NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 27, 2010
Print ISSN 1222-4227; Online ISSN 2067-5720

SSR MARKERS ASSOCIATED WITH THE CAPACITY FOR OSMOTIC ADJUSTMENT IN WHEAT (*TRITICUM AESTIVUM* L.)

Matilda Ciucă[1], Constantina Bănică[1], Monica David[1], Nicolae N. Săulescu[1]

[1] National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania
E-mail: matilda72001@yahoo.co.uk

ABSTRACT

Sixty two doubled haploid (DH) lines from the cross between cultivars Izvor (high osmotic adjustment) and Jiana (medium osmotic adjustment), obtained using the „Zea system", were used to study the association between the capacity for osmotic adjustment, as estimated by the pollen grain test developed by Morgan (1999), and several SSR markers located on chromosome 7A. SSR m arkers Xwmc9, Xwmc596 and Xwmc603 were significantly associated with pollen grain response to immersion in solution of 55% PEG + KCl, being located at approximately 9.1 cM estimated distance from the osmotic adjustment gene. These markers, which had a relatively low recombination frequency with the osmotic adjustment locus, can prove useful for increasing the frequency of progenies with better performance under drought in a wheat breeding program.

Key words: wheat, SSR markers, osmotic adjustment, drought resistance.

INTRODUCTION

Improving yield under water stress is one of the main wheat breeding objectives in Romania and its importance is expected to increase with the expected climate changes (Săulescu et al., 1998).

Selection for physiological traits related to drought tolerance is essential as it can increase selection efficiency by reducing the effect of the large year to year variation in water availability and the effects of soil un-uniformities.

Many mechanisms and traits, potentially useful for improving plant performance under drought have been described (Blum, 1996, 1998; Ginkel et al., 1998). Among them, osmotic adjustment (OA) is receiving increased recognition as a major mechanism of drought resistance in crop (Zhang et al., 1999). The capacity to adjust osmotically is an inherited trait, which in wheat is controlled by alternative alleles at one locus on chromosome 7A, that controls primarily differences in potassium accumulation (Morgan, 1983, 1991). Other genes might be involved, as shown by Galiba (2002), who reported that genes controlling OA were mainly located on chromosomes 5A and 5D.

As OA is a cellular mechanism it is expressed in all plant cells, including pollen, and this offers a convenient way to characterize germplasm for this trait (Morgan, 1999; Moud and Yamagishi, 2005).

There are many reports documenting the association between osmotic adjustment and crop production (yield and/or biomass) under water-limited conditions, in several crops, including wheat (see Blum, 1996), but also authors questioning the importance of osmotic adjustment (Serraj and Sinclair, 2002). The fact that the cultivar Izvor, which showed the best performance under drought (Mustăţea et al., 2003, 2009) also had the highest osmotic adjustment capacity (Bănică et al., 2008), suggests that osmotic adjustment plays an important role in determining the yield in dry years in Romania.

Molecular markers proved to be an important way to increase selection efficiency and there are good prospects for marker-assisted selection in improving drought responses in wheat (Quarrie et al., 2003). However, despite many recent reports of markers associated with drought resistance, as far as we know, the only published marker for the „*or*" gene remains the RFLP locus Xpsrll9, located at approximately 13 cM towards the

Received March 2, 2010; accepted March 31, 2010

centromere from the „*or*" gene (Morgan and Tan, 1996). This paper presents preliminary results about the association of several SSR markers with genetic differences in the osmotic adjustment capacity, estimated using pollen grain expression of OA.

MATERIAL AND METHODS

Sixty two doubled haploid (DH) lines from the cross between cultivars Izvor (drought resistant, characterized by high OA capacity) and Jiana (medium drought resistance, with medium OA capacity), were obtained using the „*Zea* system" (Giura, 1993, 2007).

The osmotic adjustment capacity was estimated using the pollen test developed by Morgan (1999). Pollen grains of matured but unopened anthers, collected from spikes sampled from field plots in 2006, were soaked in 55% polyethylene glycol (PEG 6000) solutions, over microscope slides, with 10 mM KCl added to the solutions (Bănică et al., 2008). After a little agitation to release the pollen grains, the anther sections were removed and the solution covered with a cover slip. Slides were incubated at 20°C for 2 days. Microscopic observations were made using a magnification of 100X and 200X. Modification of pollen grains shape (shrinking) was estimated visually to discriminate the lines according to their OA capacity. The test was repeated in 2007 and 2009, to resolve uncertain classifications.

Total DNA was isolated from leaves and purified following the protocol proposed by Saghai-Maroof et al. (1984), using a CTAB based protocol.

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

The Applied Biosystem 9600 thermal cycler, was programmed for: 3 minutes at 94ºC, followed by 35, 40 or 45 cycles, each consisting of: 1 minute at 94ºC, 1 minute at 50ºC, 53ºC, 55ºC, or 61ºC (according to the primer), 2 minutes at 72ºC and a final extension of 10 minutes at 72ºC. PCR products were evaluated by electrophoresis, on 2% agarose (High resolution-Sigma) gels in 0.5X TBE buffer. The bands were visualized by ethidium bromide staining. Images were taken with the help of a BioPrint documentation system.

Primers were selected from the database www.graingenes.org based on their location on chromosome 7A, as previous information associated this chromosome with osmotic adjustment and/or drought resistance (Morgan and Tan, 1996; Galiba, 2002; Cattivelli et al., 2002; Quarrie et al., 2006; Kordenaeej, 2008). Data were analyzed using the Demo version of JoinMap®4 software (Van Ooijen, 2006).

RESULTS AND DISCUSSION

Out of the 62 DH lines from the cross between a cultivar with high OA (Izvor), and one with intermediate OA (Jiana), 31 had high OA, similar to the cultivar Izvor, and were considered carriers of the recessive allele „*or*" and 31 had the intermediate pollen expression of osmotic adjustment, characteristic for the cultivar Jiana, and were considered carriers of the dominant allele „*Or*" (Figure 1).

This is in complete agreement with the results of Morgan (1991) who found that differences in osmotic adjustment detected between studied wheat cultivars were controlled by alleles at one locus.

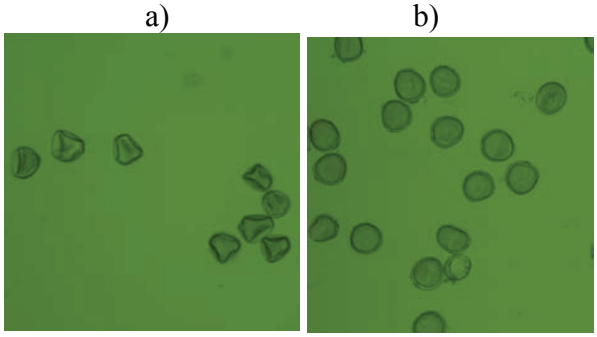

Figure 1. Polen grains shape and volume after immersion in 55% PEG solution + KCl in Jiana – a genotype with low osmoregulation capacity (a), and in Izvor – a genotype with high osmoregulative capacity (b)

Based on analysis of linkage with the RFLP loci, Morgan and Tan (1996) suggested a probable position of the „*or"* gene on the short arm of chromosome 7A, approximately

13 cM towards the centromere from RFLP locus Xpsrll9.

The authors indicated that their findings, which were based on a relatively small sample, are of a preliminary nature and require confirmation with a larger set of genetic stocks.

Microsatellite (also called SSR) markers are recognized as combining a number of advantages for use in breeding: they are co-dominant and multi-allelic, they are highly variable, being in most cases able to detect a higher level of polymorphism per locus than RFLP or AFLP markers, and they are amenable to high throughput analysis (Rőder et al., 1995).

This is why we considered desirable to identify SSR markers associated with the „*or*" gene, that would be easier to use in marker assisted selection. In our search, we started from the information given by Morgan and Tan (1996) about the probable location of this gene.

SSR primers for Xbarc108, Xbarc121, Xwmc9, Xwmc596, Xwmc603 and Xgwm260 showed clear polymorphism between the parents, and among DH lines, while other primers like Xwmc65 showed no polymorphism.

Classifications of DH lines according to the alleles at the Xwmc9, Xwmc596 and Xwmc603 loci were in complete agreement, confirming the very close linkage between these loci. Xbarc108 Xgwm260 and especially Xbarc121 gave different classification.

For all these primers segregation of DH lines was in agreement with one gene hypothesis, the observed slight differences from 1:1 ratio being not significant according to χ^2 test ($\chi^2 < 1.47$).

Genetic distances between the tested markers and the „*or*" gene, estimated by JoinMap®4 software for the calculation of genetic linkages (Van Ooijen, 2006), are presented in table 1.

We previously established a weak but significant association of markers Xwmc9, Xwmc596, Xwmc603 and Xbarc108 with cell membrane stability after water stress, and, based on the association found by Bănică et al. (2008) between membrane stability and genetic differences in the capacity of osmotic adjustment expressed in pollen grains, suggested that these markers might be associated with the „*or*" gene (Ciucă and Petcu, 2009). Our present results confirm that hypothesis and show that markers Xwmc9, Xwmc596 and Xwmc603 have the lowest recombination frequency with the „*or*" gene.

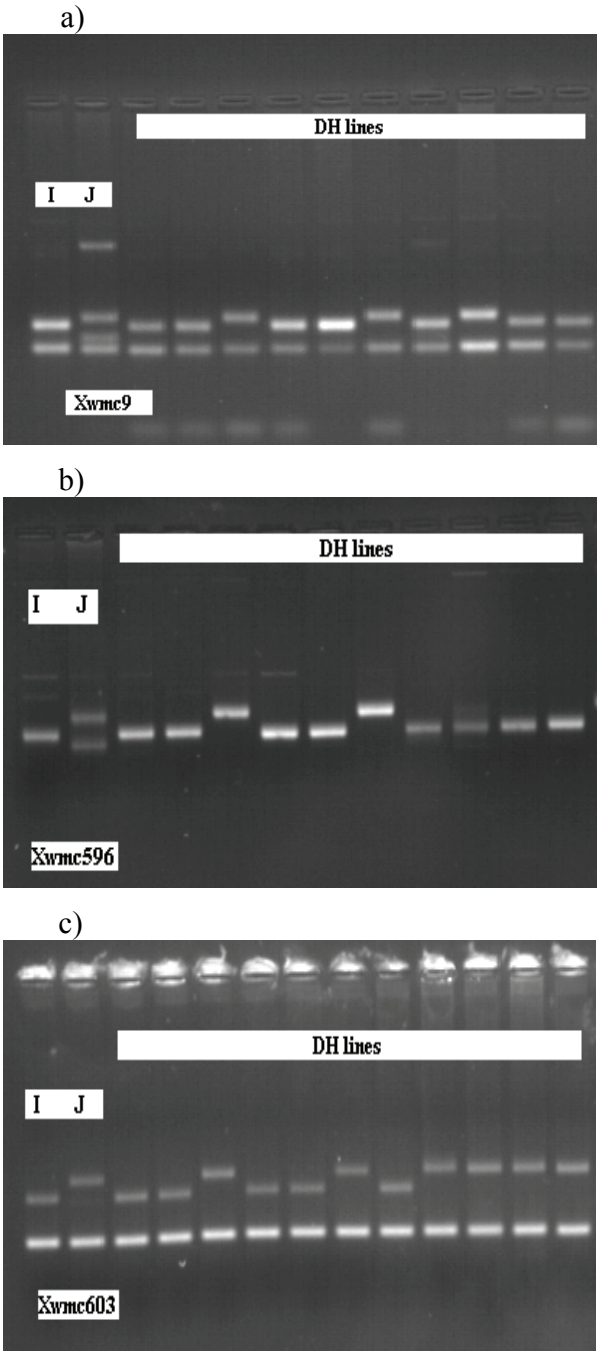

Figure 2. Polymorphism between parental forms Izvor (PR) and Jiana (PS), and among DH lines revealed by SSR markers Xwmc9 (a), Xwmc596 (b) and Xwmc603 (c), as detected by electrophoresis in agarose gel

Table 1. Genetic distances between markers Xwmc9, Xwmc596, Xwmc603, Xbarc108, Xgwm260 and Xbarc121, and the „*or*" gene, estimated by JoinMap®4 software

Marker	Estimated distance from the osmotic adjustment gene	Recombination frequency	LOD
Xwmc9	9.1	0.0806	11.44
Xwmc596	9.1	0.0806	11.44
Xwmc603	9.1	0.0806	11.44
Xbarc108	9.3	0.0833	9.46
Xgwm260	13.0	0.1321	7.61
Xbarc121	29.1	0.2182	4.12

The relatively small mapping population and the subjective nature of the pollen grain test for osmotic adjustment capacity might have affected the precision of our estimation of linkages between the SSR markers and the osmotic adjustment gene. Definitely, our data require confirmation with a larger set of genetic stocks and a more objective estimation of osmotic adjustment. However, we consider that even present results indicate a possibility of using one of the markers Xwmc9, Xwmc596 and Xwmc603 in breeding wheat for one important component of drought resistance.

CONCLUSIONS

SSR markers Xwmc9, Xwmc596 and Xwmc603 proved to be significantly associated with the capacity of osmotic adjustment expressed in pollen grains and can be useful for increasing the frequency of progenies with better performance under drought in a wheat breeding program.

REFERENCES

Bănică, C., Ciucă, M., Giura, A., 2008. *Pollen grain expression of osmotic adjustment in Romanian winter wheat*. European Wheat Aneuploid Co-operative Newsletter 2008: 100-102.

Bănică, C., Petcu, E., Giura, A., Săulescu, N.N., 2008. *Relationship between genetic differences in the capacity of osmotic adjustment and other physiological measures of drought resistance in winter wheat (Triticum aestivum L.).* Romanian Agricultural Research, 25: 7-11.

Blum, A., 1996. Yield *potential and drought resistance: Are they mutually exclusive*?. In: M.P. Reynolds et al. (eds.) Increasing yield potential in wheat: Breaking the barriers: 90-100. CIMMYT, Mexico, D.F.

Blum, A., 1998. *Improving wheat grain filling under stress by stem reserve mobilization.* In: Wheat: Prospects for Global Improvement (Editors H.-J. Braun, F. Altay, W. E. Kronstad, S.P.S. Beniwal, A. McNab). Kluwer Academic Publishers, Netherlands: 135-141.

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.

Fischer, R. A., Maurer, R., 1978. *Drought resistance in spring wheat cultivars.* I. Grain responses. Aust. J. Agric. Res. 29: 897-912.

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

Ginkel, M. van, Calhoun, D. S., Gebeyehu, G., Miranda, A., Tian-you, C., Pargas Lara R., Trethowan, R.M., Sayre, K., Crossa, J., Rajaram, S., 1998. *Plant traits related to yield of wheat in early, late or continuous drought conditions*. In: Wheat: Prospects for Global Improvement" (Editors H.-J. Braun, F. Altay, W.E. Kronstad, S.P.S. Beniwal, A. McNab). Kluwer Academic Publishers, Netherlands: 167-179.

Giura, A., 1993. *Progress in wheat haploid production.* Proc. 8th Int. Wheat Genet. Symp. Beijing, China: 741-745.

Giura, A., 2007. *The use of biotechnological Zea system in wheat genetics and breeding at NARDI Fundulea*. An. INCDA Fundulea, LXXIV-Omagial: 179-182.

Kordenaeej, A., 2008. *Mapping QTLs for yield and yield components under drought stress in bread wheat.* Dissertation for a doctorate degree, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria: 97. http://zidapp.boku.ac.at/abstracts/download.php?property id=107&dataset_id=6636

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

Morgan, J.M., 1983. *Osmoregulation as a selection criterion for drought tolerance in wheat.* Aust. J. Agric. Res., 34: 607-614.

Morgan, J.M., 1991. *A gene controlling differences in osmoregulation in wheat*. Aust. J. Agric. Res., 18: 249-257.

Morgan, J.M., 1999. *Pollen grain expression of a gene controlling differences in osmoregulation in wheat*

leaves: a simple breeding method. Aust. J. Agric. Res., 50: 953-962.

Moud, A.A.M., Yamagishi, T., 2005. *Application of projected pollen area response to drought stress to determine osmoregulation capability of different wheat (Triticum aestivum L.) cultivars.* International Journal of Agriculture and Biology, 7(4): 604-605.

Mustățea, P., Săulescu, N.N., Ittu, Gh., Păunescu, Gabriela, Stere, Ioana, Tanislav, N., Zamfir, M. C., Voinea, I., 2003. *Diferențe genotipice în rezistența grâului la secetă, evidențiate în condițiile anului 2002*. Analele ICDA Fundulea, 70 : 7-16.

Mustățea, P., Săulescu, N.N., Ittu, Gh., Păunescu, Gabriela, Voinea, Leliana, Stere, Ioana, Mîrlogeanu, Sorina, Constantinescu, E., Năstase, D., 2009. *Grain yield and yield stability of winter wheat cultivars in contrasting weather conditions*. Romanian Agricultural Research, 26: 1-8.

Quarrie, S.A., Dodig, D., Pekiç, S., Kirby, J., Kobiljski, B., 2003. *Prospects for marker-assisted selection of improved drought responses in wheat.* Bulg. J. Plant Physiol., Special Issue 2003: 83-95.

Quarrie, S.A., Quarrie, S.P., Radosevic, R., Kaminska, A., Barnes, J.D, Leverington, M., Ceoloni, C., Dodig, D., 2006. *Dissecting a wheat QTL for yield present in a range of environments: From the QTL to candidate genes.* Journal of Experimental Botany, 11: 2627-2637.

Röder, M.S., Plaschke, J., König, S.U., Börner, A., Sorrells, M.E., Tanksley, S.D., and Ganal, M.W., 1995. *Abundance, variability and chromosomal location of microsatellites in wheat.* Mol. Gen. Genet., 246: 327-333.

Sagai-Maroof, M.A., Soliman, K., Jorgensen, R. A., Allard, R.W.M., 1984. *Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location and population dynamics*. PNAS 81: 8014-8018.

Săulescu, N.N., Ittu, Gh., Balotă, Maria, Ittu, Mariana, Mustățea, P., 1998. *Breeding wheat for lodging resistance, earliness and tolerance to abiotic stresses.* In: Wheat: Prospects for Global Improvement. (Editors H.-J. Braun, F. Altay, W.E. Kronstad, S.P.S. Beniwal, A. McNab), Kluwer Academic Publishers, Netherlands: 181-188.

Serraj, R. and Sinclair, T.R., 2002. *Osmolyte accumulation: can it really help increase crop yield under drought conditions?* Plant, Cell and Environment, 25: 333-341.

Van Ooijen, J.W., 2006. *JoinMap®4, Software for the calculation of genetic linkage maps in experimental populations*. Kyazma B.V., Wageningen, Netherlands.

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