Characterization of genetic diversity and linkage disequilibrium of *ZmNAC33* in maize

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Genet. Mol. Res. 17 (1): gmr16039879

Received January 02, 2018

Accepted February 18, 2018

Published February 20, 2018

DOI http://dx.doi.org/10.4238/gmr16039879

ABSTRACT. The maize *NAC* gene, encoding a *NAC* domain transcription factor, plays vital roles in the growth process of maize under abiotic stresses. To investigate the response of *ZmNAC33* to salinity stress, we analyzed the expression of the *ZmNAC33* gene in roots and leaves of three-leaf stage seedlings among maize inbred lines. The result showed that the expression of *ZmNAC33* was up-regulated in the salinity tolerant inbred line Ji853 and down-regulated in the salinity susceptible inbred line Chang7-2. The cDNA and the full sequence from maize inbred line Ji853 were amplified to analyze genetic structure. *ZmNAC33* gene comprises three exons and two introns. We compared genetic diversity of *ZmNAC33* among 69 elite maize inbred lines, a total of 144 sequence variants, including 113 SNPs and 31 Indels. Neutrality tests were negative selection by Tajima's D and Fu and Li's test. Analysis of polymorphism sites showed that at least 28 recombination have occurred. This finding provides insight into the development of breeding programs, germplasm management and association genetic studies.

Key words: Maize; *NAC*; Transcription factor; Salinity; Haplotype

INTRODUCTION

Environmental stress conditions are major factors limiting growth, productivity, and the distribution of plants in the world, especially, salinity and drought (De Oliveira et al. 2011). Growth process of maize is frequently impacted by salinity and drought, so improving drought and salinity tolerance is an important goal in breeding programs (Liu et al. 2013). The molecular mechanisms underlying plant adaptation to drought and salinity stresses have been researched intensively, and transcriptional regulation of gene expression play an important role in this process (Mao et al. 2016). Some have been used as targets for engineering stress tolerance in plants. When plant was subjected to abiotic stresses, the expression of some important functional proteins was

regulated by transcription factors (TFs). TFs can regulate the expression of target genes through binding to specific *cis*-acting elements in the promoters of stress-related genes (Fan et al. 2014, Shao et al. 2015).

NAC (*NAM*, *ATAF1/2*, *CUC2*) transcription factors family is a plant-specific transcription factor superfamily and not only associated with diverse biological processes, including shoot apical meristem development and response to many biotic stress, but also, they have a key function in abiotic stress tolerance inclusive drought and salinity (Ernst et al. 2004, Golldack et al. 2011). *NAC* domain genes represent one of the key regulatory gene families involved in plant development and abiotic stress responses (Wu et al. 2009, Zheng et al. 2009, Wei et al. 2016). Detailed phylogenetic analysis has reveal that *ZmNAC33* belonged to stress-responsive group (*ZmNAC33* was different number in different article) (Fan et al. 2014, Shiriga et al. 2014, Peng et al. 2015), which included *ZmSNAC1* (Lu et al. 2012), *ZmNAC55* (Mao et al. 2016), *ZmNAC111* (Mao et al. 2015). When *AtNAC* genes of *Arabidopsis* and *ZmNAC* genes were used to constructed phylogenetic tree, the stress- group included *ANAC2*, *ANAC19*, *ANAC32*, *ANAC55*, *ANAC72*, *ANAC81*, *ANAC102* (Fan et al. 2014).

OsNAC genes of rice and *ZmNAC* genes were used to construct phylogenetic tree, the stress-group included *OsNAC3*, *OsNAC5*, *OsNAC6*, *OsNAC88* (Fan et al. 2014). The expression of *ANAC2*, *ANAC19*, *ANAC55*, *ANAC72* induced by *NaC*l, drought, over-expression of *Arabidopsis* plant showed significantly increased drought and salinity tolerance (Fujita et al. 2004, Tran et al. 2004, Wu et al. 2009, Zhu et al. 2015, Liu et al. 2016). *ANAC2* in *Arabidopsis* was homologous gene of *ZmNAC33*, it indicated that *ZmNAC33* gene would play a vital role in response to abiotic stresses.

In rice, *OsNAC5* and *OsNAC6* were stress-responsive *NAC* gene, the transgenic plants enhanced significantly tolerances to multiple abiotic stresses (Nakashima et al. 2012, Puranik et al. 2012). *NAC* genes were specifically up- regulated in the drought tolerance line Han21 but maintained an unaltered expression level in the drought susceptible line Ye478 using Affymetrix Maize Genome Array (Zheng et al. 2010). Genotyping method especially genetic diversity of candidate gene makes the SNPs and Indels more attractive as genetic marker. An indel of *ZmNAC111* sequence variants in the promoter region was found by sequenced entire sequence of *ZmNAC111* gene from TST maize inbred lines, the indel as a marker was confirmed in the three bi-parental $F_{2:3}$ populations (Mao et al. 2015). However, no report on studies have addressed expression of *ZmNAC33* gene under salinity stress and the genetic diversity of the *ZmNAC33* is not known yet, a study of the genetic diversity of *ZmNAC33* gene in the relevant maize germplasm would provide a better understanding of the genetic diversity.

In this study, we cloned transcription factor *ZmNAC33* gene and chose 69 elite maize inbred lines in the Northeast region of China. The expression of *ZmNAC33* gene under salinity treatment, which was responsive to salinity stress, was characterized. We analyzed genetic diversity of *ZmNAC33* gene in the 69-elite maize inbred lines and described haplotype diversity and recombination events in this gene in maize.

MATERIALS AND METHODS

Collection *ZmNAC* proteins and At*NAC* proteins

To identify members of maize *NAC* transcription factor gene family, multiple database searches were performed. The online Plant Transcription Factor Database (TFDB) (http://planttfdb.cbi.pku.edu.cn/), maize genetics and genomics database (http://www.maizegdb.org/), the Plant Genomics Resource (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Zmays), Gramene database (http://ensembl.gramene.org/Zea_mays/Info/Index), National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) were used to search for *NAC* genes. We gathered *NAC* genes in maize by using keywords (*NAC*, *NAM*) and domain (PF02365) search. All maize *ZmNAC* genes were confirmed by Pfam (http://pfam.sanger.ac.uk/) and SMART (http://smart.embl-heidelberg.de/). To order to collect *Arabidopsis thaliana NAC* gene family, we used the Plant Transcription Factor Database (http://planttfdb.cbi.pku.edu.cn/family.php?sp=Ath&fam=*NAC*)to search for *Arabidopsis thaliana*. As a query sequence, we used maize *NAC* protein to BLASTP program.

Phylogenetic analysis and sequence alignment

All *ZmNAC* proteins were aligned by means of Clustal W, and all *ZmNAC* sequences were checked manually to remove redundant sequences. Phylogenetic trees were constructed by using *ZmNAC* protein, and an uprooted tree was generated by ClustalX version 2.1 (Thompson et al. 1997) by the neighbour-joining (NJ) method with the pairwise-deletion option and the Poisson correction. Bootstrap analysis was performed using 1000 replicates.

The tree was analyzed and displayed by using MEGA software version 5. Another two uprooted trees were constructed by the same method to align *ZmNAC* and *AtNAC* protein.

Expression analysis of *ZmNAC33* under salinity treatment

Gene expression patterns can provide important clues for determining gene function. We carried out qRT-PCR analysis of *ZmNAC33* gene subjected to salinity stress. Two maize inbred lines, Ji853 (salt tolerant) and chang7-2 (salt susceptible), were used. The seeds were sown into pots containing vermiculite in a greenhouse under normal conditions. Plants in the three-leaf stage were subjected to salt treatment. Seedlings were subjected to 200 mM *NaC*l, shoots and roots were harvested separately at the time points indicated, then immediately frozen in liquid nitrogen and stored at -70°C. Total RNA was isolated using TRIZOL reagent. The concentration of total RNA was estimated using a Nanodrop 1000 (Thermo Scientific product, USA).

Quantitative real-time PCR was used to quantify *ZmNAC33* expression for real-time RT-PCR, 2 μg of total RNA was reverse transcribed into first-strand cDNA with M-MLV reverse transcriptase (Trans) according to the manufacturer's protocol. A mixture of PCR reaction components was prepared according to the manufacturer's protocol of the SYBR Premix Ex Taq^{TM}. Thermocyclin and fluorescence detection were performed with Quant Studio™ 6 (Applied Biosystems). PCR was performed using a two-step method: 94°C for 30s, followed by 40 cycles of 94°C for 10 s and 60°C for 30 s. The relative expression level was determined based on the $2^{-\Delta\Delta CT}$ method (K.J. Livak 2001) by using maize 18S rRNA as an internal control. The primers for real-time PCR are listed in Table 1.

ZmNAC33 isolation and sequence analysis

We performed a search of the *ZmNAC33* ID (accession No. GRMZM2G014653) in the maize sequence database (http://www.maizesequence.org),and found a corresponding genome sequence in B73. Specific primers were designed to amplify the full-length cDNA sequence *ZmNAC33* from the maize inbred line Ji853. Two pairs were designed from the sequence of B73 inbred line to produce overlapping fragments covering 2385 bp from Ji853, which contained 5'UTR region, coding region, intron and 3'UTR region. All primer sequences are listed in Table 1. Extracted and purified PCR products by sequencing. The PCRs were carried out as follows: 5 min at 94°C; 35 cycles of 30s at 94°C, 30 s at 60°C, and 1 min at 72°C; and then 10 min at 72°C. The Contig Express software (version 6.0) was used to assemble sequences.

Table 1. Primers designed for PCR amplification of cDNA and genomic sequence of ZmNAC33

Primers	Sequence (5'→3')	PCR product (bp)
1F (DNA)	CGCGCGTCCGTCCAAGAAAC	1287
1R (DNA)	TGTAGATGCGGCACAGGACCCAG	
2F (DNA)	ACCGTGCCCAATGATTCGAC	1538
2R (DNA)	TATCAACGTGTTCAGTGTCTCGC	
3F (cDNA)	CAGCAGGGAGGAACACGATGAG	1078
3R (cDNA)	TGCCGCCTCTCCATGTAATC	
4F (qPCR)	CCAAGGGCGAGAAGACCAACT	132
4R(qPCR)	CGCCGCCCTTCTTGTTGTAG	
18S rRNA-F	CCTGCGGCTTAATTTGACTC	174
18S rRNA-R	GTTAGCAGGCTGAGGTCTCG	

Plant materials and sequencing *ZmNAC33* gene

A total of 69 maize inbred lines were used to construct elite maize inbred lines (Table S1). These inbred lines represented most of the genetic diversity available to breeding and research programs in the Northeast region of China. DNA was extracted from fresh leaf tissues of 3-week inbred lines after germination using the Maxi CTAB method (Saghai-Maroof MA 1984). Sequences of *ZmNAC33*, including the 5'- and 3'-untranslated regions (UTRs), were sequenced from the maize elite inbred lines. Two pairs of primers of *ZmNAC33* were designed using inbred line Ji853 gene sequence. PCR reactions were carried out in 50 μl volumes under the following conditions: 100 ng template DNA, 250 nM of each primer, 250 nM dNTPs, 2 U Taq polymerase and 250 μM $MgCI_2$. Reaction were performed as follows: an initial 94°C denaturation step for 5 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 90 s. The final extension step was followed by 72°C incubation for 10 min. Amplified DNA fragments were sequenced.

Sequence variation analysis for *ZmNAC33* gene

The sequences were assembled using Contig Express software. Full alignments were built for *ZmNAC33* gene by using default settings of the CLUSTALX program (Thompson et al. 1997). The software DNASP version 5 (Rozas et al. 2003) was used to analyze the nucleotide diversity (π) and average number of nucleotide differences (k) within each group and each defined region. The neutrality of the polymorphisms of *ZmNAC33* was analyzed by using Tajima's D statistical test (Tajima 1989) and Fu and Li's statistical tests (Fu and Li 1993).

Recombination events, haplotype, and salinity tolerance of elite maize inbred lines

DNASP soft was used to analyze the haplotype diversity among *ZmNAC33* gene based on the amplified sequences of the elite maize inbred lines. Recombination events of *ZmNAC33* gene among these inbred lines were estimated with the methods developed by Hudson and Hey (Hudson and Kaplan 1985). To estimate salinity tolerance, the 69-elite maize inbred lines were salinity treatment 100 mmol/L *NaC*l, the five-appropriate physiological and biochemical indices were tested and used to evaluate salinity tolerance, which included germination percentage, relative conductivity, SOD activity, MDA content and proline content (Yang et al. 2011).

RESULTS

Identification of *ZmNAC* family and *AtNAC* family and phylogenetic analysis

The keywords (*NAC*, *NAM*) and domain (PF02365) were regarded as a query to search five public databases: NCBI, TFDB, maize DB, phytozome, and Gramene. Therefore, there were at least 214 *ZmNAC* genes. By removing redundant sequences from the five databases, the longest protein sequence was selected if a single Zm*NAC* was subject to differential splicing. Then the putative protein sequences of *ZmNAC* were confirmed by Pfam and SMART searches for the presence of the *NAC* domain. After removing duplicate sequences and identification of Pfam and SMART, a total of 133 *ZmNAC* genes were identified. The number of *NAC* proteins in maize is greater than in *Arabidopsis* (105 members).

To clarify the phylogenetic relationships among the *ZmNAC* genes and *AtNAC* genes, multiple alignment, and phylogenetic analyses of the proteins of *ZmNAC* and *AtNAC* were performed using ClustalX 2.1 and MEGA 5 software. An unrooted tree was constructed to examine the phylogenetic relationships.

To classify subgroups and identify the evolutionary relationships between Zm*NAC*s and At*NAC*, phylogenetic analysis was performed (Figure 1). Most of stress-responsive *NAC* could be classified into the same groups. Studies have shown that at least 10 *NAC* genes, *ZmNAC111* (Mao et al. 2015), *ZmNAC1* (Lu et al. 2012), *ZmNAC70* (Mao et al. 2016), AT1G69490 (*ANAC029*), AT1G52890 (*ANAC019*), AT3G15500 (*ANAC055*), AT4G27410 (*RD26*), AT1G77450 (*ANAC032*), AT5G63790 (*ANAC102*), AT1G01720 (*ANAC2*) (Wu et al. 2009, Le et al. 2011). In the QTL of salinity resistance in maize, *ZmNAC33* was candidate gene among these candidate genes of the QTL. *ZmNAC33* was up-regulated 18-fold in the maize drought tolerant inbred line (HKI577), *ZmNAC33* was *ZmNAC 51* (Shiriga et al. 2014). Therefore, *ZmNAC33* was identified to play a role in maize under salt stress.

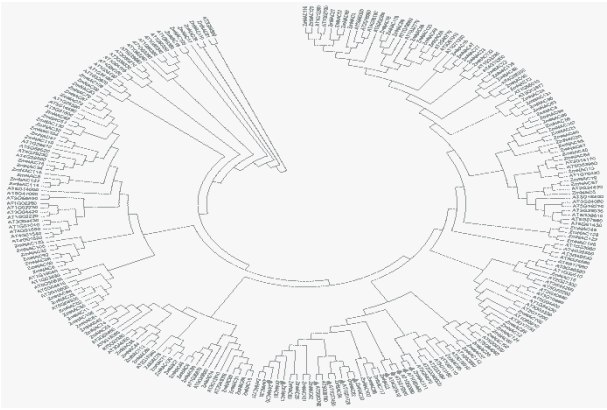

Figure 1. Phylogenetic analysis of maize and Arabidopsis *NAC* domain proteins

Induced *ZmNAC33* expression by salinity stress

To investigate the response of *ZmNAC33* to salinity stress, we performed qRT-PCR using RNA isolated from *NaC*l treated maize inbred lines Ji853 and Chang7-2. The result showed that the expression of *ZmNAC33* was induced significantly by high salinity (200 mM *NaC*l) and was up-regulated in the salinity tolerant inbred line Ji853 and down-regulated in the salinity susceptible inbred line Chang 7-2 (Figure 2). *ZmNAC33* transcript level increased gradually in leaves and roots, the relative level of *ZmNAC33* expression increased by more than 6-fold in roots.

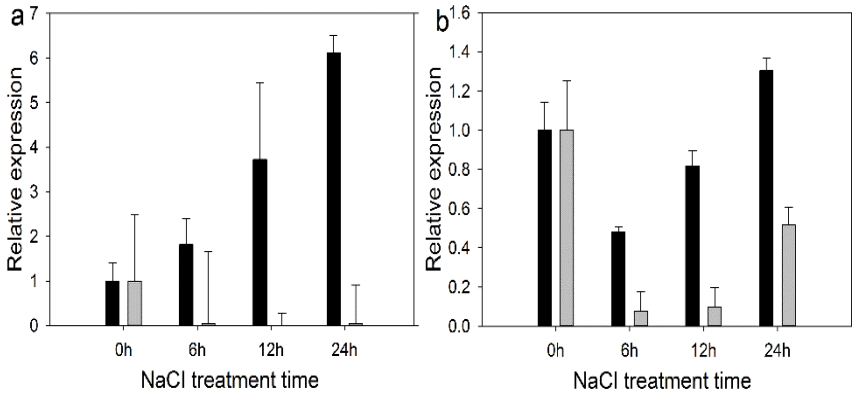

Figure 2. The expression patterns of *ZmNAC33* gene under high salinity treatment

Cloning *ZmNAC33* gene

We cloned *ZmNAC33* gene from maize inbre line Ji853. Overlapping sequences of 2385 bp was generated through reference sequence of B73. When aligned with reference sequence of cDNA sequence, *ZmNAC33* gene is composed of 3 exons spaced by 2 introns with 2385 bp in total (Figure 3), which spanned the 5'-UTR (283bp) to the 3'-UTR region (750 bp). The full length open reading frame (ORF) of *ZmNAC33* is 882 bp, and encodes a polypeptide of 292 amino acid residues. Sequence alignment revealed that *ZmNAC33* contains a typical *NAC* structure with a conserved *NAC* domain. The nucleotide sequences of the maize *ZmNAC33* gene in inbred line Ji853, whose genome has been sequenced, were used as the references to amplify the sequences of this gene in 69 inbred lines.

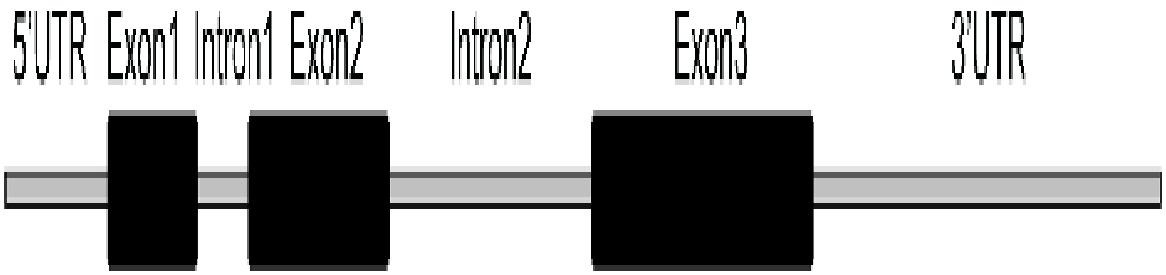

Figure 3. Schematic diagram showing two sets of primers based on the reference sequence of *ZmNAC33*

ZmNAC33 genetic diversity

Sequence polymorphisms were analyzed among 69 maize inbred lines across 2385 bp of sequence, which includes a 5' untranslated region (UTR), a coding region, a intron region, and a 3' UTR. The SNPs and indel of *ZmNAC33* gene were identified and summarized (Table 2). From genomic sequences of *ZmNAC33* gene of the elite inbred lines, a total of 113 SNP sites and 31 indel were identified, revealing the frequency of SNPs and indels (1 SNP every 21.1 bases and 1 indel every 76.9 bases on average). The frequency of SNPs was highest in the 3'UTR region, intron region was second subsequencely, but the frequency of indels was highest in the intron region. The frequencies of SNP and indel in the coding region were less than other region, one SNP occurred every 58.2 bp in the coding region. Polymorphism analysis found the frequency of SNPs higher than indels.

Table 2. Summary of the frequency of polymorphisms of ZmNAC33 gene

Parameters	5'-UTR	Coding region	Intron	3'-UTR	Entire region
Total length of amplicons (bp)	283	815	537	750	2385
Number of all sequence variants (SNPs and indels)	16	18	45	65	144
Frequency of all sequence variants	0.0565	0.0221	0.0838	0.0867	0.0604
Number of SNPs	14	14	34	51	113
Frequency of SNPs per bp	0.0495	0.0172	0.0633	0.068	0.0474
Number of indels	2	4	11	14	31
Frequency of indels per bp	0.0071	0.0049	0.0205	0.0187	0.013

From the elite maize inbred lines, the entire nucleotide diversity of *ZmNAC33* gene was 0.01469 (Table 3), followed by the 5'-UTR region (πU0.02382), coding regions (π=0.00598), intron regions (π=0.2264) and the 3'-UTR region (ing regions Among 4 regions of *ZmNAC33* gene, the coding region showed much lower nucleotide polymorphism than others, while the intron region showed the highest frequency of all sequence region. The result was caused because of the number of SNPs, it had the highest frequency of SNPs per bp. Most of the nucleotide changes in the intron regions and the 3'-UTR regions.

Table 3. Summary of nucleotide diversity and divergence in each region of ZmNAC33 in maize

Regions	5'-UTR	Exon	Intron	3'-UTR	Total
S	22	26	41	173	238
π	0.02382	0.00598	0.02264	0.02661	0.01469
k	6.454	4.792	10.483	17.614	32.266
D	1.26008	-0.35923	0.74645	-1.77318	-1.2169
D*	1.37946	-1.50212	0.15291	-5.03434*	-3.87249*
F*	1.59187	-1.28962	0.45283	-4.46225*	-3.35412*

Note: S, number of segregating sites; π, nucleotide diversity; k, average number of nucleotide differences; D, Tajima's D; D*, Fu and Li's D*; F*, Fu and Li's F*; * P<0.05.

The neutrality of the polymorphisms of *ZmNAC33* was evaluated by Tajima's D test and Fu and Li's D* and F* test. Separate Tajima's D tests for each region (5'-UTR, exon, intron and 3'-UTR) revealed positive and non-significant departures from the expectation, but significant deviation in the 3'-UTR and the entire gene sequence from the neutral expectation (P<0.05) occurred with the Fu and Li D* and F* tests (Table 3). When the 3'-UTR regions were excluded, the results of Fu and Li D* and F* tests were positive and non-significant. It might easily change in the 3'-UTR regions. These results could not reject the hypothesis, a lack of footprint of positive selection in most region of *ZmNAC33* was suggested. The sliding window analysis of Tajima's D showed that the elite maize inbred lines had both positive and negative values at different regions of *ZmNAC33 gene* (Figure 4). Negative D values were detected in the 5'UTR region (70-126 bp), exon1 (333-358 bp), intron1 (472 bp), exon2 (675 bp, 750-838 bp), intron2 (941-1020 bp, 1139-1209 bp), exon3 (1259-1284 bp, 1365-1421 bp, 1497-1523 bp, 1624-1649 bp), 3'UTR (1996-2452 bp). These regions are mainly located in the exon3 region. The observed pattern of variability under the neutral different from expected variability, Tajima's D test was not significant.

The full-length sequence of *ZmNAC33* in the elite inbred lines contained 238 segregating sites. However, most of segregating sites were detected across the region of 3'-UTR region. Moreover, the 3'-UTR region showed higher nucleotide diversity (of segregating sites were detected across the region of 3'-UTR region.

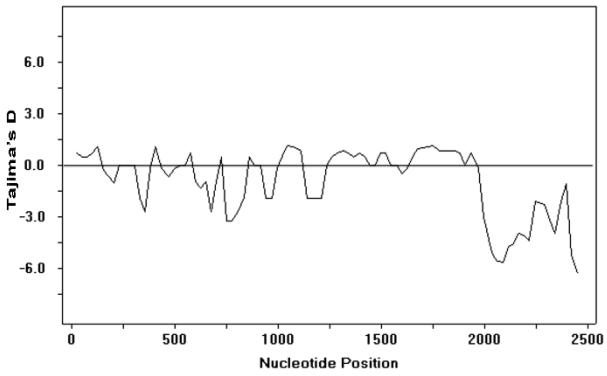

Figure 4. Tajima

Evidence of recombination, haplotype diversity and salinity tolerance of elite maize inbred lines

The polymorphic sites in the entire *ZmNAC33* sequence were used to analyze the evidence of recombination. According to Hudson and Kaplan, at least 28 recombination events were found to be responsible to the polymorphism of *ZmNAC33* gene. The recombination events were analyzed in the informative sites of every region, there was one in the 5'-UTR positions and between and intron2 and exon3, respectively. There were two in the positions between 5'-UTR and intron1, there were five in the positions between intron1 and intron2, there were nineteen in the 3'-UTR positions.

Based on the entire sequence of *ZmNAC33* gene sequence sequenced in 69 maize inbred lines, a total of 34 haplotypes were detected with a Hd equal to 0.9646. The tested elite maize inbred lines were unbalanced distributed in these haplotypes. Among the haplotypes identified in this analysis, the most frequent haplotype was Haplotype2 and Haplotype3, which contained 7 maize inbred lines, respectively, 19 contained only one maize inbred line. In addition, we also noticed that two frequent haplotypes, including Haplotype9 and Haplotype19, contained 5 maize inbred lines, respectively. Haplotype10 and Haplotype21 contained 4maize inbred lines, respectively. Others contained 2 maize inbred lines, respectively. In the CDS region, 16 were synonymous sites, and the other 10 were non-synonymous sites. In the CDS region of the maize *ZmNAC33* gene, a total of 4 indels were identified. When we translated the CDS region into amino acid sequence, one indel could result in frame shift during translation some of elite maize inbred lines, the code appeared stop code in advance.

Based on results of physiological and biochemical indices, there were 5 inbred lines with high salinity tolerance, 15 inbred lines with salinity tolerance, 32 inbred lines with medium salinity tolerance, 10 inbred lines with susceptible tolerance, 7 inbred lines with high susceptible (Table S1). Combined to haplotypes and salinity tolerance, inbred line Ji853 belonged to the Haplotype3, which consisted salinity tolerance. It included 1 high salinity tolerance inbred line, 4 medium salinity tolerance inbred lines, 2 salinity tolerance inbred lines. The Haplotype19 also consisted salinity tolerance inbred lines.

DISCUSSION

TFs play important roles in the regulation of gene expression in response to salinity and drought stresses, and their molecular engineering is proposed as a potential strategy for the genetic improvement of stress tolerance in crop (Mao et al. 2015). When we analyzed expression of *ZmNAC33* gene by qRT-PCR in the tolerance inbred line and susceptible inbred line, the result showed that *ZmNAC33* gene appeared higher expression under salinity stress in tolerant inbred line than that in the susceptible inbred line. When maize inbred line HKI577 (drought tolerant) and HKIPC3-3 (drought susceptible) were drought treatment, *ZmNAC33* expression was up-regulated in the drought tolerant inbred line HKI577 and down-regulated in the drought susceptible inbred line HKIPC3-3(Shiriga et al. 2014). The expression showed that *ZmNAC33* gene response abiotic stress. Its homologous gene of *Arabidopsis NAC, AtNAC2*, was induced by drought, high salinity, abscisic acid (ABA) and overexpression plants in *Arabidopsis* remarkably enhanced plant tolerance to drought (Wu et al. 2009).

The nucleotide polymorphisms of *ZmNAC33* gene were a non-significant difference in the frequencies analyzed among 5'UTR, CDS, intron and 3' UTR region. Because Tajima's D test for the total sequence and every region were also not significant, this suggests that the population is evolving as predicted by infinite site models. Both the entire length of the *ZmNAC33* sequence and every region had positive Tajima's D values, indicating that a deficiency in the number of low frequency alleles in the population could be the result of a demographic process. Based on the non-significant results of the Tajima's D test, selection might not act on the *ZmNAC33*

gene. Although nucleotide polymorphisms of *RSUS3* gene showed that Tajima's D test was not significant, 2 markers based on the indel and SNP found by haplotypes and genetic diversity (Lestari et al. 2009, Lestari et al. 2011).

The identification of SNPs and indels associated with resistance phenotype would be helpful for developing resistant varieties (Kadam et al. 2016). The nucleotide variations of *ZmNAC33* gene in the 69-elite maize inbred lines identified 34 different haplotypes, however, Haplotype3 and Haplotype19 contained salinity tolerance inbred lines. *GmSALT3* gene of soybean was divided into 5 haplotypes, two indels were found salinity tolerance and salinity susceptible haplotypes (Guan et al. 2014). Nucleotide polymorphism is a major origin of heritable phenotypic variation, the abundant genetic diversity is the foundation for crop improvement. Sequence polymorphism of *TaGW2-6A* gene was analyzed by sequenced 207 Indian wheat, association of these SNPs and that of the corresponding haplotypes identified a novel SNP, the SNP was converted into CAPS (Cleaved Amplified Polymorphism Sequences) marker to distinguish the alleles of *TaGW2-6A* (Jaiswal et al. 2015). We analyzed the genetic diversity and the haplotype diversity of *ZmNAC33* gene, which was induced expression by drought and salinity treatment. The nucleotide polymorphisms of *ZmNAC33* gene would be helpful in identifying alleles for further genetic analysis, the nucleotide polymorphism of *ZmNAC33* gene would allow for more effective markers development and can be prospective for breeding program. Hence, the nucleotide polymorphism might be prospective and useful as marker assisted selection.

CONCLUSION

The study revealed that the expression of *ZmNAC33* was induced in salinity treatment. *ZmNAC33* gene includes three exons and two introns. A total of 113 SNP and Indels were obtained from 69 maize inbred lines. The results of Tajima's D and Fu and Li's test showed that neutrality of *ZmNAC33* was not significant in population. Combined to haplotypes and salinity tolerance, the Hap_3 belonged to salinity tolerance, it included 1 high salinity tolerance inbred line, 4 medium salinity tolerance inbred lines, 2 salinity tolerance inbred lines.

ACKNOWLEDGEMENTS

The study was conducted with financial support from Agricultural Science and Technology Innovation Program of Jilin Province, Discovery of excellent germplasms and cultivation of inbred lines suitable for mechanized harvesting in maize and the Key Project of Science and Technology Department of Jilin Province "The creation of maize lines for alkali tolerance by GWAS and QTL mapping".

CONFLICT OF INTEREST

None declared.

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