POLYMORPHISM OF SSR MARKERS LOCATED ON CHROMOSOME 7A, IN SEVERAL WHEAT CULTIVARS GROWN IN ALGERIA

Yousfi Younes[1]

ABSTRACT

Water stress is one of the main limiting factors for wheat yield in the semi-arid plains of Algeria, and this recommends wheat cultivars grown there as potential donors of genes, controlling traits that can improve performance under stress. Fourteen wheat cultivars grown on significant acreage in Algeria were analyzed to explore the polymorphism of several SSR markers located on chromosome 7A, which was associated with drought resistance in many previous studies. Markers Xwmc9, Xwmc596 and Xwmc603 showed polymorphism among both *T. durum* and *T. aestivum* Algerian wheat cultivars. None of the studied Algerian cultivars produced an electrophoretic profile similar to the less drought resistant Romanian cultivar used as a check, but some cultivars had profiles similar to the drought resistant Romanian check, for some of the markers. For most Algerian cultivars, the observed polymorphism was different from that found in Romanian cultivars. The polymorphism found among Algerian cultivars for markers located on chromosome 7A offers chances of finding useful associations between these markers and traits that can contribute to improved drought resistance.

Key words: wheat, SSR (Simple sequence repeat) molecular markers, drought.

INTRODUCTION

Water stress is one of the main limiting factors for wheat yield in many regions, including the semi-arid plains of Algeria (Boulal et al., 2007).

The cereals are cultivated through the whole of the agro-ecological zones of the country, but they are primarily localized in the semi-arid areas and even arid (where precipitations varies between 200 and 500 mm) and thus are subjected to the climatic risks which strongly penalize the production (Boulal et al., 2007).

The weakness of precipitations, their irregularity and raised evaporation in this area, cause water deficits at the various stages of the culture, which, during the vegetative period and even at the stage of reproduction and grain development, negatively influence the physiological processes of the plant and thereafter the yield (Abed et al., 2000).

Breeding drought resistant cultivars is an important way to increase yield under water stress (Zhang et al., 1999). Progress in breeding for drought resistance depends on identifying genetic sources for traits which can improve performance under stress. Wheat cultivars grown in the semi-arid part of Algeria are potential donors of genes controlling such traits. Transfer of these genes can be accelerated by identification of molecular markers associated with drought resistance traits and their use in marker assisted selection.

Several studies associated chromosome 7A with drought resistance in wheat (Morgan and Tan, 1996; Galiba, 2002; Cattivelli et al., 2002). Morgan (1991) located a gene for osmoregulation (*„or")* on chromosome 7A, and later Morgan and Tan (1996), using RFLP analysis, established the location of this gene on the short arm, at about 13 cM distance from the centromere.

The objective of this work was to explore the polymorphism of several SSR (Simple sequence repeat) markers located on chromosome 7A, in fourteen wheat cultivars, grown on significant acreage in Algeria.

MATERIAL AND METHODS

Nine durum wheat cultivars - *Triticum durum* (Table 1) and five common wheat cultivars - *Triticum aestivum* (Table 2), grown in various regions of Algeria, kindly provided by the Technical Institute for Field Crops (Institut Technique des Grandes Cultures), Alger, were planted at the National Agricultural Research and Development Insitute Fundulea, Romania. Three of these are local populations and the rest were introduced from other countries, based on their adaptation to the growing areas of Algeria (Boufenar et al., 2006).

Two Romanian wheat cultivars (Izvor and Jiana), characterized by Bănică et al. (2008) as different for osmotic adjustment ability, were also included as checks in the analysis of DNA polymorphism.

[1] National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania. E-mail: ymhg2001@yahoo.fr

Table 1. Name, origin, earliness and drought response of durum wheat cultivars (*Triticum durum*), included in the study (Boufenar et al., 2006)

Cultivar	Origin	Earliness	Drought response
Waha	ICARDA/Syria	Early	Susceptible
Chen's'	ICARDA/Syria	Early	Tolerant
Bidi 17	Local	Late	Resistant
Vitron	Spain	Medium early	Susceptible
Hedba 3	Local	Late	Susceptible
Mohamed ben bachir	Local	Late	Tolerant
Gtadur	CIMMYT/Mexico	Early	Resistant
Bousselem	ICARDA/Syria	Late	Resistant
Mexicali	CIMMYT/Mexico	Late	-

Table 2. Name, origin earliness and drought response of common wheat cultivars (*Triticum aestivum*), included in the study (Boufenar et al., 2006)

Cultivar	Origin	Earliness	Drought response
Hiddab	CIMMYT/Mexico	Medium early	Tolerant
Arz	CIMMYT/Mexico	Medium early	Resistant
Anza	USA	Early	Resistant
Mahon demias	Spain	Late	Tolerant
Ain abid	Spain	Medium early	Tolerant

DNA extraction was achieved using a CTAB (Cetyl trimethyl ammonium bromide) based protocol adapted from Saghai-Maroof et al. (1984) and Henry (1997).

The amplification was performed in a 25 µl final volume of a reaction mixture, which included reaction buffer 1x, 0.2 mM each deoxinucleotide, 0.25 mM each primer, 1.5 mM $MgCl_2$, 1U of enzyme Taq polymerase Promega and 50-100 ng genomic DNA matrix.

The PCR reaction was performed using a thermo-cycler Applied Biosystem GeneAmp 9600, programmed for: 3 minutes initial denaturation at 94ºC, followed by 35 cycles, each consisting of: 1 minute at 94ºC, 1 minute at 61ºC, 2 minutes at 72ºC and a final extension of 10 minutes at 72ºC. PCR products were evaluated by electrophoresis, on 2% agarose gels in 0.5 x TBE buffer, stained with ethidium bromide and were recorded as BioPrint images.

Here we present the results obtained with three pairs of primers, located on chromosome 7A (www.graingenes.org), which showed polymorphism among Romanian cultivars differing in drought response (Ciucă and Petcu, 2009) (Table 3).

Table 3. SSR markers and corresponding primers used in this study (Somers and Isaac, 2004)

Marker	Primer	
Wmc603 SSR-7A	Forward	ACAAACGGTGACAATGCAAGGA
	Reverse	CGCCTCTCTCGTAAGCCTCAAC
Wmc9 SSR-7A	Forward	AACTAGTCAAATAGTCGTGTCCG
	Reverse	GTCAAGTCATCTGACTTAACCCG
Wmc596 SSR	Forward	TCAGCAACAAACATGCTCGG
	Reverse	CCCGTGTAGGCGGTAGCTCTT

According to Somers and Isaac (2004) these markers are closely linked in a position close to the centromere of wheat chromosome 7A (Figure 1).

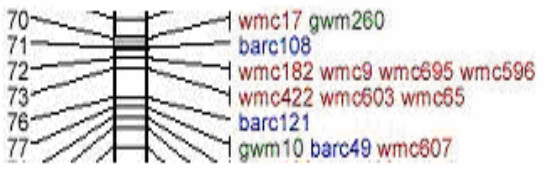

Figure 1. Position of markers Xwmc9, Xwmc596 and Xwmc603 on chromosome 7A (Somers and Isaac, 2004)

RESULTS

The PCR products obtained using the primers for the marker Xwmc603 showed polymorphism among Algerian wheat cultivars. None of these cultivars was similar with the less drought resistant check Jiana, but cultivars Hedba3 (*T. durum*), Bousselem (*T. durum*) and Mohamed Ben Bachir (*T. durum*) produced amplification products similar with the drought resistant check Izvor. The other cultivars, and especially Mahon Demias (*T. aestivum*), Mexicali (*T. durum*), Vitron (*T. durum*), Chen'S' (*T. durum*) etc., showed a polymorphism not found in Romanian cultivars (Figure 2).

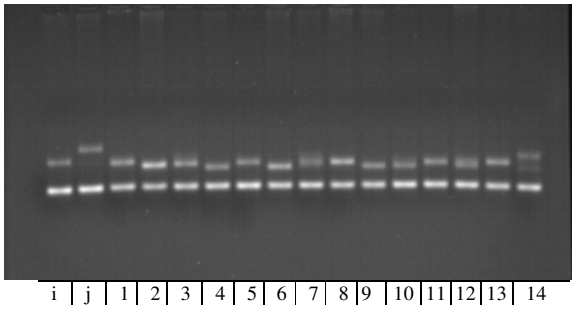

Figure 2. Electrophoretic profiles obtained with the primers for marker Xwmc603:
(i. Izvor; j. Jiana; 1. Hedba3; 2. Mexicali; 3. Ain Abid; 4. Vitron; 5. Bousselem; 6. Chen's'; 7. Arz; 8. Mohamed Ben Bachir; 9. Anza; 10. Bidi17; 11. Waha; 12. Gtadur; 13. Hiddab; 14. Mahon Demias)

The PCR products obtained using the primers for marker Xwmc9 also showed significant polymorphism among Algerian cultivars (Figure 3). None of these cultivars was similar with the less drought resistant check Jiana, nor with the drought resistant check Izvor. One of the two band observed in the resistant check was similar with the band present in cultivars Hedba3 (*T. durum*), Mexicali (*T. durum*), Bousselem (*T .durum*), Chen's' (*T. durum*), Bidi 17 (*T. durum*), Arz (*T. aestivum*) or Hiddab (*T. aestivum*), but the second band cannot be seen in these cultivars. Other cultivars show specific amplification products, not found in the checks. It is to be noted however that marker Xwmc9 is located not only on chromosome 7A, but also on chromosome 1A, so that part of the observed polymorphism might not be due to differences on chromosome 7A.

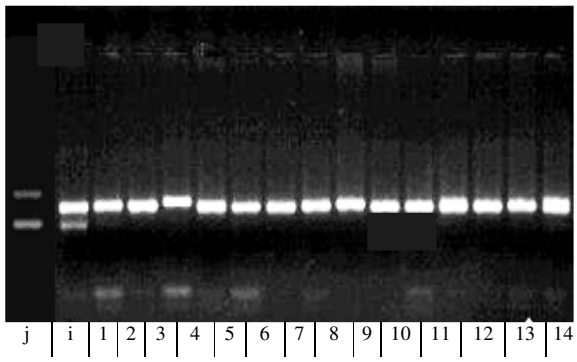

Figure 3. Electrophoretic profiles obtained with the primers for marker Xwmc9:
(j. Jiana; i. Izvor; 1. Hedba3; 2. Mexcicali; 3. Ain Abid; 4. Vitron; 5. Bousselem; 6. Chen's'; 7. Arz; 8. Mohamed Ben Bachir; 9. Anza; 10. Bidi17; 11. Waha; 12. Gtadur; 13. Hiddab; 14. Mahon Demias)

Electrophoretic profiles obtained with the primers for marker Xwmc596 also showed polymorphism among the wheat cultivars from Algeria (Figure 4). Again, none of these profiles was similar with the check cultivar Jiana, but cultivars Mexicali (*T. durum*), Ain Abid (*T. aestivum*), Anza (*T. aestivum*), Bidi17 (*T .durum*) and Gtadur (*T. durum*) were similar with check Izvor. Several cultivars, including Bousselem (*T. durum*), Mohamed Ben Bachir (*T. durum*) and Mahon Demias (*T. aestivum*) had specific profiles, significantly different from the checks.

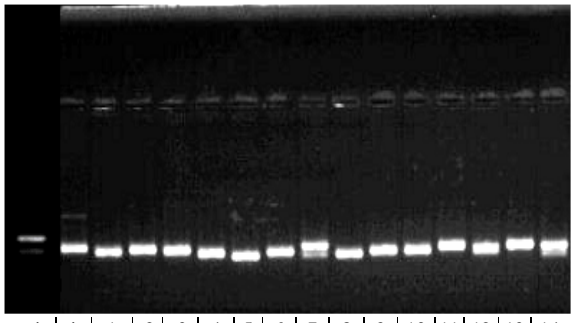

Figure 4. Electrophoretic profiles obtained with the primers for marker Xwmc596:
(j. Jiana; i. Izvor; 1. Hedba3; 2. Mexcicali; 3. Ain Abid; 4. Vitron; 5. Bousselem; 6. Chen's'; 7. rz; 8.Mohamed Ben Bachir; 9. Anza; 10. Bidi17; 11. Waha; 12. Gtadur; 13. Hiddab; 14. Mahon Demias)

DISCUSSION

Our results show a considerable polymorphism among wheat cultivars grown in Algeria, for all three markers presented in this paper. The polymorphism was present both in *Triticum durum* and *T. aestivum* cultivars.

None of the studied Algerian cultivars produced an electrophoretic profile similar to the less drought resistant Romanian cultivar used as a check, but some cultivars had profiles similar to the drought resistant Romanian check, for some of the markers.

Ciucă and Petcu (2009), studying the same markers in doubled haploid lines derived from a cross between cultivars Izvor and Jiana, used as checks in our study, found that markers Xwmc9, Xwmc596 and Xwmc603 co-segregated, showing a complete correspondence between grouping the lines according to the alleles of the three markers. In contrast,

Algerian cultivars having electrophoretic profiles similar to Izvor for one marker, did not have the same similarity for the other markers. This suggests that the Algerian cultivars carry various combinations of alleles at the three marker loci, not found in the Romanian cultivars.

Further studies should establish to what extent the polymorphism found in Algerian cultivars for markers Xwmc9, Xwmc596 and Xwmc603 is associated with traits contributing to drought resistance.

CONCLUSIONS

SSR markers Xwmc9, Xwmc596 and Xwmc603 showed polymorphism among the tested Algerian *T. durum* and *T. aestivum* wheat cultivars.

The observed polymorphism was, to a large extent, different from that found in Romanian cultivars. Cultivars that had an electrophoretic profile similar to the drought resistant Romanian cultivar Izvor for one marker had different profiles for the other markers.

The polymorphism found among Algerian cultivars for markers located on chromosome 7A offer chances of finding useful associations between these markers and traits that can contribute to improved drought resistance.

REFERENCES

Abed, R., Badis, M., Mekliche, R., Larbi, A., 2000. *Effet du déficit hydrique sur la production de deux variétés de blé dur (Triticum turgidum L. var. durum) en région semi-aride.* CIHEAM - Options Mediterraneennes: 295-297.

Bănică, C., Petcu, E., Giura, A., Săulescu, N.N., 2008. *Relationship between genetic differences in the capacity of osmotic adjustement and other physiological measures of drought resistance in wheat (Triticum aestivum L.).* Romanian Agricultural Research, 25: 7-13.

Boufenar, Z. F., Zaghouan, O., 2006. *Guide des principales variétés de céréales à paille en Algérie (Blé Dur, Blé Tender, Orge et Avoine*). ITGC, ICARDA: 154.

Boulal, H., El Mourid, M., Rezgui, S., Zaghouan, O., 2007. *Guide pratique de la conduite des céréales d'automne (Blé et Orge) dans le Maghreb (Algérie, Maroc et Tunisie)*. ITGC. INRAA. ICARDA: 176.

Cattivelli, L., Baldi, P., Crosatti, C., Di Fonzo, N., Faccioli, P., Grossi, M., Mastrangelo, A. M., Pecchioni, N. and Michele, Stanca, A., 2002. *Chromosome regions and stress-related sequences involved in resistance to abiotic stress in Triticeae.* Plant Molecular Biology, 48 (5-6): 649-665.

Ciucă, Matilda and Petcu, Elena, 2009. *SSR markers associated with membrane stability in wheat (Triticum aestivum L.)*. Romanian Agricultural Research, 26: 21-24.

Galiba, G., 2002. *Mapping of genes regulating abiotic stress tolerance in cereals*. Acta Agronomica Hungarica, 50(3):235-247.

Henry, RJ., 1997. *Practical applications of plant molecular biology.* 1st ed, Chapman and Hall, London.

Morgan, J. M. 1991. *A gene controlling differences in osmoregulation in wheat*. Australian Journal of Plant Physiology, 18: 249-257.

Morgan, J. M., Tan, M. K., 1996. *Chromosomal location of a wheat osmoregulation gene using RFLP analysis.* Australian Journal of Plant Physiology, 23(6): 803-806.

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A., and Allard, R. W. 1984. *Ribosomal DNA spacer-length polymorphism in barley: mendalian inheritance, chromosomal location and population dynamics.* Proc Natl Acad USA 81: 8014-18.

Somers, D. J. and Isaac, P. 2004. *A high-density wheat microsatellite consensus map for bread wheat (Triticum aestivum L.).* Theoretical and Applied Genetics, 109: 1105-1114.

Zhang, J., Nguien, H. T., Blum, A., 1999. *Genetic analysis of osmotic adjustement in crop plants*. Journal of Experimental Botany, 50 (332): 291-302.