Bulgarian Journal of Agricultural Science, 19 (No 2) 2013, 181-185
Agricultural Academy

IDENTIFICATION OF SNP MUTATIONS IN *MYBE-1* GENE INVOLVED IN DROUGHT STRESS TOLERANCE IN MAIZE

B. ASSENOV[1], V. ANDJELKOVIC[2], D. IGNJATOVIC-MICIC[2], J. VANCETOVIC[2], A. NIKOLIC[2], N. K. CHRISTOV[1], S. TSONEV[1], N. ABU-MHADI[1], D. VASSILEV[1], Y. MUHOVSKI[3], M. ILCHOVSKA[4] and E. TODOROVSKA[1]*
[1] *AgroBioInstitute, BG - 1164 Sofia, Bulgaria*
[2] *Maize Research Institute "Zemun Polje", Slobodana Bajica 1, 11185 Zemun Polje, Belgrade, Serbia*
[3] *Département Sciences du vivant Centre wallon de Recherches Agronomiques, Gembloux, Belgium*
[4] *Maize Research Institute, BG - 5000 Kneja, Bulgaria*

Abstract

ASSENOV, B., V. ANDJELKOVIC, D. IGNJATOVIC-MICIC, J. VANCETOVIC, A. NIKOLIC, N. K. CHRISTOV, S. TSONEV, N. ABUMHADI, D. VASSILEV, Y. MUHOVSKI, M. ILCHOVSKA and E. TODOROVSKA, 2013. *Bulg. J. Agric. Sci.,* 19: 181-185

As one of the most important agricultural crops, maize is a staple diet for a large portion of the continuously growing world's human population. Unfortunately, its production is severely affected by adverse environmental stresses such as drought, salt, low and high temperatures. The mechanisms of drought stress tolerance in maize are quite complex and involves a signaling network of genes that controls its adaptation to the stress. Recently, many transcription factors (TFs) for tolerance to drought stress have been identified in maize. In this study, specifically designed primers were used to identify functional single nucleotide polymorphisms (SNPs) in the MYB-like protein E1 (*MYBE1*) gene of 26 tolerant and sensitive maize inbred lines from the gene banks of Maize Research Institute "Zemun Polje", Serbia and Maize Research Institute, Kneja, Bulgaria by PCR amplification and direct sequencing. Multiple alignments of the sequenced fragments to the drought sensitive US maize inbred B73 and other inbred lines, representing the functional diversity of maize, from the Panzea database (http://www.panzea.org/) was performed. The sequence alignment revealed several SNPs in inbred lines object of this study, one of which unique to the drought tolerant Serbian inbreds T3, T5, T6, T7 and T8. In these lines, the A→G transition, located in the acidic *Ser/Thr* – rich region of the gene, leads to amino acid change from Tryptophan to Alanine at the corresponding position of the protein. This replacement does not affect the binding site of *MYBE-1* transcription factor, but possibly reflects the spatial conformation through changing of its functional activity.

This study will contribute the identification of functional markers in genes implicated in the development of drought stress tolerance and their further use for genomic improvement in the national maize selection programs.

Key words: maize, drought, *MYBE1* gene, SNPs

Introduction

Maize is one of the most important agricultural crops and a staple diet for a large portion of the continuously growing world's human population. However, its production is severely affected by adverse environmental stresses such as drought, salt, low and high temperatures. To overcome these limitations, the contemporary breeding programs in maize comprise a lot of knowledge about the mechanisms of how plants overcome these stress resistance at both physiological and biochemical level (Byrt and Munns, 2008; Munns and Tester, 2008). Therefore, the identification and functional study of stress responses and the complex net-

*E-mail: b.assenov@abv.bg; e.g.todorovska@gmail.com

works of genes is a keystone for better understanding the molecular mechanisms of the plant stress response and tolerance, and will ultimately lead to improvement of stress tolerance in maize.

In plants, drought-tolerance results from the action of a complex signalling network that involves a number of metabolic pathways and multiple genes. Cloned genes involved in drought resistance encode proteins such as transcription factors (TFs), kinases, late embryogenesis abundant proteins and oxidative enzymes that synthesise osmoprotectants, anti-oxidant compounds, molecular chaperones and other molecules. Numerous reports show that TFs, which are necessary for plant development and are important regulators of stress-responsive genes expressed in the signal transduction network (Zhang et al., 2012). Among the transcription factors, the MYB TFs comprise one of the largest gene families (Riechmann et al., 2000). The distinguishing characteristic of MYB TFs is possession of a MYB domain, which consists of 1–4 imperfect tandem repeats (MYB repeat) located near the N-terminus. The *MYB* gene family is divided into 4 different types according to the number of repeat(s) in the MYB domain (Dubos et al., 2010). Typically, the MYB repeat consists of 50–53 amino acids and contains three regularly distributed tryptophan (or phenylalanine) residues, which together form a hydrophobic core. Each MYB repeat forms three a-helices: the two that are located at the C-terminus adopt a variation of the helix–turn–helix (HLH) conformation that recognizes and binds to the DNA major groove at the specific recognition site C/TAACG/TG (Ogata, 1998).

The first plant *MYB* gene, C1 which is involved in anthocyanin biosynthesis, was isolated in *Zea mays* (Paz-Ares et al., 1987). So far, large number of *MYB* genes has been identified in different plant species. Among the MYB transcription, regulatory factors *R1-MYB* play vital role in transcriptional modification during plant metabolism and development. A *R1-MYB* gene (*ZmMYBE1*, GeneBank Acc.No FJ024049) was isolated from maize (*Zea mays* L.). The ZmMYBE1 protein contains a conserved MYB domain near the N-terminus as well as an acidic Ser/Thr-rich area in the downstream region. *ZmMYBE1* is involved in the regulation of growth rate, plant height and photoperiod in maize (Jia et al., 2008).

In this study, a direct PCR sequencing was used to identify and characterize single nucleotide polymorphisms (SNPs) in *ZmMYBE1* gene in several drought tolerant and drought sensitive maize inbred lines. The aim of the current work was to identify SNP mutations associated with genes involved in drought stress tolerance in maize, in order to develop functional markers for efficient use in marker-assisted selection for drought tolerance breeding selection.

Materials and Methods

Plant material

Twenty six drought tolerant and drought sensitive maize inbred lines from the gene banks of Maize Research Institute "Zemun Polje", Serbia and Maize Research Institute, Kneja, Bulgaria as expressed by the difference in their behaviour, survival and yield potential under stress were included in this study.

DNA isolation

DNA was isolated from bulk samples (5 plants) using CTAB method according to Murray and Tompson (1980) with few modifications.

PCR amplification, sequencing, and SNPs characterization

The available information on the STS marker PZB02023.1 (http://www.panzea.org/) was used for amplification of the region located downstream from the conservative MYB domain of the *MYBE1* gene. M13 Forward and Reverse tails were added at 5' end of the original primers (F 5'CCATGCTCAGAAGTTTTTCTCC3' and R 5'CCAGGATGTTTGCATCACTTCT3') to allow direct sequencing of the PCR products.

PCR amplification of gDNA of the selected maize lines was performed on a Veriti Thermal cycler (Applied Biosystems) using 1 unit of High fidelity *Taq* DNA polymerase (Fermentas) in a total volume of 20µl containing 100 ng of gDNA, 1xPCR buffer, 200µM of each dNTP, 10 pmol of each PCR primer, 1.5mM $MgCl_2$. Two step PCR reaction was performed with different annealing temperatures as follows: an initial denaturation for 3 min at 94°C followed by 5 cycles amplification step each including: denaturation at 94°C for 40 sec, annealing at 55°C for 45 sec, extension at 72°C for

1 min and 25 cycles where the annealing temperature was 62°C instead 55°C.

PCR products were gel electrophoresed, excised, purified by Gene Jet Purification Kit (Fermentas) and then subjected to double stranded sequencing on ABI 3130xl platform. The row sequencing data was basecalled and assembled in contigs using phred (Ewing et al., 1998) and phrap (Green, 1996) with default parameters. The contigs were aligned and analysed for SNP detection using BioLign software (Hall, 2001). Nucleotide sequences were converted into amino acid sequences and were compared with MYBE1 protein sequences (http://www.ncbi.nlm.nih.gov; http://www.panzea.org/) to verify if the identified SNPs were able to produce functional mutations with amino acid changing.

Results

In this study, a direct PCR sequencing was used to identify and characterize single nucleotide polymorphisms (SNPs) in the *ZmMYBE1* gene in 26 drought tolerant and drought sensitive maize inbred lines from both Serbian and Bulgarian maize germplasm collection.

Using the STS primer pair for the marker PZB02023.1 (http://www.panzea.org/) a 769 bp fragment that corresponds to the region located between exon 3 and exon 5 of the *ZmMYBE1* gene was amplified. The region covers the last 1/3 part of the MYB conserved domain (R1) and the downstream located regions (Nuclear Localization Signal, *NSL* and acidic *Ser/Thr* – rich area). The sequenced *MYBE1* fragments of both tolerant and sensitive Serbian and Bulgarian maize inbred lines were aligned to identify SNP mutations. Multiple alignment of the sequenced fragments to the drought sensitive US maize inbred B73 and other inbred lines, representing the functional diversity of maize, from the Panzea database (http://www.panzea.org/) revealed SNPs in several of the inbred lines object of this study, one of which unique to the drought tolerant Serbian inbreds T3, T5, T6, T7, T8. In these lines, an **A→G** transition was observed in the acidic *Ser/Thr* – rich area located in the forth exon of the gene (Figure 1). The transition leads to amino acid change from Tryptophan to Alanine at the corresponding position of the protein (Table 1). This replacement does not affect the binding site (*TFIIIB'* DNA-binding domain) of *MYBE-1* transcription factor, but might possibly reflect the spatial conformation through changing of its functional activity. Additional mutations were also found in the exon 4 of the *MYBE1* gene - one synonymous (CG**T**→CG**C**) which results in the same amino acid (Arg) being incorporated in the protein sequence of several tolerant and sensitive lines (Table 1) at that position and one non-synonymous (**A**CA→**G**CA) resulting in a codon which encodes a different amino acid (Thr→Ala) in lines S3, S5, T1, T2, T3, T5, T6, T7, T8, T15. SNPs as C->A, A->T, G->A and A->G were found in the introns 3 and 4 of several of the inbred lines (Table 1). Both synonymous and non-synonymous mutations (SNPs) were identified only in Serbian maize inbreeds.

Discussion

Maize presents greater variability for tolerance to abiotic stresses, and in particular to drought, than do

Table 1
Summary of the SNP mutations identified in the *MYBE1* gene sequences of 26 maize inbred lines from both Serbian and Bulgarian germplasm collections

SNP position (bp)	Exonic mutations	Genotypes with SNP's
116	CG**T**→CG**C** (Arg→Arg)	S3, S5, T1, T2, T3, T5, T6, T7, T8, T15
213	**A**CA→**G**CA (Thr→Ala)	S3, S5, T1, T2, T3, T5, T6, T7, T8, T15
533	**A**CT→**G**CT (Thr→Ala)	T3, T5, T6, T7, T8
SNP position (bp)	Intronic mutations	Genotypes with SNP's
23	C→A	S3, S5, T1, T2, T3, T5, T6, T7, T8, T15
57	A→G	S3, S5, T1, T2, T3, T5, T6, T7, T8, T10, T11, T15
76	A→T	S3, S5, T1, T2, T3, T5, T6, T7, T8, T15
533	G→A	S3, S5, T1, T2, T3, T5, T6, T7, T8, T15

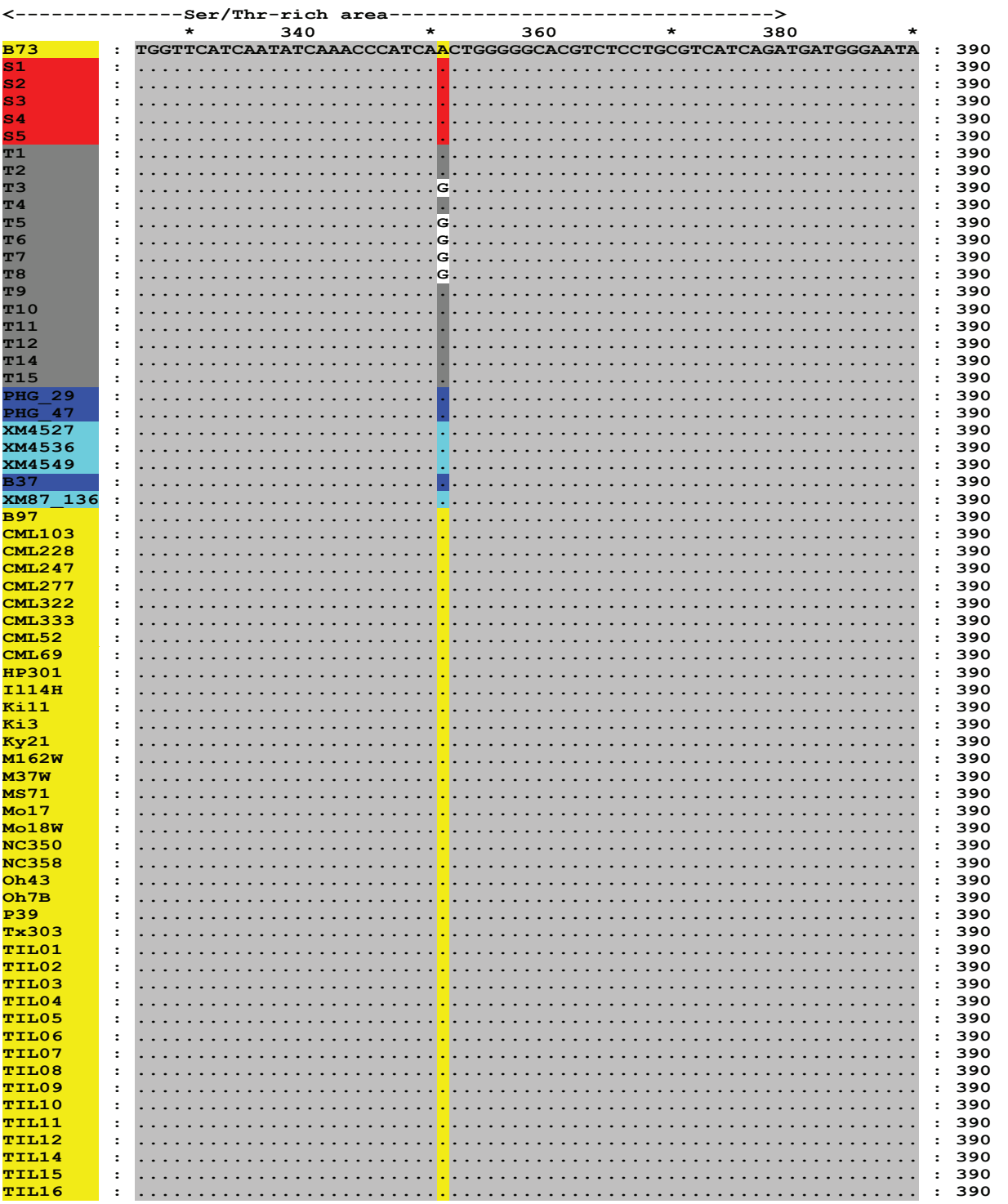

Fig. 1. Nucleotide sequence alignment of 67 regions downstream located from the N typical maize R1-MYB domain in the *MYBE1* gene of Serbian, Bulgarian and Panzea maize database. The shading of the alignment represents different degrees of conservation among sequences; the dark shading indicates identical residues, the light shading indicates nucleotide changes

rice and barley. However, systematic studies of genes concerning abiotic stress tolerance in this crop are still limited. The available maize SNP databases (http://www.panzea.org/) provide some information about SNP mutations in genes associated with drought but still it is not clear, which of them can be used as potential functional markers in breeding for tolerance.

In this study, direct PCR sequencing was utilized to detect SNP mutations in *MYBE1* transcription factor gene, involved in drought stress tolerance pathways. The reported here SNPs, both synonymous and non-synonymous, were found in the region located downstream from the conserved domain (*R1-MYB*) of the *MYBE1* gene. Even though the observed mutations were not located in the conserved *R1* domain of the MYBE1 protein, one of them was detected in the *Ser/Thr* – rich area. This area possesses a biological function, which is not, yet fully determined (Jia et al., 2008). The observed mutation is unique to the drought tolerant Serbian inbred lines T3, T5, T6, T7 and T8 and to our knowledge is the first one reported for this position of the *MYBE1* gene as compared to the other inbred lines, representing the functional diversity of maize, from the Panzea database (http://www.panzea.org/). The observed SNPs were found only in Serbian maize inbreds, which is an evidence for the higher genetic diversity as compared to Bulgarian maize inbreeds.

Conclusion

In this study, a direct PCR sequencing was utilized to identify SNP mutations in *MYBE1* transcription factor gene, involved in drought stress tolerance pathways. A unique SNP was found in several droughts tolerant Serbian inbreeds as compared to all available sequences in the Panzea database. Additional analysis of the conservative domain (R1) of this protein will allow exploration of SNPs in the protein substrate binding sites that could generate variations in protein function. Further, the SNPs identified in the tolerant maize genotypes could be used to explain the difference in behaviour of these genotypes under drought stress conditions. The applied methodology will allow the development of functional markers able to be efficiently used in marker-assisted selection for drought tolerance breeding selection.

Acknowledgements

This study is supported by the grant **TR31028** of the Ministry of Education, Science and Technological Development, Republic of Serbia**.**

References

Byrt, C. S. and R. Munns, 2008. Living with salinity. *New Phytologist*, **179**: 903–905.

Dubos, C., R. Stracke, E. Grotewold, B. Weisshaar, C. Martin and L. Lepiniec, 2010. MYB transcription factors in Arabidopsis. *Trends Plant Sci.*, **15**: 573–581.

Ewing, B., L. Hillier, M. Wendl and P. Green, 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Research,* **8**:175-185.

Green, P., 1996. Phrap documentation. http://www.phrap.org/phredphrap/phrap.html.

Hall, T., 2001. Biolign alignment and multiple contig. Editor. http://en.bio-soft.net/dna/BioLign.html.

Jia, G. Bo Li, D. Zhang, T. Zhang, Z. Li, J. Dai and S. Wang, 2009. Cloning and characterization of a novel R1-MYB transcription factor in maize. *Progress in Natural Science*, **19**: 1089–1096.

Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, **59**: 651–681.

Murray, M. G. and W. F. Thompson, 1980. Rapid isolation of high-molecular-weight plant DNA. *Nucl Acids Res* **8**: 4321-4325.

Ogata, K., 1998. Structure and dynamics of the transcription factor, Myb, in DNA-sequence recognition. *Seikagaku*, **70**: 1233–1250.

Paz-Ares, J., D. Ghosal, U. Wienand, P. A. Peterson and H. Saedler, 1987. The regulatory c1 locus of Zea mays encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. *EMBO Journal*, **6**: 3553–3558.

Riechmann, J. L., J. Heard, G. Martin, et al., 2000. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science*, **290**: 2105–2110.

Zhang, L., G. Zhao, J. Jia, X. Liu and X. Kong, 2012. Molecular characterization of 60 isolated wheat MYB genes and analysis of their expression during abiotic stress. *J. Experimental Botany*, **63**: 203–214.

Received November, 2, 2012; accepted for printing March, 2, 2013.