DOI: 10.1007/s10535-015-0499-9 BIOLOGIA PLANTARUM 59 (2): 291-297, 2015

Mapping of QTLs associated with abscisic acid and water stress in wheat

M.N. BARAKAT[1,2]*, M.S. SALEH[3], A.A. AL-DOSS[1], K.A. MOUSTAFA[1], A.A. ELSHAFEI[1,4], A.M. ZAKRI[1], and F.H. AL-QURAINY[3]

Plant Production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh, Saudi Arabia[1]
Crop Science Department, Faculty of Agriculture, University of Alexandria, P.O. Box 21545, Alexandria, Egypt[2]
College of Pure Science, King Saud University, P.O. Box 2455, Riyadh, Saudi Arabia[3]
Genetic Engineering and Biotechnology Division, National Research Centre, P.O. Box 12622, El-Dokki, Cairo, Egypt[4]

Abstract

A segregating F_4 population from the cross between drought sensitive (Yecora Rojo) and drought tolerant (Pavon 76) genotypes was made to identify molecular markers linked to a wheat (*Triticum aestivum* L.) abscisic acid (ABA) content at two water regimes. The parents and 150 F_4 lines were evaluated phenotypically for drought tolerance using two irrigation treatments [0.25 and 0.75 $m^3(H_2O)$ m^{-2}(soil)]. Forty different target region amplification polymorphism (TRAP) primer combinations, 98 different sequence-related amplified polymorphism (SRAP) primer combinations, and 400 simple sequence repeat (SSR) primers were tested for polymorphism among the parental genotypes and the F_4 lines. Seven loci in the F_4 lines treated with the drought stress were identified. Single quantitative trait loci (QTLs) were located on chromosomes 1B, 2A, 3A, 5D, and 7B and each of them explained from 15 to 31 % of phenotypic variance with a LOD value of 7.2 to 15.7. Five QTLs were located on chromosome 4A and six QTLs on chromosome 5A. In control (well-watered) F_4 lines, two QTLs were mapped on chromosome 3B and one QTL on each chromosome 5B and 5D. Statistically the most significant groups of QTLs for the ABA content were identified in the regions of chromosomes 3B, 4A, and 5A mostly near to Barc164, Wmc96, and Trap9 markers. Therefore, these markers linked to QTLs for the drought-induced ABA content can be further used in breeding for drought tolerance in wheat.

Additional key words: SRAP, SSR, TRAP, *Triticum aestivum*.

Introduction

Drought tolerance is a quantitative trait with a complex phenotype and a genetic control (McWilliam 1989). Therefore, understanding the genetic and physiological bases of drought tolerance in crop plants including wheat (*Triticum aestivum* L.) are a prerequisite for developing superior genotypes through conventional breeding. Recently, the identification of new simple sequence repeat (SSR), target region amplification polymorphism (TRAP), and sequence-related amplified polymorphism (SRAP) markers linked to a chlorophyll content, leaf senescence, and cell membrane stability in water-stressed wheat have been reported (Barakat *et al.* 2013, Elshafei *et al.* 2013, Saleh *et al.* 2014). Therefore, the application of a quantitative trait loci (QTLs) analysis to study physiological and agronomical traits will improve our understanding genetic factors that influence these complex traits.

A plant hormone abscisic acid (ABA) regulates responses to various environmental stresses and diverse physiological and developmental processes. It is a principal regulator of plant response to drought by inducing stomatal closure, therefore reducing water loss *via* transpiration (Davies and Zhang 1991, Zhu 2002). Further, ABA induces gene expression in response to drought, salinity, and chilling (Chandler and Robertson 1994). Thus, ABA content has been recognized as a useful characteristic of drought tolerance in cereal crops (Quarrie *et al*. 1994).

Submitted 25 November 2013, last revision 23 November 2014, accepted 24 November 2014.
Abbreviations: BSA - bulk segregant analysis; CIM - composite interval mapping; LOD - likelihood ratio; QTL - quantitative trait locus; RIL - recombinant inbred line; SSR - simple sequence repeat; SRAP - sequence-related amplified polymorphism; TRAP - target region amplification polymorphism.
Acknowledgements: The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project no. RGPVPP- 161.
* Corresponding author; fax: (+96) 6114678467, e-mail: mnrbarakat@yahoo.com

It has been confirmed that an ABA accumulation is under the control of several genes and is inherited in a quantitative manner. Several highly significant QTLs for ABA accumulation were identified (Quarrie *et al*. 1997) and the results of QTL mapping have been reported (Iuchi *et al.* 2002, You *et al*. 2006). Identification of QTL for ABA responsiveness at a seedling stage associated with an ABA-regulated gene expression in common wheat has been reported by Kobayashi *et al.* (2010). The QTL analysis of drought-related traits and of grain yield in relation to variation in leaf ABA content in field-grown maize has been reported by Sanguineti *et al*. (1999). A molecular mapping of loci associated with ABA accumulation in triticale anthers in response to a low temperature stress has been reported by Żur *et al.* (2012). The objectives of this investigation were to identify TRAP, SRAP, and SSR markers linked to an ABA content in control and water-stressed wheat and to map QTLs for ABA content in a F_4 RIL population.

Materials and methods

Plants and experimental design: A set of 150 recombinant wheat (*Triticum aestivum* L.) inbred lines (RILs, at F_4) was developed from the cross between Pavon76 (a drought tolerant cultivar introduced from *CIMMYT* with a high ABA accumulation) and Yecora Rojo (a drought sensitive dwarf cultivar with a low ABA accumulation developed in USA and recommended for the environment of Saudi Arabia since 1981; Barakat *et al.* 2010).

The 150 RILs and the two parents (Pavon76 and Yecora Rojo) were tested for tolerance to drought under field conditions. Seeds were sown in plots consisting of three rows 3 m long and 0.2 m apart with a seeding rate of 14 g m^{-2} for each wheat genotype. Fertilizers were applied at the rate of 1.2 kg(N) and 0.8 kg(P_2O_5) m^{-2}. Two watering regimes were established after germination on the basis of free-surface water evaporation monitored at the weather station next to the field at the Agricultural Research Station of King Saud University (Dierab, near Riyadh, 24° 42' N, 44° 46' E, 400 m a.s.l.). A control treatment was irrigated frequently when a cumulative evaporation reached 50 mm to a 100 % field capacity, whereas a drought treatment was irrigated to 33 % field capacity when a cumulative evaporation reached 150 mm. The irrigation regimes were applied two weeks after sowing. Six agronomic traits (time of heading, plant height, spike number, kernel number, kernel mass, and grain yield) were determined.

The experiment was laid out in a split-plot design with three replications. The water treatments were assigned to the main plots, whereas the wheat genotypes were distributed randomly over the sub-plots. The analysis of variance (*ANOVA*) was performed using the *SAS 9.1* program. *ANOVA* was estimated according to Steel and Torrie (1980). Pearson's correlation coefficients between the ABA content and the six agronomic traits were obtained with the *CORR* procedure of *SAS*. A phenotypic frequency distribution was performed using the *Excel* program.

Quantification of ABA by HPLC: The accumulation of ABA was measured according to Krochko *et al*. (1998), and Muthurajan *et al*. (2011). Ten leaves (the first leaf below the flag leaf) from 10 different plants for each genotype were collected from well-watered and drought-stressed plants in the milk stage. Tissues were freeze-dried for 24 h and stored at -80 °C. The tissues were then ground using a liquid N_2, dissolved in 80 % (v/v) methanol, and incubated at 4 °C for 12 h. The debris were pelleted by centrifugation at 1 006 *g* for 5 min and methanol in the supernatant was evaporated using a rotary flash evaporator. To this extract, an equal volume of a phosphate buffer (pH 8.0) was added and pH was adjusted between 8 and 9 using 0.1 M KOH. The mixture was then extracted twice with ethyl acetate by adding an equal volume of ethyl acetate and centrifuged at 1 006 *g* for 5 min. The ethyl acetate fraction containing chlorophyll was discarded and the pH of the pooled extract was adjusted between 2 and 3 using 0.2 M HCl and evaporated in a rotary evaporator. Then, the residue was dissolved in 4 cm^3 of methanol and used for the HPLC [*Agilent Technologies*, Lexington, MA, USA; column *SB-C1B* (1.8 μm, 4.8 × 150 mm)] analysis of ABA. Peak areas were measured, and the ABA content was quantified using a standard curve obtained using a chemical-grade ABA (*Sigma*, London, UK).

DNA extraction and PCR amplification: Frozen young leaves (500 mg) were ground to powder in a mortar and a pestle with a liquid nitrogen. DNA extraction was done using the cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof *et al.* 1984). Ten leaves from five different plants were sampled for DNA extraction for each line at F_4 generation.

Forty different TRAP primer combinations (Hu and Vick 2003), 98 different SRAP primer combinations (Li and Quiros 2001), and 400 SSR loci (Röder *et al.* 1998, Gupta *et al*. 2002) were used in this study. Two hundred primer pairs were developed by P. Cregan and Q. Song in the USDA-ARS, Beltsville Agricultural Research Center, USA (BARC, www. scabusa.org). A PCR reaction mixture consisted of 20 to 50 ng of genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM each dNTP, 0.5 μM primer, and 1 U *Taq* polymerase in a volume of 0.025 cm^3. After incubation at 94 °C for 5 min, 5 cycles were performed at 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 1 min 40 s. Further, similar 35 cycles were performed, with the exception of the annealing temperature at 50 °C and a final extension at 72 °C for 7 min, for SRAP and TRAP programs of PCR. The

program of PCR for the SSR analysis included an initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50, 55 or 60 °C (depending on the individual microsatellite primer) for 1 min, and extension at 72 °C for 2 min followed by a final extension at 72 °C for 17 min. Amplification products were electrophore-tically resolved on 2 - 3 % (m/v) agarose gels containing 0.1 μg cm^{-3} ethidium bromide and photographed on a UV trans-illuminator.

Data and linkage analysis: *Map Manager QTX v. 0.22* (http://manager.roswellpark.org/mm QTX.html) was used to analyze the linkage relationship of the detected TRAP, SRAP, and SSR markers. A linkage was detected when the log of the likelihood ratio (LOD) threshold was 3.0 and a maximum distance was 50 cM. The Kosambi's mapping function was used. QTLs were identified using a composite interval mapping provided by *Map Manager QTX*. Genetic loci with the most significant effect for each QTL were assembled into multiple regression models using *PROC REG* of *SAS v. 9.1* software packages (*SAS Institute*, Cary, NC, USA) to determine the total amount of the phenotypic variation explained (Nelson 1997).

Results

ANOVA indicates that there were statistically highly significant differences ($P < 0.01$) for the ABA content and all the agronomic traits among the water treatments and the wheat genotypes. The interaction between the water treatments and the wheat genotypes was also highly significant. The parent Pavon76 had a higher ABA content [0.22 ± 0.02 and 0.745 ± 0.003 μg g^{-1}(f.m.) under the well-watered and drought-stressed conditions, respectively] than the parent Yecora Rojo [0.139 ± 0.010 and 0.491 ± 0.020 μg g^{-1}(f.m.)]. The mean ABA content of the F_4 lines was midway between Pavon76 and Yecora Rojo [0.333 ± 0.089 and 0.577 ± 0.12 μg g^{-1}(f.m.) in the well-watered and water-stressed plants, respectively] and these values were significantly different from those in both the parents. The F_4 lines showed a continuous distribution of ABA from 0.210 to 0.894 μg g^{-1}(f.m.) under the drought stress, which is in agreement with distribution expected for a polygenic and quantitatively inherited trait. Among the F_4 lines, 22 F_4 lines had a lower ABA content than the parent Yecora Rojo, and 9 F_4 lines had a higher ABA content than the parent Pavon 76 under the drought stress (Fig. 1). A transgressive segregation was found between the F_4 lines indicating that favorable alleles governing target traits had been widely separated in the F_4 lines. Therefore, the distribution character of phenotypic data was suitable for a QTL analysis.

Significant and positive correlations were observed between the ABA content and the plant height ($r = 0.28$, $P < 0.01$), the ABA content and the kernel mass ($r = 0.18$, $P < 0.01$), and the ABA content and the grain yield ($r = 0.32$, $P < 0.01$) under the drought stress indicating that an increased ABA content also increased tolerance to drought. The mean grain yields of Pavon76, Yecora Rojo,

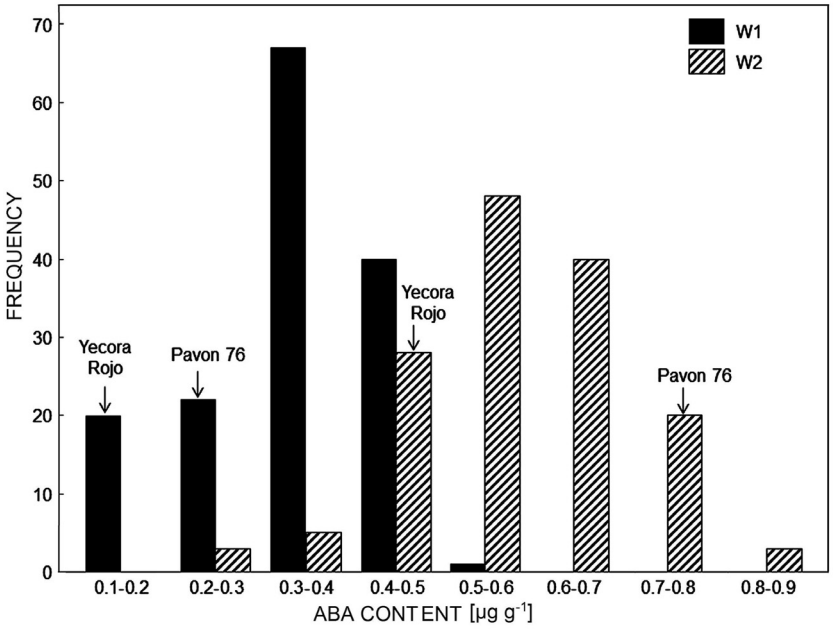

Fig. 1. Frequency distribution of ABA content [μg g^{-1}(f.m.)] in two parents and 150 F_4 lines derived from the cross between Pavon76 and Yecora Rojo under well-watered (W1) and drought-stress (W2) conditions.

and the F_4 lines grown in the well-watered conditions were 0.797 ± 0.096, 0.492 ± 0.022, and 0.55 ± 0.165 kg m^{-2}, respectively. Under the drought, the mean grain yield of the F4 lines (0.44 ± 0.107 kg m^{-2}) was significantly higher than that of Yecora Rojo (0.31 ± 0.037 kg m^{-2}), but significantly lower than that of Pavon76 (0.57 ± 0.076 kg m^{-2}). However, a significant and negative correlation was observed between the ABA content and the spike number per m^2 (r = -0.40, $P < 0.01$) under the drought stress. On the other hand, under the well-watered treatment, no significant correlation was observed between the ABA content and the six agronomic traits.

A total of 20 associated markers were obtained, comprising 3 TRAP, 5 SRAP, and 12 SSR that were used to assemble genetic linkage maps. The TRAP markers [Trap1 (F: TGAGTCCAAACCGGAAT and R: TCA CCCGCACCTTCTTCC), Trap9 (F: TGAGTCCAA ACCGGAGC and R: TCACCCGCACCTTCTTCC), and Trap14 (F: GAGTCCAAACCGGAGC and R: CCC TCCACCAATCACAAT)] were dominant. The SRAP markers [Srap19 (F: TGAGTCCAAACCGGTCC and R: GACTGCGTACGAATTCAG), Srap5 (F: TGAGTC CAAACCGGTAG and R: GACTGCGTACGAATT CAG), Srap89 (F: GAGTCCAAACCGGAAG and R: GACTGCGTACGAATTCAG), Srap93 (F: GAGTCC AAACCGGAAG and R: GACTGCGTACGAATTCAA), and Srap95 (F: GAGTCCAAACCGGAAG and R: GAC

Table 1. The characteristic of basic parameters of QTLs related to ABA content as indicator of drought tolerance in 150 F_4 lines derived from the Pavon76×Yecora Rojo cross under drought-stressed and well-watered conditions (H - homologous chromosome number, LG - linkage group. Positive additive effects indicate that an allele from the tolerant parent Pavon76 contributed to a higher ABA content.

Treatment	Marker	H/LG	QTL [cM]	LOD	R^2 [%]	P value	Additive effect
Drought-stressed	Barc81	1B/LG1	16.2	15.3	15	0.00001	0.07
	Wmc177	2A/LG2	16.2	14.9	16	0.00001	0.08
	Barc45	3A/LG3a	22.1	15.7	31	0.00001	0.08
	Srap5	4A/LG4	23.7	8.2	18	0.00001	0.08
	Trap1	4A/LG4	21.7	8.9	22	0.00001	0.09
	Barc52	4A/LG4	17.5	13.4	21	0.00001	0.08
	Wmc161	4A/LG4	19.2	11.1	17	0.00001	0.08
	Wmc96	4A/LG4	16.1	13.7	26	0.00001	0.10
	Srap93	5A/LG5a	20.2	10.3	22	0.00001	0.09
	Trap14	5A/LG5a	17.2	10.8	25	0.00001	0.11
	Srap89	5A/LG5a	15.6	13.8	30	0.00001	0.11
	Trap9	5A/LG5a	13.5	14.4	26	0.00001	0.11
	Barc10	5A/LG5a	19.8	9.9	27	0.00001	0.10
	Srap95	5A/LG5a	19.0	10.2	24	0.00001	0.10
	Barc110	5D/LG5d	24.3	7.2	18	0.0001	0.09
	Barc72	7B/LG6	17.3	13.7	22	0.0001	0.09
Well-watered	Barc164	3B/LG3b	14.5	8.5	16	0.00001	0.08
	Srap19	3B/LG3b	15.5	8.3	24	0.00001	0.08
	Barc142	5B/LG5b	17.7	6.3	20	0.00001	0.09
	Barc161	5D/LG5d	25.4	4.1	14	0.00001	0.06

Table 2. The list of SSR markers used in this study. Primer sequences from USDA (www.scabusa.org) or from Gupta *et al*. (2002) marked by *asterisks.*

Primer name	Forward sequence	Reverse sequence
Barc10	GCGTGCCACTGTAACCTTTAGAAGA	GCGAGTTGGAATTATTTGAATTAAACAAG
Barc45	CCCAGATGCAATGAAACCACAAT	GCGTAGAACTGAAGCGTAAAATTA
Barc52	GCGCCATCCATCAACCGTCATCGTCATA	GCGAGGAAGGCGGCCACCAGAATGA
Barc72	CGTCCTCCCCCTCTCAATCTACTCTC	CGTCCCTCCATCGTCTCATCA
Barc81	GCGCTAGTGACCAAGTTGTTATATGA	GCGGTTCGGAAAGTGCTATTCTACAGTAA
Barc110	CCCGAACAATGGCTTTGGTGTCGTAAT	CATGGTGACGGCAAGTGTGAGGT
Barc142	CCGGTGAGAGGACTAAAA	GGCCTGTCAATTATGAGC
Barc161	GCGAAAGGGAAAGCTAAGTAACACTAA	GCGCAGAACACAGGGTATCGTC
Barc164	TGCAAACTAATCACCAGCGTAA	CGCTTTCTAAAACTGTTCGGGATTTCTAA
Wmc96*	TAGCAGCCATGCTTAGCATCAA	GTTTCAGTCTTTCACGAACACG
Wmc161*	ACCTTCTTTGGGATGGAAGTAA	GTACTGAACCACTTGTAACGCA
Wmc177*	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA

Table 3. The presentation of the most significant QTLs for ABA content.

QTLs	Marker interval	Nearest marker	Position [cM]	Confidence interval	LOD score	R^2 [%]	Additive effect
QABA-ww-3B	Barc164-Srap19	Barc164	14.5	8.4 - 22.3	8.5	39.4	0.15
QABA-ds-4A	Wmc161-Wmc96	Wmc161	16.1	9.2 - 23.6	13.7	41.8	0.16
QABA-ds-5A	Srap89-Trap9	Trap9	13.5	8.1 - 21.45	14.4	46.1	0.15

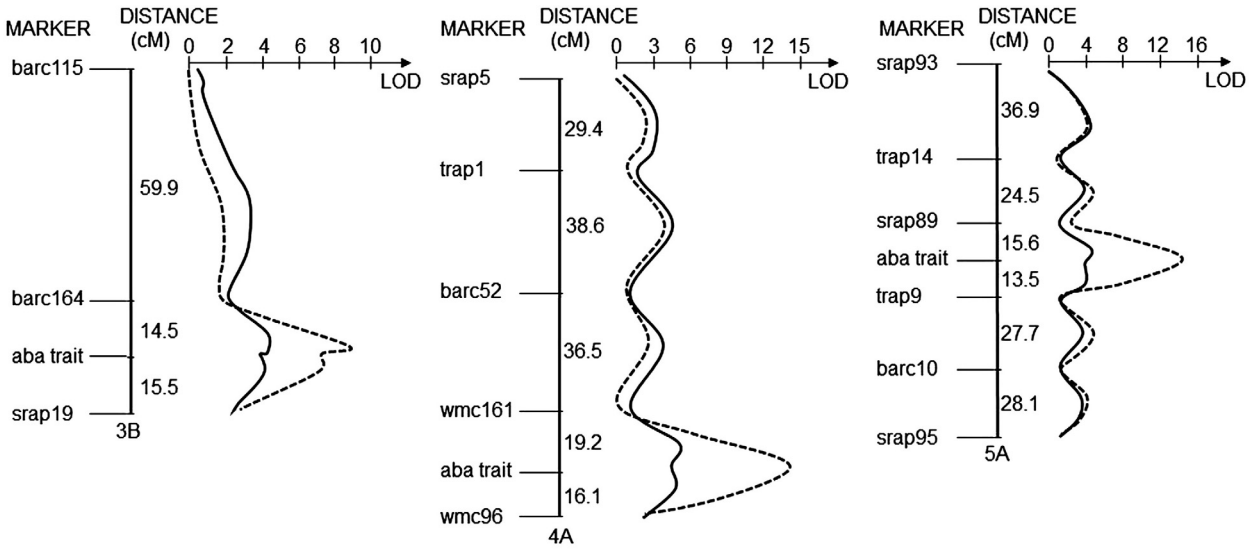

Fig. 2. QTL-likelihood curves of LOD scores showing the locations of QTLs for ABA content [μg g^{-1}(f.m.)] on chromosomes 3B, 4A and 5A.

TGCGTACGAATTGAC)] were also dominant. Twelve SSR markers were identified for ABA content at the two water regimes, and only one (Barc45) co-dominant marker was observed (Table 1). The sequences of the used SSR markers are listed in Table 2.

On the basis of ABA content (Fig. 1), 20 markers with LOD from 4.1 to 15.7 were identified using a composite interval mapping (CIM) (Table 1). Among many loci identified by the CIM method, a total of 16 markers were identified for the F_4 lines grown in the water-stressed conditions, and 4 markers for the F_4 lines grown in the well-watered conditions (Table 1).

The genome scan identified seven loci in the F_4 lines grown under the water stress. Single QTLs were located on chromosomes 1B, 2A, 3A, 5D, and 7B and each of them explained from 15 to 31 % of phenotypic variance with a LOD value of 7.2 to 15.7 (Table 1). Five associated markers were located on chromosome 4A and six associated markers on chromosome 5A. The highest LOD score (14.4) was calculated for Trap9-5A locus, and it represented 26 % of the variability of the trait examined. In the well-watered F_4 lines, two markers were mapped on chromosome 3B. The highest LOD score (8.5) was calculated for Barc164-3B locus, and it represented 16 % of the variability of the ABA content. Two loci were located on chromosomes 5B and 5D with LOD values of 6.3 and 4.1, and with 20 and 14 % contributions to phenotypic variations for 5B and 5D, respectively (Table 1). No common QTL for ABA content under both the watering regimes was found (Table 1).

All of the QTLs for ABA content had a positive additive effect indicating the contribution of alleles increasing the ABA content by the tolerant parent Pavon76 (Table 1). In addition, the positive additive effects indicated the relative importance of genes controlling the abscisic acid content for drought tolerance in the F_4 lines.

In the experiments conducted in the current study, statistically the most significant groups of QTLs for ABA content were identified on three chromosomes (Table 3, Fig. 2). One of the QTLs was located on chromosome 3B and linked closely to Barc164 (Table 3, Fig. 2). This was a major QTL (LOD > 3) that explained 39.4 % of the phenotypic variance. The second major QTL located on chromosome 4A next to Wmc96 explained 41.8 % of the phenotypic variance. The third major QTL located on chromosome 5A next to Trap9 explained 46.1 % of the phenotypic variance (Table 3, Fig. 2).

Discussion

ABA induces stomatal closure and reduces water loss, which leads to an increased tolerance to water stress (Zeevaart and Creelman 1988, Davies and Zhang 1991). Water stress causes ABA accumulation (Ünyayar *et al.* 2004), and Saeedipour and Moradi (2012) observed a higher ABA content under water deficit in two wheat cultivars differing in drought tolerance. Our results show that the two parents Pavon76 and Yecora Rojo differed considerably in their drought tolerance and also in their ABA content under both the drought stress and well-watered conditions.

In the present study, the TRAP markers were assigned to chromosomes 4A and 5A in agreement with a previous report (Li *et al.* 2007). Also, the SRAP markers were assigned to chromosomes 3B, 4A, and 5A in agreement with a previous report (Li *et al.* 2007). In addition, the 12 SSR markers were assigned to chromosomes 1B, 2A, 3A, 3B, 4A, 5A, 5B, 5D, and 7B in agreement with previous reports (Gupta *et al.* 2002, Röder *et al.* 1998, Song *et al.* 2005). Homologous groups of chromosomes 2, 3, 5, and 7 of wheat contain a number of genes that are important for tolerance to abiotic stresess (Dubcovsky *et al.* 1995, Golabadi *et al.* 2011). Previously, Sanguineti *et al*. (1999) detected 17 QTLs controlling leaf ABA content in a maize population of 80 F_4 random lines tested for 2 years under drought in field conditions. Several studies have mapped QTLs for leaf ABA content in maize under drought stress (Tuberosa *et al.* 1998, Sanguineti *et al*. 1999). Sixteen QTLs for ABA content corresponded with QTLs for at least one of the following traits: stomata conductance, drought sensitivity index, leaf temperature, leaf relative water content, anthesis-silking interval, and grain yield. An increase in ABA content is generally associated with a decreased stomata conductance and grain yield but an increased leaf temperature. However, an opposite effect was observed for a QTL on chromosome 7 in maize that aligned with a QTL regions influencing root pulling resistance at flowering suggesting that elevated ABA stimulated the development of a more extensive root system (Lebreton *et al*. 1995). A QTL for ABA content in leaves on the long arm of chromosome 5A is associated with a better performance under drought stress (Quarrie *et al.* 1994, 1997). Recently, five QTLs located on chromosomes 1B, 2A, 3A, 6D, and 7B were reported (Kobayashi *et al.* 2010). A QTL with the greatest effect is located on chromosome 6D and explains 11.12 % of variance in ABA responsiveness. Żur *et al*. (2012) reported that a CIM analysis indicated one QTL localized on chromosome 5A (LOD = 2.5, R^2 = 9.3 %) which is associated with ABA content in unstressed triticale, and four QTLs on chromosomes 2A, 1B, and 5R (LOD 2.6 - 5.7, R^2 = 9.5 - 24 %) regulating ABA accumulation in response to a low temperature.

In the present investigation, we were able to identify several types of molecular markers associated with ABA content in wheat at both the water regimes. We identified 3 TRAP (Trap1, Trap9, and Trap14), 5 SRAP (Srap5, Srap89, Srap93, Srap95, and Srap19) and 12 SSR markers. In previous studies, Elshafei *et al*. (2013) identified Srap95-5A marker linked to flag leaf senescence in wheat under water stress conditions. Saleh *et al.* (2014) identified Trap9-5A and Trap14-5A markers linked to cell membrane stability and chlorophyll content, respectively, in water-stressed wheat. QTLs for ABA content were associated with above mentioned markers and explained from 14 to 31 % of phenotypic variation for ABA content. These markers should be useful for a marker-assisted selection. Two QTLs on chromosomes 3B and 4A closely flanked SSR markers Barc164-Srap19 and Wmc161-Wmc96 by intervals 14.5 and 16.1 cM, respectively. The QTL on chromosome 5A was flanked by TRAP markers Srap89 and Trap9 that are 30 cM apart. Molecular markers that are closely linked with target alleles present a useful tool in plant breeding since they can help to detect the tolerant genes of interest without the need of carrying out field evaluation. Also, they allow screening large breeding materials at early growth stages and in a short time.

The present study indicates that the TRAP, SRAP, and SSR markers were linked to the QTLs for ABA content as indicator of drought tolerance in wheat. The markers presented in this study might be further considered in wheat breeding programs.

References

Barakat, M.N., Al-Doss, A.A., Moustafa, K.A., Ahmed, E.I., Elshafei, A.A.: Morphological and molecular characterization of Saudi wheat genotypes under drought stress. - J. Food Agr. Environ. **8**: 220-228, 2010.

Barakat, M.N., Wahba, L.E., Milad, S.I.: Molecular mapping of QTLs for flag leaf senescence under water stressed conditions in wheat (*Triticum aestivum* L). - Biol. Plant. **57**: 79-84, 2013.

Chandler, P.M., Robertson, M.: Gene expression regulated by abscisic acid and its relation to stress tolerance. - Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 113-141, 1994.

Davies, W.J., Zhang, J.: Root signals and the regulation of growth and development of plants in drying soil. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 55-76, 1991.

Dubcovsky, J., Luo, M.C., Dvorák, J.: Linkage relationships among stress-induced genes in wheat. - Theor. appl. Genet. **91**: 795-801, 1995.

Elshafei, A.A., Saleh, M.S., Al-Doss, A.A., Moustafa, K.A., Al-Qurainy, F.H., Barakat, M.N.: Identification of new SRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat under water-stress condition. - Aust. J. Crop Sci. **7**: 887-893, 2013.

Golabadi, M., Arzani, A., Maibody, S.M., Tabatabaei, B.S.,

Mohammadi, S.A.: Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. - Euphytica **177**: 207-221, 2011.

Gupta, P., Balyan, H., Edwards, K., Isaac, P., Korzun, V., Röder, M. Leroy, P.: Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. - Theor. appl. Genet. **105**: 413-422, 2002.

Hu, J., Vick, B.A.: Target region amplification polymorphism: a novel marker technique for plant genotyping. - Plant mol. Biol. Rep. **21**: 289-294, 2003.

Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., Tabata, S., Kakubari, Y., Qin, X.Q., Zeevaart, J.A.D.: Overexpression of a 9-*cis*-epoxycarotenoid-dioxygenase gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. - Plant Physiol. **128**: 544-551, 2002.

Kobayashi, F., Takumi, S., Handa, H.: Identification of quantitative trait loci for ABA responsiveness at seedling stage associated with ABA-regulated gene expression in common wheat. - Theor. appl. Genet. **121**: 629-641, 2010.

Krochko, J.E., Abrams, G.D., Loewen, M.K., Abrams, S.R., Cutler, A.: (+)-abscisic acid 80-hydroxylase is a cytochrome P450 monooxygenase. - Plant Physiol. **118**: 849-860, 1998.

Lebreton, C., Lazic-Jancic, V., Steed, A., Pekic, S., Quarrie, S.A.: Identification of QTLs for drought responses in maize and their use in testing causal relationships between traits. - J. exp. Bot. **46**: 853-865, 1995.

Li, G., Quiros, C.F.: Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. - Theor. appl. Genet. **103**: 455-461, 2001.

Li, S.S., Jia, J.Z., Wei, X.Y., Zhang, X.C., Li, L.Z., Chen, H.M., Fan, Y.D., Sun, H.Y., Zhao, X.H., Lei, T.D., Xu, Y.F., Jiang, F.S., Wang, H.G., Li, L.H.: A intervarietal genetic map and QTL analysis for yield traits in wheat. - Mol. Breed. **20**: 167-178, 2007.

McWilliam, J.: The dimensions of drought. - In: Baker, F.W.G. (ed.): Drought Resistance in Cereals. Pp. 1-11. CAB International, Wallingford 1989.

Muthurajan, R., Shobbar, Z.S., Jagadish, S.V.K., Bruskiewich, R., Ismail, A., Leung, H., Bennett, J.: Physiological and proteomic responses of rice peduncles to drought stress. - Mol. Biotechnol. **48**: 173-182, 2011.

Nelson, J.C.: *QGENE*: software for marker-based genomic analysis and breeding. - Mol. Breed. **3**: 239-245, 1997.

Quarrie, S.A., Gulli, M., Calestani, C., Steed, A., Marmiroli, N.: Localization of drought-induced abscisic acid production on the long arm of chromosome 5 A of wheat. - Theor. appl. Genet. **89**: 794-800, 1994.

Quarrie, S.A., Laurie, D.A., Zhu, J., Lebreton, C., Semikhodskii, A., Steed, A., Witsenboer, H., Calestani, C.: QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. - Plant mol. Biol. **35**: 155-165, 1997.

Röder, M.S., Korzun, V., Gill, B.S., Ganal, M.W.: The physical mapping of microsatellite markers in wheat. - Genome **41**: 278-283, 1998.

Saeedipour, S., Moradi, F.: Relationship of endogenous ABA and IAA to accumulation of grain protein and starch in two winter wheat cultivars under post-anthesis water deficit. - J. agr. Sci. **4**: 147-156, 2012.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W.: Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. - Proc. nat. Acad. Sci. USA **81**: 8014-8018, 1984.

Saleh, M.S., Al-Doss, A.A., Elshafei, A.A., Moustafa, K.A., Al-Qurainy, F.H., Barakat, M.N.: Identification of new TRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat under water-stress conditions. - Biol. Plant. **58**: 64-70, 2014.

Sanguineti, M.C., Tuberosa, R., Landi, P., Salvi, S., Maccaferri, M., Casarini, E., Conti, S.: QTL analysis of drought-related traits and grain yield in relation to genetic variation for leaf abscisic acid concentration in field-grown maize. - J. exp. Bot. **50**: 1289-1297, 1999.

Song, Q., Shi, J., Singh, S., Fickus, E., Costa, J., Lewis, J., Cregan, P.: Development and mapping of microsatellite (SSR) markers in wheat. - Theor. appl. Genet. **110**: 550-560, 2005.

Steel, R.G., Torrie, J.H.: Principles and Procedures of Statistics, a Biometrical Approach. - McGraw-Hill, Kogakusha 1980.

Thompson, D.S., Wilkinson, S., Bacon, M.A., Davies, W.J.: Multiple signals and mechanisms that regulate leaf growth and stomatal behavior during water deficit. - Physiol. Plant. **100**: 303-313, 1997.

Tuberosa, R., Sanguineti, M.C., Landi, P., Salvi, S., Casarini, E., Conti, S.: RFLP mapping of quantitative trait loci controlling abscisic acid concentration in leaves of drought-stressed maize (Zea mays L.). - Theor. appl. Genet. **97**: 744-755, 1998.

You, J., Li, Q., Yue, B., Xue, W.-Y., Luo, L.-J., Xiong, L.-Z.: Identification of quantitative trait loci for ABA sensitivity at seed germination and seedling stages in rice. - Acta genet. sin. **33**: 532-541, 2006.

Ünyayar, S., Keles, Y., Ünal, E.: Proline and ABA levels in two sunflower genotypes subjected to water stress. - Bulg. J. Plant Physiol. **30**: 34-47, 2004.

Zeevaart, J.A.D., Creelman, R.A.: Metabolism and physiology of abscisic acid. - Annu. Rev. Plant Physiol. Plant mol. Biol. **39**: 439-473, 1988.

Zhu, J.K.: Salt and drougtht stress signal transduction in plants. - Annu. Rev. Plant Biol. **53**: 247-273, 2002.

Żur, I., Krzewska, M., Dubas, E., Gołębiowska-Pikania, G., Janowiak, F., Stojałowski, S.: Molecular mapping of loci associated with abscisic acid accumulation in triticale (×*Triticosecale* Wittm.) anthers in response to low temperature stress inducing androgenic development. - Plant Growth Regul. **68**: 483-492, 2012.