGCTTTATAGGCGCGAG
        CGAGCACGGCCGAGGGCGGGGCTCTGACATGCGCTCGGGGCGTGCACGAC
        GAACGCCAGTGCGCGCTCTGGCGCCTGGCACAGCGTCGAGCACGAGGCAG
        CGCAGAGAGAGGTCAAGTTCAAACGCGGTTTGGGCCCAAATCTTTGAGAT
        TAAGGCCACGATCCCGTTGAAAGATCTCTTCCTCTCAGGTCCCTTTGTCG
        TTTGTGTGTGGAGGCTAGCAAGTTTCGTTGACTGGATTAAGAGATAGAGA
        GGGGAGAAATCCTGTTTGTCTCACTGCCATACACAGGGAGAAAATCTCTG
        GTTTGACGTGTCCGAGGCACCGGTCCAATCTTTCTAGAAGATCTCCTACA
        ATATTCTCAGCTGCCATGGAAAATCGATGTTCTTCTTTTATTCTCTCAAG
        ATTTTCAGGCTGTATATTAAAACTTATATTAAGAACTATGCTAACCACCT
        CATCAGGAACCGTTGTAGGTGGCGTGGGTTTTCTTGGCAATCGACTCTCA
        TGAAAACTACGAGCTAAATATTCAATATGTTCCTCTTGACCAACTTTATT
        CTGCATTTTTTTTGAACGAGGTTTAGAGCAAGCTTCAGGAAACTGAGACA
        GGAATTTTATTAAAAATTTAAATTTTGAAGAAAGTTCAGGGTTAATAGCA
        TCCATTTTTTTGCTTTGCAAGTTCCTCAGCATTCTTAACAAAAGACGTCTC
TTTTGACATGTTTAAAGTTTAAACC

>DMF3
        CCGGAAATTCGGATGGCTCGAGTTTTTCAGCAGATTGGACCGGTGAGGCG
        ACCAGAGGGAGGGCGAATAGGAGCCACTAAAAATTCTCTTGCGAGAACAA
        CAACATGACTTGATTCCCAATTTAACCCAGAACCTCTAGGGACTCACAAA
        GCACCAGTTCACTGTGTAATTCAAACCATTGTCATTTGAATACACCAAAA
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        ACCTAGGAGAGAAACAAACCAACTATCTACCAAAATCAGAGACAAAATCA
        TATAACAGAGGGCGCGAAAGTGCCGGGCACCAAAACTGAGGCAGATGTGC
        CTCAAACTTTGCAAAAGTTTGTGTGTGGAGAAAGATGTCTAGTAGTGAAG
        TATCAACCCAAGCAACACAAAAACCAAATCACAAATCAAAAATCTCATTT
        AGTGTGTGTTCTGCCAGTGAGTATTGCACCGTGTGCGTGTCCCTCACGTC
        CTAGAAATCTGTCCCACCGGACCAATCTTTCTAGAAGATCTCCTACAATA
        TTCTCAGCTGCCATGGAAAATCGATGTTCTTCTTTTATTCTCTCAAGATT
        TTCAGGCTGTATATTAAAACTTATATTAAGAACTATGCTAACCACCTCAT
        CAGGAACCGTTGTAGGTGGCGTGGGTTTTCTTGGCAATCGACTCTCATGA
        AAACTACGAGCTAAATATTCAATATGTTCCTCTTGACCAACTTTATTCTG
        CATTTTTTTTGAACGAGGTTTAGAGCAAGCTTCAGGAAACTGAGACAGGA
        ATTTTATTAAAAATTTAAATTTTGAAGAAAGTTCAGGGTTAATAGCATCC
        ATTTTTTGCTTTGCAAGTTCCTCAGCATTCTTAACAAAAGACGTCTCTTT
TGACATGTTTAAAGTTTAAACC

>DMF4
        ATCATCCGAGTCGCATGCTCCGGCCGCCATGGCGGCCGCGGGAATTCCGA
        TTTACCCGGTCCACGTACTCCACATTGGCTTGCTGTCCGCATGCTTTACT
        CCGCCTCCTGCCTTTCCAGAAGCTAAGGATTTTACAAGGATTTCGTTTCA
        TCAAATCGTGAGGCCCATGCTGCAAGAAAGGAGGCCCATGCTGCAAGAGA
        TGAGGCGGTTGAGAGGCAGAGAAGGATGTAACGGCTGCGCTCCAAGAAAA
        TTGCAATCCATGAAAGAGGAAGGAAAAAAAACGGATAATGAGAAACGTGG
        CATCACAGGATTTCCATTCGCTCCAGTTTCGCGCACGATTCCGCAAATCT
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        GCAGTTTTGCCAAACCAGTTGAAAAGGACGTGGAGAACACATAGTACCAC
        TAAACCTCCTACATCCAGATGTGATACTGGATTTTTTTTCTTCGACAACA
        TACCCTACTGCGGTACTATTTGGTAACAAGTTGTGGCATAGAAAAGTTAC
        TGATGTGGGGAAAAAAAGTAGAGACATGTCTTCTTACTGATAATAATAGG
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        TGTGTTATTCAACTATTCAGCTCATTTGTTGGGATTAACATCGATTTGAT
        GTGCAGAAGCAGGTGATGATTTCTCCCTATCAGAGCTGATTTGGTGACCG
GGTAATCACTAGTGAATTC

>DMF5
        AGAGGGGGAGTCGCATGCTCCGGCCGCCATGGCGGCCGCGGGAATTTCCG
        ATTTACCCGGTCCACGGATTGGTATTATAGGGACTTAGAAATCGATTCCT
        AGGCACCAAAGAATCTAACAATGGTATCAGAGCCATCCTAAGAACCCTAG
        ATCTCCTAATCTGCATCGGTTTCATGAGATCACAGGAAGGTCTCGGATAA
        ATGTGATTGAACTCATTTTTTTATCATTGTTCATCACCCGAGATCAGACA
        GATTAGAATATAAAGAAGTTTAGATCTCTCGATTTAGGGTTTTAACCTAA
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        GGTTTTTAAACTGGGCCAATAGAAATCACAGTGGCCCGGGATCCAACGAT
        CCACGGCTAAGAAACAATCAAGAGGAAATAGAAACACAGATCGTGTTAGG
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      CACTGTTCGTTTAGGTTCGGTGACCGGGTAATCACTAGTGAATTC
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>DMF6
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        CTAATCTGCATCGGTTTCATGAGATCACAGGTAGGTCTCGGATAAATGTG
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        AGGGTTTTGGCCACTGACAGGCAAAACCCATGGAGTCCAAGATAGTAAAA
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        TTTAAACTGGGTCAATAGAAATCACAGTGGCTCGGGATCCAACGATCCAC
        GGCTAAGAAACAATCAAGAAGAAATAGAAACACAGATCGTGTTAGGGTTC
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GTTCGTTTAGGTTCGGTGACCGGGTAATCACTAGTGAATTC
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>DMF7
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>DMF8
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>DMF10
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>DMF11
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>DMF12
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TTGCAGAATCTGATTGCACAATCTATTCGGTTCCGGTGTAATCACTAGTGAAT
TC

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>DMF13
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AGTCTTCAATGGATAATTAAATTTTTTCATCTATAATTTATAAAACATCTGAC
AAGATAACTGACTGAAACAGTTTCAAACAACAGCATAGTTAAATTCAAAATTT
GATATCTCCCAAACTACACAACCAAAAATTATGAAATTCTGCTGGCAGTAATA
ACTTTTATCCCTCTATAAAAGTCTCCATAGTTGGAAGAGCCAATTCGGCCATT
TACTAGATGAAACCCACTAGTGAACAGACCCGCTCATTTTTGCTAGACAAACA
GAAACAGCCACAGTAAAACAAACATAACTGGAGTTTAAAATTCATATGAATAC
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>DMF14
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TGAGGTGGCCGAAGCATGCCAAAGGGCCTTCAGCACAATTTGCTCTTTTATTG
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GACAACTGGGAAATGCCGAAAGAAACCATCACTAACCCTAGTGAAGGCGGCCT
GGTTCAATTGAAATACACCTTCAGGTTTGGAGACCAGTTTGTCGAACCAGATG
ATGACTGGCTGAAGTGTGTTGAAAATACTAGCGAAGAGCTACTTGGAGTATAT
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CCACCGGTCCA

>DMF15
GNNNNTCGCGGGAATTCGATTGNCCGGAGTGCTGCCNGATAGGGCACATGATG
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CCCANAGCGAGCACCGAAGCTGTAACAACCAAAGTTGTTATGATATTGCTCCT
TACCTCANGGTTTTGTCTCATATATTAACTCATGNATATTGCANAAGCTTTTC
NCACTTGGNTCTAGGCTCTAGCTCTTCNCGGCCCAGACGAAGAGTCCCATTCT
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NNNGCCNNNANCNNNNNNNTATCCNNANNNATTNNNNTNNNNNNGTNNNNANG
NNGACTGNGNANTCNACTNNAAAANTTTANNNNTCNNCNNNNANATTGCNNNN
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TGNTANTCCNCACAGTAAACTATGAATACTCNCTNTTTGCNANACACAGTANN
CGNCCNNNNAAANCNTTANNTATNNCNCCTGGGNAGTACNCCGGCAACNGTGA
NACTCANNNGAATTGACNGGGNCCCNCNCAANCCNNANNAACATNNNGTTTTA
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     NTTCNNNGATNCCNCNNNNA

     >DMF16
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     AATAGTTAGTTGATTACAGTTGTATGACATAATTAGTGGAGATTTATCCGTTT
     ACATGATATATTGTAAAGTTACCCGGCTAATCACTAGTGAATTC

>DMF17
     GGCCGCCATGGCGGCCGCGGGANTTCGATTAGCCGGGTAATAAGAAGCTCAGC
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     ACATGTTGAGGGCATGTGTGCTACACTATGGAAAGGATTGGGACAAGTGTCTT
     TCATTGGTAGAATTCTCCTATAATAACAGCTACTAGTCCAGTTTGAAGATGGC
     ACCTTTTGAAGCCTTATATGGGAGAAGGTGTAGGACCCCGCTAAATTGGTCTC
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     CTATCATGACAAGAGAAGGAATCCTCTACAGTTTGAAATGGGAGATCATGTTT
     ATCTTAAGGTGTCACCCACCAAAGGTGTCCAGAGATTTGGACTCAAGGGCAAG
     TTAGCTCCTCGCTACATTGGACCCTACGAGATCAAGGAAACTTGTGGACTTGT
     GGCATATCAATTGAAGTTGCCACCCCACATGTCAGCTGTTCATGATGTGTTCC
     ATGTATCCCAGCTACGGAAGTGCGTCCGCTTACCCGGCTAATCACTAGTGAAT
     TC

     >DMF18
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     ATGCCACAACAACAAGGTATCACCAACCAAAGATCTATGGAAGGATACGTTCA
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     TTATACAACGAAGGATATTGAAGCATAAAAGGTTGGTTCCCAATTCAGCGGTC
     TTCCAAGAATCTGATCTGAGTCCCATTTTTCAAGATGAAGGAACCTAGCTCAA
     GGAAGGTGTCCTTTACTTTCATTAACTTCGTCCAGAAGTGAGAGTCCCCTGGC
     TTTCTTGTTACTTTGACAACGTATACCTGCTTAGGTACTTTTTCCTTAGTAAG
     TTTTGCCGCAACCCATTCTCATTAATCATCTTAAAGAGACATTTACTGAGCAA
     ACCCTTGTTTTGGACGTCTATATTCTGAATTCCTAACCCTTCTTACTCTTTTA
     GAAGGCATAGAATATTCCATTTACCCGGCTAATCACTAGTGAATTC
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LncRNAs act in the regulation of development and stress responses and also participate in many biological processes, such as cellulose, lignin, and gibberellin biosynthesis, regulation of MYB genes and demethylation of transposable elements (Liu *et al.,* 2012; Chen *et al.,* 2015; Erturk *et al.,* 2015; Zhao *et al.,* 2018; Yan *et al.,* 2019). DMF4, DMF10, and DMF13 were found to be homologous to lncRNA genes. LncRNAs can function in response to abiotic stresses such as drought, salinity and high temperature (Xin *et al.,* 2011; Karlik *et al.,* 2018). Zhang *et al.,* (2014) identified 664 RNAs transcript from 8449 drought-responsive transcripts as drought-responsive lncRNAs. They found that 567 from the 664 lncRNAswere upregulated, while the remaining lncRNAs 97 weredownregulated in the leaves of maize under drought stress. Stable methylation of non-coding RNA genes, which regulate gene expression in response to abiotic stress in

Populus simonii plant was previously studied (Song *et al.,* 2016). They isolated and sequenced 1376 stress-specific differentially methylated regions which classified into 1123 encoding protein genes, 16 miRNA genes, and 17 long non-coding RNA (lncRNA) genes.

Protein DEHYDRATION- INDUCED 19 (Di19) homolog 3, a zinc finger gene, is represented by DMF3. Di19 protein family comprises of several Di19-like proteins, which contain two conserved Cys2/His2 zinc-finger domains (Milla *et al.,* 2006). Di19 protein family was reported to be functioned in response to abiotic stresses (Gosti *et al.,* 1995; Milla *et al.,* 2006; Wang *et al.,* 2014). Li *et al.,* (2010) reported that transgenic plants overexpressing the AtDi19 gene exhibited greater tolerance to drought stress. They suggested that Di19 family genes would be useful in generating stress-resistant crops.

Differentially methylated fragment 18 was demethylated under drought stress and located downstream nudix hydrolase 2 gene. Nudix hydrolase (NUDX) genes take part in homeostatic control of energy during stresses. Overexpression of ZmNUDX2 and ZmNUDX8 confer drought tolerance in Arabidopsis plants (Njuguna *et al.,* 2018).

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