

SSR MARKERS ASSOCIATED WITH MEMBRANE STABILITY IN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Membrane stability has been suggested as a useful measure of drought tolerance in wheat breeding programs. We studied the association between membrane stability, as estimated by conductivity test after exposing plants to water stress in the field, and several SSR markers located on chromosome 7A. SSR markers wmc9, wmc596, wmc603 and barc108 were weakly but significantly associated with cell membrane stability after water stress and can be used for increasing the frequency of progenies with better performance under drought in a wheat breeding program.

Key words: wheat, SSR markers, membrane stability.

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). In addition to selection for field performance, selection for physiological traits related to drought tolerance is essential because of large year to year variation in water availability. Among the physiological traits that are correlated with performance under drought, membrane stability has been recognized as a useful measure of drought and heat tolerance in wheat (Blum and Ebercon, 1981).

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).

This paper presents preliminary results about the association of several SSR markers with membrane stability in a set of doubled haploid (DH) lines derived from a cross between two cultivars, different for membrane stability after water stress.

MATERIAL AND METHODS

Sixty two doubled haploid (DH) lines from the cross between cultivars Izvor (drought resistant) and Jiana (medium drought resistant), were obtained at NARDI Fundulea (Giura, 2007),

and characterized for several traits related to drought resistance, including membrane stability.

Membrane stability after water stress was estimated by a conductance test, following the method described by Saadalla et al. (1990) and modified by Petcu and Țerbea (1995), using naturally water stressed plants from the field. Medium part of flag leaves, collected from field plots on May 21, 2007 when wilting was noticeable on medium drought resistant parent Jiana, were cut in 2 cm segments, washed and then placed in Falcon tubes with 25 ml distilled water. Each rep consisted of nine leaf segments. The tubes were shaken for 24 hours to mix the content and an initial conductance reading (C1) was made. The tubes were then autoclaved at a pressure of 1.5 atm for 15 min to completely destroy the tissue and a second conductance reading (C2) was made.

Membrane injury index was calculated by formula:

$$\% \text{ injury} = (C1/C2) \times 100,$$

and membrane stability using the formula:

$\text{MemSt} = 1 - \% \text{ injury} = 1 - (C1/C2) \times 100,$
where C1 and C2 are the first and the second reading of conductance.

Total DNA was isolated from leaves and purified following the protocol proposed by Saghai-Marooof 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, 0.25mM primer, 1.5 mM MgCl₂, 1U of Taq polymerase Promega and 50-100 ng genomic DNA matrix.

The Applied Biosystem 9600 thermal cycler, was programmed for: 3 minutes at 94°C, followed by 35, 40 or 45 cycles, each consisting of: 1 minute at 94°C, 1 minute at 50°C, 51°C, 55°C, 60°C or 61°C (according to the primer), 2 minutes at 72°C and a final extension of 10 minutes at 72°C. PCR products were evaluated by electrophoresis, on 2% agarose

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gels in 0.5 x TBE buffer, stained with ethidium bromide and recorded as BioPrint images.

The following primers were selected from the database www.graingenes.org, based on their location on chromosome 7A, as previous information associated this chromosome with drought resistance (Morgan and Tan, 1996; Galiba, 2002, Cattivelli et al., 2002): Xbarc108, Xbarc121, Xwmc9, Xwmc596, Xwmc603, Xwmc65, Xwmc695, Xwmc17, Xwmc182, Xwmc233, Xgwm130, Xgwm260, Xgwm 573, Xgwm635, Xgwm350, Xgwm170, Xgwm332, Xgwm276, Xcwm18, Xcwm46, Xcwm55.

Data were analyzed using ANOVA and Qgene software.

RESULTS AND DISCUSSION

Parental cultivars Izvor and Jiana were significantly different in membrane stability (78.1% versus 51.0%), and the DH lines derived from the cross Izvor/Jiana showed a variation of the membrane stability from 39.3% to 90.2%.

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 (figure 1a, b and c).

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

ANOVA for membrane stability shows a significant effect of the grouping according to alleles at Xwmc9, Xwmc596, Xwmc603 or Xbarc108 loci, but not of the grouping according to Xbarc121 or Xgwm260 (Table 1).

There was overlapping between distributions of DH lines carrying Xwmc9, Xwmc596, Xwmc603 or Xbarc108 marker alleles of Izvor or Jiana, but most lines carrying the Izvor allele had less membrane injury and better membrane stability after water stress than lines with Jiana allele (Figure 2). On average, lines carrying the allele of Izvor had membrane stability of 73.7% as compared with 65.6% in

lines carrying the Jiana allele. The existent overlapping and the multimodal distributions, both suggest a possible implication of more than one QTL in the control of membrane stability in this cross.

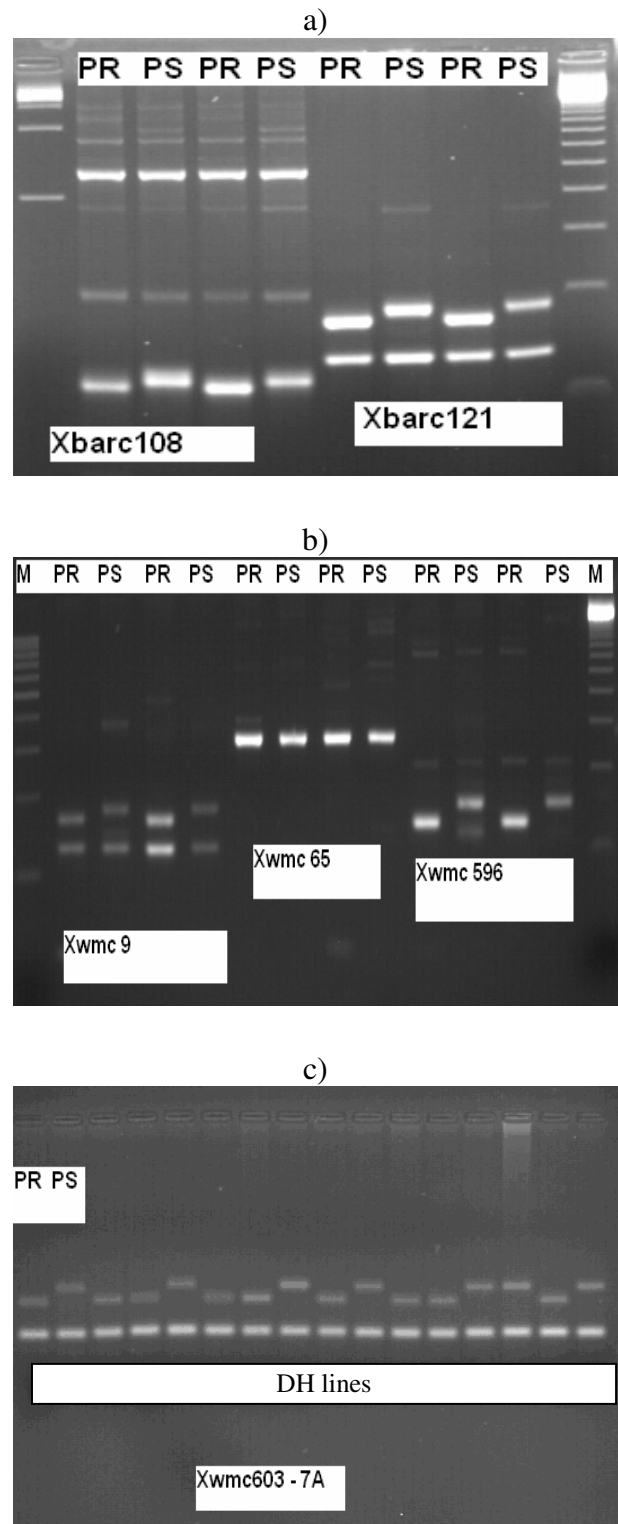


Figure 1. Polymorphism between parental forms Izvor (PR) and Jiana (PS), revealed by SSR markers Xbarc108, Xbarc121 (a); Xwmc9, Xwmc65, Xwmc596 (b) and Xwmc603 (c), as detected by electrophoresis in agarose gel

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Table 1. ANOVA for membrane stability after water stress

Sources of variation	DF	MS when grouped according to alleles at:		
		Xwmc9, Xwmc596, Xwmc603 or Xbarc108 loci	Xbar121 locus	Xgwm260 locus
Between groups	1	786.4*	876.2n.s.	981.7n.s.
Within groups	60	131.4	230.7	269.0

* significant at P<5% level



Figure 2. Distribution of DH lines carrying the Izvor (A) or Jiana (B) allele at loci Xwmc9, Xwmc596, Xwmc603 or Xbarc108, according to membrane stability after water stress

Analysis of the results using the Qgene program suggests the existence of a QTL for membrane stability and injury index, located close to Xwmc9, Xwmc596, Xwmc603 and Xbarc108 (figure 3). Although the LOD score (logarithm of the likelihood ratio for linkage) is rather low, these markers can still prove useful in increasing the frequency of progenies with better performance under drought in a wheat breeding program.

The location of this QTL on chromosome 7A, close to the location of the „or” gene controlling osmotic adjustment (Morgan and Tan, 1996) and the association found by Bănică et al. (2008) between membrane stability and genetic differences in the capacity of osmotic adjustment expressed in pollen grains, suggest that markers Xwmc9, Xwmc596, Xwmc603 and Xbarc108 might be associated with the „or” gene.

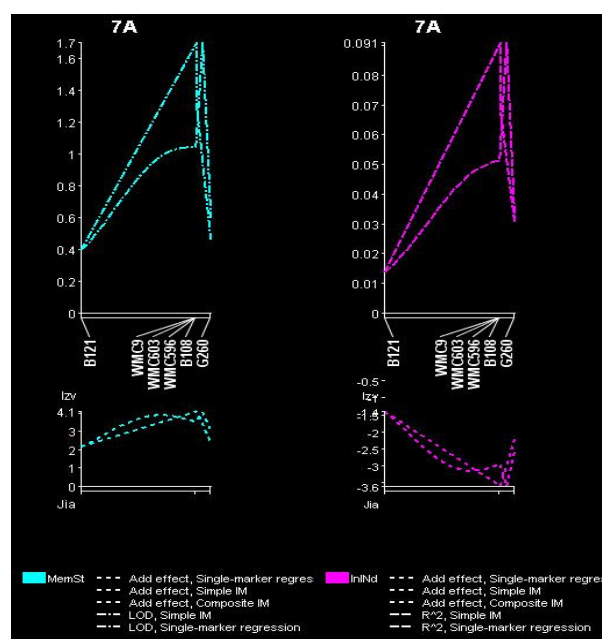


Figure 3. Detection of a QTL for membrane stability (MemSt) and injury index (InINd) using the Qgene program

CONCLUSIONS

SSR markers wmc9, wmc596, wmc603 and barc108 are weakly but significantly associated with cell membrane stability after water stress and can be used for increasing the frequency of progenies with better performance under drought in a wheat breeding program.

Acknowledgements

This study was financed by the CNCSIS grant 787 (2006-2008). The authors are grateful to Prof. Hermann Buerstmayr for his support during the main author working stage at IFA Tulln, Austria.

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