UDC 575.22: 633.15
Original scientific paper

S1 FAMILIES AS A SOURCE OF BENEFICIAL ALLELES FOR BREEDING DROUGHT TOLERANT MAIZE GENOTYPES

Ksenija MARKOVIĆ, Ana NIKOLIĆ, Dragana IGNJATOVIĆ-MICIĆ, Violeta ANĐELKOVIĆ and Vesna LAZIĆ-JANČIĆ

Maize Research Institute „Zemun Polje“, Belgrade, Serbia

Marković K, A. Nikolić, D. Ignjatović-Micić, V. Anđelković and V. Lazić-Jančić (2008): *S1 families as a source of beneficial alleles for breeding drought tolerant maize genotypes.*– Genetika, Vol. 40, No. 1, 83 -93.

As water resources for agronomic use become more limiting development of drought tolerant genotypes become increasingly more important. In maize, under drought stress conditions, an increase in the length of anthesis- silking interval and a decrease of grain yield is observed. Different strategies have been proposed to overcome negative effects of drought on maize. Breeders at Maize Research Institute (MRI) have created and used adapted drought tolerant population - DTP-A (DTP79xDTP12xDwarf), that was shown to be a good source of drought tolerance for locally adapted germplasm. In this research s1 families from DTP-A x B84 were scored for anthesis-silking interval and genotyped with SSR markers using BSA approach, with the aim to create a new gene pool for breeding drought tolerant hybrids. SSR analysis was performed in order to identify putative genomic segments and allele pattern differences responsible for expression of ASI, thus

Corresponding author: Vesna Lazić-Jančić,Maize Research Institute „Zemun Polje“
S. Bajića 1, 11185 Belgrade,Serbia
Tel: 011/3756704, Fax: 011/3756707,e-mail: vlazic@mrizp.co.yu

providing an insight into the genetic structure of s1 families. Four SSR loci, different in allelic structure between short and long ASI families, were identified. The main characteristic of these differences was the presence or absence of DTP79/DTP12 alleles. It seems that combination of several different genes and alleles influence ASI expression, with DTP79 alleles being beneficial and DTP12 alleles detrimental. Two chromosome regions identified in this work were congruent with qASI regions reported in Maize Genetics and Genomics Database. Clustering revealed that all sASI families grouped with DTP79, but one of the long ASI grouped with these genotypes too. This could indicate that, although total participation of DTP79 genome was similar in all the analyzed families, only a small portion of its genome influence anthesis-silking interval.

Key words: ASI, BSA, DTP, maize, SSR

INTRODUCTION

Drought is the primary abiotic stress, causing not only differences between the mean yield and the potential yield, but also causing maize yield instability. When drought stress occurs just before or during the flowering period, a delay in silking is observed resulting in an increase in the length of the anthesis-silking interval (ASI). This asynchrony between male and female flowering has been recognized as a major source of grain yield decrease (HALL et al., 1982; WESTGATE and BASSETTI, 1990; BOLANOS and EDMEADES, 1996). In routine breeding nurseries, poorly synchronized plants are automatically eliminated. Selection against silk delay has been the most effective method of breeding for drought tolerance and has been shown to be well correlated with improved yields under drought stress (TROYER, 1983). While ASI is a simple trait and has a relatively high heritability, selection for ASI is best achieved under proper drought conditions, which severely limits its use in many breeding programs. Considering these limitations to efficient selection, the use of molecular markers identifying genomic segments responsible for expression of ASI could provide a useful tool to complement phenotypic selection.

During the last two decades, development of molecular marker systems has been one of the most dynamic areas in applied molecular genetics. Techniques which are particularly promising in assisting selection for desirable characteristics involve the use of molecular markers such as *restriction fragment length polymorphism* (RFLP) and PCR (*polymerase chain reaction*) based DNA markers. Different types of molecular markers have been used for identifying genomic segments responsible for expression of ASI. RFLP markers were used for detection of quantitative trait loci (QTL) for ASI under drought conditions in tropical maize and putative QTLs were identified under drought on chromosomes 1, 2, 4, 5, 8, 9 and 10. (RIBAUT et al., 1996). Analyzing maize populations with RFLP, SSR (*simple sequence repeats*) and AFLP (*amplified*

fragment length polymorphism) markers, diverse QTLs appeared to be expressed under controlled and stressed conditions (SARI-GORLA et al., 1999). Molecular markers were used by LI et al. (2003) for identification of quantitative trait loci for flowering time and yield components in maize populations. Using SSR markers they showed that under well-watered and drought-stressed regimes, three and two QTL loci involved in the expression of ASI were on chromosomes 1, 2 and 3, i.e. 2 and 5, respectively.

The usual method for locating loci controlling QTLs is associated with a mapping population, where each plant has to be genotyped with all the markers selected to cover the genome and phenotyped for the traits of interest. A modification of QTL analysis, bulk segregant analysis (BSA), is a valuable alternative and reliable approach to identify marker alleles, from a complex gene pool, that are consistently associated with good expression of the drought tolerance trait. This technique involves pooling of DNA from plants with extreme phenotypic expression, forming two pools contrasting for a trait. These bulks are then analyzed to identify markers that distinguish between them. BSA has been successfully used in different studies (QUARRIE et. al., 1998; QUARRIE et. al., 1999; IGNJATOVIĆ-MICIĆ et al., 2006; MARKOVIĆ et al., 2007a; MARKOVIĆ et al., 2007b).

The objective of this research was to identify s1 families obtained from DTP-A population (Drought Tolerant Population-Adapted) for creating a new gene pool for breeding drought tolerant hybrids. To fulfill this task, s1 families were scored for anthesis-silking interval and genotyped with SSR markers using BSA approach. Marker analysis was performed in order to identify putative genomic segments and allele pattern differences responsible for expression of short ASI, thus providing an insight into the genetic structure of s1 families.

MATERIAL AND METHODS

Material

Secondary drought tolerance trait - ASI was scored for 76 selfed families obtained from DTP-A (DTP79xDTP12xDwarf) x B84 cross. Replicated field trials were conducted in Zemun Polje, in two different years (2005 and 2006), on 20 individual plants per family. ASI was calculated as difference between silking date (the number of days from sowing until 50% of the plants show silks) and anthesis date (the number of days from sowing until 50% of the plants have extruded anthers). Families with extreme ASI values (long and short ASI) were chosen for further analysis with SSR markers. Families with no more than 1 day difference were considered as short ASI, whereas families with no less than 5 days difference comprised for long ASI.

Methods

DNA was isolated from leaf samples of parental components and chosen families of the cross, according to modified method of SAGHAI-MAROOF

et al. (1984). Survey for polymorphic markers of parental components was preformed with 100 SSR primer pairs covering all 10 chromosomes. Identified polymorphic markers were used for bulk segregant analysis of families selected according to the extreme ASI phenotypic values. Throughout SSR analysis special emphasis was on the regions that encompass known QTLs for ASI. These regions were identified according to data from Maize Genetics and Genomics Database (http://maizegdb.org).

PCR reaction was carried out in 15 µl reaction volume containing 1x betaine SCR, 1x enzyme buffer, 2 mM $MgCl_2$, 100 µM dNTPs, 0.5 µM primers, 0.5U *Taq* polymerase and 50 ng of DNA. SSR amplification was performed using Touchdown PCR programme: 1. 95ºC/2 min; 2. 20 cycles of 95ºC/1min, 65ºC/1min (decreasing 1ºC/2 cycles), 72ºC/1min; 3. 20 cycles of 95ºC/1min, 55ºC/1min, 72ºC/1min; 4. final extension 72ºC/2min. Products of PCR reaction were separated on 4% MetaPhor agarose gels, stained with ethidium-bromide and photographed. SSR profiles for each primer were scored as presence/absence of individual bands. Allele pattern differences for each locus were determined visually. Data analyses were performed using statistical package NTSYSpc2.1.

RESULTS

According to extreme ASI phenotypic expression only five families met defined criteria – three families for short ASI (sASI) and two families for long ASI (lASI) (Table 1). Most of the other families exhibited ASI between two and four days, whereas ASI expression in some families was discrepant. Therefore, these families could not be assorted in either short or long ASI families.

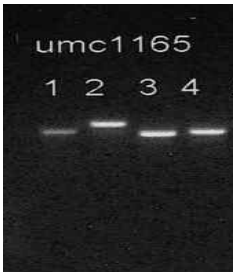
Table 1. Anthesis-silking interval (days) for identified short and long ASI families

Families	ZP2005	ZP2006	Average
sASI11	0	1	0.5
sASI14	1	1	1
sASI25	1	1	1
lASI27	6	5	5.5
lASI57	5	6	5.5

Parental molecular analysis identified 64 polymorphic and 29 monomorphic SSRs, while seven primer pairs gave poor signal and the profiles could not be read. Polymorphic SSR markers were divided into three groups: informative (23), semi-informative (21) and non-informative (20). This categorization was done on the basis of possibility to distinguish between the alleles from one or both DTP lines and the other two parental components. Informative SSR markers are those whose profile has DTP bands (alleles) different from both B84 and dwarf genotypes, thus enabling alleles originating from DTP79 and DTP12 to be easily recognized in the progeny. On the other

hand semi-informative SSRs distinguish only one of the two DTP lines (i.e., one of DTP lines has a common allele with either B84 or dwarf). The remaining 20 polymorphic markers named as non-informative could not distinguish alleles from DTP79 and DTP12 from either B84 or dwarf (Picture 1).

a) informative bnlg1523 marker (DTP alleles different from B84 and dwarf)



b) semi-informative umc1165 (one of DTP lines has a common allele with either B84 or dwarf)

c) Non-informative bnlg1884 (alleles from DTP lines cannot be distinguished from either B84 or dwarf)

Picture 1. Illustration of parental polymorphic SSR marker types - informative, semi-informative and non-informative (1-DTP79, 2-DTP12, 3-B84 and 4-dwarf)

Informative SSRs were used for allelic pattern (number and position of bands) analysis of sASI and lASI families (Table 2). All twenty-three markers were multiallelic and a total of 84 bands were recorded. Four SSR loci, different in allelic structure between sASI families and lASI families, were identified. The four loci are located on the following chromosomes region: bin3.03, bin3.08, bin3.09 and bin8.03 When compared with qASI (identified quantitative trait loci for ASI) from MGDB, two chromosome regions identified in this work were congruent with MGDB data.

Table 2. Identified chromosome regions involved in ASI expression and their comparison with regions comprising qASI reported in MGDB

	Probe	Bin	qASI / MGDB	sASI/ lASI
1	bnlg1014	1.01	X	
2	phi039	1.08	X	
3	phi064	1.11		
4	umc165	2.01		
5	bnlg1018	2.04		
6	bnlg2077	2.07	X	
7	umc1394	3.01		
8	bnlg1523	3.02		
9	bnlg1325	3.03		X
10	bnlg1350	3.08	X	X
11	umc1136	3.09		X
12	umc2048	3.1		
13	bnlg1890	4.11		
14	bnlg609	5.06	X	
15	umc1014	6.04		
16	bnlg1732	6.05	X	
17	phi082	7.05		
18	bnlg1863	8.03	X	X
19	bnlg2181	8.05		
20	umc1384	8.07	X	
21	bnlg1810	9.01	X	
22	bnlg1724	9.01	X	
23	bnlg1525	9.07	X	

Informative and semi-informative SSRs (a total of 126 bands) were used for genetic similarity (GS) and cluster analysis. Genetic similarity values were in the range from 0.1 (DTP79 and DTP12) to 0.88 (sASI-11 and sASI-14). In comparison with the four parental components all the families showed highest similarities with DTP79 and lowest with DTP12. The only exception was lASI-57, with genetic similarities almost even with all four parental components. GS between lASI27 and all three sASI families are higher than GS between lASI57 and the sASI families (0.4, 0.37 and 0.3 in respect to 0.28, 0.29 and 0.24). Results are presented in Table 3.

Table 3 Genetic similarities between parental components, sASI and lASI families
GS calculated using *Jaccard* coefficient

	Dtp79	Dtp12	dwarf	B84	sASI11	sASI14	sASI25	lASI27	lASI57
Dtp79	1								
Dtp12	0.1	1							
dwarf	0.12	0.15	1						
B84	0.21	0.19	0.29	1					
sASI11	0.37	0.17	0.28	0.17	1				
sASI14	0.34	0.12	0.29	0.14	0.88	1			
sASI25	0.31	0.09	0.27	0.22	0.3	0.3	1		
lASI27	0.38	0.16	0.19	0.15	0.4	0.37	0.3	1	
lASI57	0.3	0.25	0.28	0.3	0.28	0.29	0.24	0.25	1

The results of the cluster analysis are presented in the form of dendogram (Figure 1). All the families, as well as the parental lines, are grouped in two sub-clusters. The only exception is DTP12 line, which does not cluster with any of the analyzed genotypes. In sub-cluster A all three sASI families and lASI27 are grouped with DTP79. The lASI57 groups with B84 and dwarf in sub-cluster B.

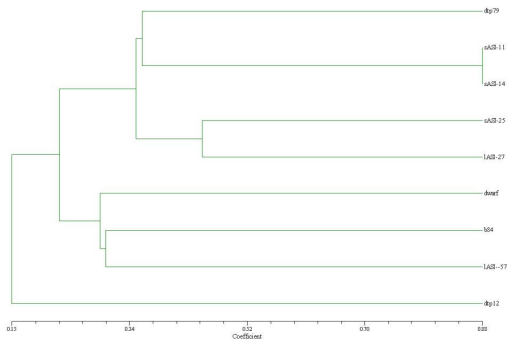


Figure 1. Dendogram of parental components, sASI and lASI families constructed using UPGMA cluster analysis of Jaccard similarity values obtained by SSR analysis

DISCUSSION

Some of the devastating effects of drought on maize could be overcome by exploiting existing genetic variation in drought tolerance in order to develop genotypes better adapted to cope with water stress. Plant breeders attempt to select lines/hybrids that perform well or are stable across environments, including stress environment. Various studies suggest that different loci and different sets of alleles are being expressed under different levels of stress (VELDBOOM and LEE, 1996). However, it is unknown if plant tolerance is due to the presence of specific favorable alleles or different genes not directly involved in control of the trait. Different strategies have been proposed to overcome negative effects of drought on maize. Breeders at MRI have created and used adapted drought tolerant population (DTP-A), that was shown to be a good source of drought tolerance for locally adapted germplasm. This population is being used as a donor of beneficial alleles to improve inbred line B84. Parallel with this improvement s1 families were analyzed with the aim to create a new gene pool for breeding drought tolerant genotypes. The trait upon which s1 families were assorted was ASI, one of the most important secondary traits for drought tolerance. When ASI is less than -5 or more than 5 days, grain yield in maize declines due to poor pollen supply (BASSETTI and WESTGATE, 1994). Greater losses can be expected when silks are exposed for more than 6 days due to silk senescence (BASSETTI and WESTGATE, 1993). These findings were the criteria for selecting families with sufficient difference in phenotypic expression. The families with ASI from 2 to 4 days difference were discarded for further analysis, because of the assumption that the shorter ASI means the better adaptation to the drought stress. The discarded families did not show stability in ASI expression over locations, what was opposite to the chosen short ASI families. Some of the families even behaved as sASI on one and as lASI on the other location.

Based on the field results SSR analysis was performed on the chosen families and four chromosome regions different in allelic structure were revealed between sASI and lASI families (Table 2). The main characteristic of these differences is the presence or absence of DTP79/DTP12 alleles. It seems that combination of several different genes and alleles influence ASI expression, with DTP79 alleles being beneficial and DTP12 alleles detrimental. Results from previous experiments on DTP79 and DTP12 (data not published) revealed ASI expression stability of DTP79 (1 day) and DTP12 instability (1 to 6 days) over different locations. Although both lines were developed from DTP population as tolerant to drought stress, DTP79 performed better for majority of measured characteristics, such as total water use, water potential, relative water content and root characteristics (QUARRIE et al., 1999). It could be assumed from the foregoing data that DTP79 line carries alleles beneficial for drought tolerant traits.

Identified chromosome regions could be potentially involved in sASI expression. The identified regions were also compared to 10 chromosome regions reported for qASI loci in the Maize Genetics and Genomics Database – MGDB (www.maizegdb.org). Two chromosome regions identified in this work (bin3.08 and bin 8.07) were congruent with MGDB data (AGRAMA and MOUSSA, 1996; VELDBOOM et al., 1994; VELDBOOM and LEE, 1996). The discrepancies with other MGDB data could be explained by several factors, such as different genetic background, different experimental conditions, different marker system used, as well as different approach in identifying chromosome regions (QTL mapping vs. BSA).

Genetic similarities between the families and parental components did not identify significant difference in the participation of different parental components in short and long ASI families. Clustering revealed that all sASI families group with DTP79, but lASI27 grouped with these genotypes too. This could indicate that, although total participation of DTP79 genome was similar in all the analyzed families, only a small portion of its genome influence anthesis-silking interval.

Selection of s1 families for creating a new gene pool for future breeding programs on maize drought tolerance could be done based upon criteria used in this work. BSA approach with SSR gave a good insight in the genetic structure indicating beneficial alleles. Results of SSR analysis should be used in future selection for breeding drought tolerant hybrids.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Science of Republic of Serbia, through the project TR-6828B.

Received February 28th, 2008
Accepted March 24th, 2008

REFERENCES

AGRAMA, H.A.S. and M.E. MOUSSA (1996): Mapping QTLs in breeding for drought tolerance. Euphytica *90*:89-97.

BASSETTI, P. and M.E. WESTGATE (1993): Senescence and receptivity maize silks. Crop Sci *33:*275-278.

BASSETTI P. and M.E. WESTGATE (1994): Floral asynchrony and kernel set in maize quantified by image analysis. Agron J. *86*:699-703.

BOLANOS, J. and G .EDMEADES (1996): The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Research, Amsterdam, *31* (2), 233-252.

HALL, A.J., F.VILELLA, N.TRAPANI and C.CHIMENTI (1982): The effects of water stress and genotype on the dynamics of pollen shedding and silking in maize. Field Crops Res *5*:349-363.

IGNJATOVIĆ-MICIĆ, D., K. MARKOVIĆ and V. LAZIĆ-JANČIĆ (2006): Application of molecular markers in bulk segregant analysis of yield in maize (*Zea mays* L.) synthetic populations. Genetika *38* (1): 59-66.

LI, X.H., X.D. LIU, M.S.LI and S.H. ZHANG (2003): Identification of quantitative trait loci for anthesis-silking interval and yield components under drought stress in maize. Acta Botanica Sinica, *45*(7): 852-857.

MARKOVIĆ, K., D.IGNJATOVIĆ-MICIĆ, G. SARATLIĆ and V.LAZIĆ-JANČIĆ (2007): Identifikacija RFLP lokusa indikatora visokog sadržaja ulja analizom grupnih uzoraka dve sintetičke populacije kukuruza (*Zea mays* L.). Arh. poljopr. nauke. *68*(242): 5-15.

MARKOVIĆ, K., D. IGNJATOVIĆ-MICIĆ, G.SARATLIĆ and V. LAZIĆ-JANČIĆ (2007): Identification of chromosome regions determining kernel high oil content in maize (*Zea mays* L.) synthetic populations. Genetika *39*(2): 197-206.

QUARRIE, S., V.LAZIĆ-JANČIĆ, D.KOVAČEVIĆ, S,PEKIĆ and A.STEED (1998): Bulk segregant analysis - a method to study the function of stress induced genes. In: Crop adaptation to cold climates, Ed. M. Sanches-Dias, E.C. Brussels, 389-398.

QUARRIE, S.A., V**.**LAZIC-JANCIC**,** D.KOVACEVIC, A. STEED and S.PEKIC (1999): Bulk segregant analysis with molecular markers and its use to help improve drought resistance in maize. Journal of Experimental Botany *50:* 1299-1306. (R51)

RIBAUT, J.M., D.A. HOISINGTON, J.A. DEUTSCH, C. JIANG and D. GONZALEZ-DE-LEON (1996): Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. Theor. Appl. Genet. *92*:905-914.

SARI-GORLA, M., P. KRAJEWSKI, N.DI FONZO, M. VILLA and C. FROVA (1999): Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. Theor Appl Genet *99*:289-295.

SAGHAI-MAROOF, M., K. SOLIMAN, R. JORGENSEN and R. ALLARD (1984): Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA 81 (24): 8014-8018.

TROYER, F. (1983): Breeding corn for heat and drought tolerance. In: Thirty-eight annual corn and sorghum research conference. Chicago, IL. Proceedings… Washington, American Seed Trade Association. *38*, 128-143.

VELDBOOM, L., M. LEE and W.L. WOODMAN (1994): Molecular marker facilitated studies in an elite maize population: 1. Linkage analysis and determination of QTL for morphological traits. Theor Appl Gen *88*:7-16.

VELDBOOM, L. and LEE M. (1996): Genetic mapping of quantitative trait loci in maize in stress and non-stress environments: 2. Plant height and flowering. Crop Sci *36*: 1320-1327.

WESTGATE M.E. and P.BASSETTI (1990): Heat and drought stress in corn in corn: what really happens to the corn plant at pollination? In: Wilkinson D (ed). Proc 45th Ann Corn and Sorghum Research Conf, ASTA, Washington, pp 12-28.

S1 FAMILIJE KAO IZVOR POŽELJNIH ALELA ZA OPLEMENJIVANJE KUKURUZA NA SUŠU

Ksenija MARKOVIĆ, Ana NIKOLIĆ, Dragana IGNJATOVIĆ-MICIĆ, Violeta ANĐELKOVIĆ i Vesna LAZIĆ-JANČIĆ

Institut za kukuruz „Zemun Polje“,Beograd, Srbija

I z v o d

U uslovima stresa suše kod kukuruza dolazi do povećanja dužine intervala između svilanja i metličanja (ASI) i do smanjnja prinosa zrna. U oplemenjivanju se primenjuju različite strategije da bi se prevazišli negativni efekti suše. U Institutu za kukuruz stvorena je DTP-A (DTP79xDTP12xDwarf) populacija, koja se pokazala kao dobar izvor tolerantnosti na sušu za germplazmu adaptiranu na uslove umerenog klimatskog pojasa. U ovim istraživanjima s1 familije DTP-AxB84 su ocenjene za ASI i analizirane SSR markerima primenom analize grupnih uzoraka, radi stvaranja novog genetičkog pula za dobijanje hibrida tolerantnih na sušu. SSR analiza je omogućila identifikaciju potencijalnih genomskih segmanata i identifikaciju razlika u alelnoj strukturi, odgovornih za ekspresiju ASI. Identifikovana su četiri SSR lokusa, koja se razlikuju u alelnoj strukturi familija sa kratkim i dugačkim ASI. Glavna karakteristika ovih razlika je u prisustvu/odsustvu DTP79/DTP12 alela. Najverovatnije da kombinacija nekoliko različitih gena i alela utiče na ekspresiju ASI, pri čemu DTP79 aleli pokazuju pozitivan, a DTP12 negativan efekat. Dva hromozomska regiona koja su identifikovana u ovom radu su prijavljena za ASI i u *Maize Genetics and Genomics Database*. Rezultati dobijeni klaster analizom su pokazali da sve familije sa kratkim ASI grupišu sa DTP79, mada se i jedna dugačka ASI familija grupiše sa navedenim genotipovima.

Ovo može ukazivati da, iako je ukupno učešće DTP79 genoma slično kod svih analiziranih familija, samo mali deo njegovog genoma utiče na ekspresiju ASI.

Primljeno 28. II. 2008.
Odobreno 24. III. 2008.