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Identification of new SRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat under water-stressed condition

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Abstract

A segregating F_4 population from the cross between drought sensitive (Yecora Rojo) and drought tolerant (Pavon 76) genotypes was made to identify SRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat (*Triticum aestivum* L.) under water-stressed condition. The parents and 150 F_4 families were evaluated phenotypically for drought tolerance using two irrigation treatments (2500 and 7500 m^3/ha). Using 98 different SRAP primer combinations tested for polymorphism in testing parental and F_4 families genotypes, the results revealed that quantitative trait locus (QTL) for chlorophyll content was associated with five SRAP markers and explained phenotypic variation ranged from 38 to 53 %. The genetic distance between chlorophyll content QTL and SRAP markers ranged from 10.4 to 22.7 cM. QTL for flag leaf senescence was associated with five SRAP markers and explained phenotypic variations ranged from 35 to 45 %. The genetic distance between flag leaf senescence QTL and SRAP markers ranged from 10.7 to 23.5 cM. QTL for cell membrane stability was associated with five SRAP markers and explained phenotypic variations from 25 to 44 %. The SRAP markers for cell membrane stability had genetic distances ranged from 7.3 to 17.1 cM. We suggest that these SRAP markers linked to the QTLs of drought-induced physiological traits, leaf chlorophyll content, flag leaf senescence and cell membrane stability can be further used in breeding for drought tolerance in wheat.

Keywords: Cell membrane stability, Chlorophyll content, Flag leaf senescence, QTL, SRAP markers, Water-stress.
Abbreviations: BSA- bulked segregant analysis; QTL- quantitative trait loci; SRAP – sequence-related amplified polymorphism.

Introduction

Wheat (*Triticum aestivum* L.), the most widely adapted crop mainly grown on rainfed land, is feeding one-third of the world's population. In developing countries, almost 37% of the areas are semi-arid and little available water greatly restricts wheat production. The world demand for wheat is predicted to increase up to 40% by 2020 (Rosegrant, 1997). Wheat genotypes/cultivars tolerant to water stress have higher yield in rainfed areas (Rajaram, 2001). Drought is a major abiotic stress which limits crop production in arid and semi-arid areas. Drought tolerance is a quantitative trait with complex phenotype and genetic control (McWilliam, 1989). Therefore, understanding the genetic and physiological bases of drought tolerance in crop plants are a prerequisite for developing superior genotypes through plant breeding programs. In addition, selection for field performance is based on the selection for physiological traits related to drought tolerance. Among the physiological traits that are associated with wheat performance under drought stresses; chlorophyll content (Shen et al., 2001; Guo et al., 2008), flag leaf senescence (Verma et al., 2004; Barakat et al., 2013) and cell membrane stability (Blum and Ebercon, 1981) have been recognized as considerable indicators for drought tolerance in cereals crops. Molecular markers have several advantages over the traditional phenotypic markers that were previously available to plant breeders for improving the efficiency of conventional plant breeding by carrying out selection, not necessarily directly on the trait of interest, but on molecular markers linked to that trait. Molecular markers are especially advantageous for agronomic traits that are otherwise difficult to tag, such as tolerance to abiotic stresses, quality parameters and quantitative traits (Aneja et al., 2012). Therefore, application of quantitative trait loci (QTLs) analysis to study the physiological traits will improve our understanding of genetic factors that influence these complex traits. Marker assisted selection may reduce problems associated with genotype × environment interactions, improve the selection efficiency and facilitate combining different tolerance traits into a single efficient genotype.

Sequence-related amplified polymorphism (SRAP) is a novel, PCR-based molecular marker technique developed by Li and Quiros (2001). SRAP markers are useful for germplasm characterization, cultivar identification, molecular mapping and gene cloning in crop plants (Aneja et al., 2012). It combines simplicity, reliability, reasonable throughput rate and most importantly targets the open reading frames in

genome. Numerous co-dominant and clear high-intensity bands with rare overlapping can be generated by these markers. The SRAPs are not crop-specific and instead show easy isolation of bands for sequencing, multi-loci and multi-allelic features, which makes it potentially more efficient for genetic diversity analysis, gene mapping and fingerprinting of genotypes. They are also cost-effective and any of the forward primers can be combined with any of the reverse primers; therefore, so many primer combinations are possible. This reduces the cost of PCR, which is very important in the developing countries (Aneja et al., 2012). SRAP was developed and used to construct a genetic map of *Brassica oleracea* (Li and Quiros, 2001), kenaf (Chen et al., 2011), cucumber (*Cucumis sativus* L.) (Zhang et al., 2010), Dendrobium species (Xue et al., 2010) and in grass (Xie et al., 2011). Our report is the first to identify SRAP markers for the physiological traits under water-stressed condition in wheat. We report here some new SRAP markers linked to genes controlling three physiological traits such as chlorophyll content, flag leaf senescence and cell membrane stability in wheat under water-stress.

Results and Discussion

The development of wheat cultivars with high yield potential under drought stress conditions is a major aim for wheat breeding programs. Breeding for complex traits needs to take into account various factors, such as understanding the genetic, physiological and molecular bases of the traits, including interactions among the component traits and with the environments. Recently, progresses have been made in mapping and tagging many agriculturally important genes with molecular markers, which forms the basis for marker assisted selection (MAS) in crop plants (Aneja et al., 2012). Molecular markers linked to the drought tolerance trait represent a more reliable tool for selecting drought tolerance genotypes at early stages in wheat breeding programs.

Field data analysis

Analysis of variance revealed highly significant differences (P<0.01) in trait means among wheat genotypes and water treatments for grain yield and three measured physiological traits; chlorophyll content, flag leaf senescence and cell membrane stability (Table 1). The differences among the mean of grain yield, chlorophyll content, flag leaf senescence and cell membrane stability of the two parents were significant (P<0.05) under water stress. In general, the parent Pavon76 had higher mean values (P<0.05) for grain yield, chlorophyll content and cell membrane stability than the parent Yecora Rojo under both water treatments. However, the parent Yecora Rojo had higher mean values (P<0.05) for flag leaf senescence than the parent Pavon76 under both water treatments. Overall, grain yield of Pavon 76 under drought was reduced by 15.8%, whereas Yecora Rojo's grain yield was reduced by 22.5%. This confirms our assumption that Pavon 76 is more drought tolerant than Yecora Rojo. Giunta et al. (1993) reported that moisture stress around anthesis had a negative effect on wheat yield. Furthermore, moisture stress from anthesis to maturity reduced the grain yield (Guttieri et al., 2001; Weightman et al., 2008) through reduction in grain filling rate.

The mean of F_4 lines for the grain yield and three physiological traits were intermediate mid-value between Pavon76 and Yecora Rojo, except flag leaf senescence and the cell membrane stability under well water treatment (Table 1). The mean of the F_4 lines were significantly different from the two parents for grain yield under both water treatments. However, the mean of the F_4 lines was significantly different from the parent Yecora Rojo for the three physiological traits under well water treatment and for cell membrane stability only under drought stress condition (Table 1). In addition, the mean of the F_4 lines significantly varied from the parent Pavon 76 only for the chlorophyll content under drought stress condition and, for flag leaf senescence and the cell membrane stability under well water treatment (Table 1). Drought susceptibility index (DSI) was negatively correlated with grain yield under limited irrigation (-0.04, P<0.65), indicating that low yielding lines in dry conditions had lower grain reductions under drought. DSI was positively correlated with grain yield under the full irrigation treatment (r =0.61, P<0.01), showing the reverse relationship between DSI and grain yield under the dry conditions. Furthermore, averaging across the two irrigation treatments, significant difference between parents and a wider range of distribution for all the physiological traits among RILs were also observed (Fig. 1). The continuous distribution of the physiological traits indicated that those traits should be polygenic in nature. Transgressive segregations, also, were observed in the RIL population for all the traits investigated (Fig. 1). The distribution of the all physiological traits in the present investigation was continuous in the RILs lines, showing their quantitative nature (Fig. 1). Meanwhile, a transgressive segregation was found between the RILs lines indicating that favorable alleles governing target traits had been widely separated in the RILs lines. Therefore, the distributive character of phenotypic data was suitable for QTL analysis.

SRAP markers analysis

Out of 98 used SRAP markers, only 25 primer pairs generated polymorphisms between the parents. These polymorphic markers were used to screen drought tolerance and susceptible DNA bulks, and the parents. Five SRAP markers (SRAP11, SRAP14, SRAP15, SRAP54 and SRAP59) were identified for the leaf chlorophyll content. The SRAP markers SRAP11, SRAP14, SRAP15, SRAP54 and SRAP59 generated polymorphic fragments at 290, 305, 180, 490 and 180bp, respectively. These polymorphic patterns were present only in the tolerant bulk and Pavon76 (tolerant parent) and were missing in the sensitive bulk and Yecora Rojo (sensitive parent) (Fig. 2).

Five SRAP primers generated polymorphic patterns between the two constructed DNA bulks regarding to flag leaf senescence. These primers were SRAP20, SRAP24, SRAP29, SRAP80 and SRAP95, and generated polymorphic bands with molecular weight of 380, 150, 280, 320 and 260bp, respectively. The identified markers were present in the tolerant bulk and Pavon76 (tolerant parent) and were missing in the sensitive bulk and Yecora Rojo (sensitive parent) (Fig. 2).

The SRAP primers (SRAP8, SRAP26, SRAP64, SRAP75, SRAP64 and SRAP85) clearly differentiated the tolerant and susceptible DNA bulks with respect to cell membrane stability (Table 2). The molecular weights of identified DNA fragments were 610, 490, 300 and 605_{bp} for SRAP8, SRAP26, SRAP75 and SRAP85, respectively. These polymorphic patterns were detected only in the tolerant bulk and Pavon76 (tolerant parent). The SRAP primer SRAP64 identified only the susceptible DNA bulk and the susceptible parent at 290bp.

Recently, some markers tightly linked to genes were found using bulked segregant analysis (BSA) (Altinkut et al., 2003; Barakat et al., 2011; Milad et al., 2011; Barakat et al., 2013).

Table 1. Means±standard deviation, of the 150 RILs (F_4 families) and parents for grain yield, chlorophyll content, flag leaf senescence and cell membrane stability under well-water and drought stress conditions.

Traits	Genotype	Treatment	
		Well-watered	Drought-stressed
Grain yield	Pavon76	7.47 ± 0.96	6.29 ± 0.76
	Yecora Rojo	3.65 ± 0.08	2.83 ± 0.37
	F_4 lines	5.43 ± 01.87	4.47 ±1.35
Chlorophyll content	Pavon76	52.5 ± 1.96	53.1 ± 0.15
	Yecora Rojo	48.4 ± 0.30	49.7 ± 0.81
	F_4 lines	51.4 ± 2.32	50.7 ± 0.19
Flag leaf senescence	Pavon76	0.12 ± 0.02	0.15 ± 0.07
	Yecora Rojo	0.24 ± 0.03	0.56 ± 0.14
	F_4 lines	0.29 ± 0.16	0.37 ± 0.18
Cell membrane stability	Pavon76	0.75 ± 0.03	0.73 ± 0.02
	Yecora Rojo	0.64 ± 0.03	0.51 ± 0.06
	F_4 lines	0.81 ± 0.11	0.66 ± 0.13

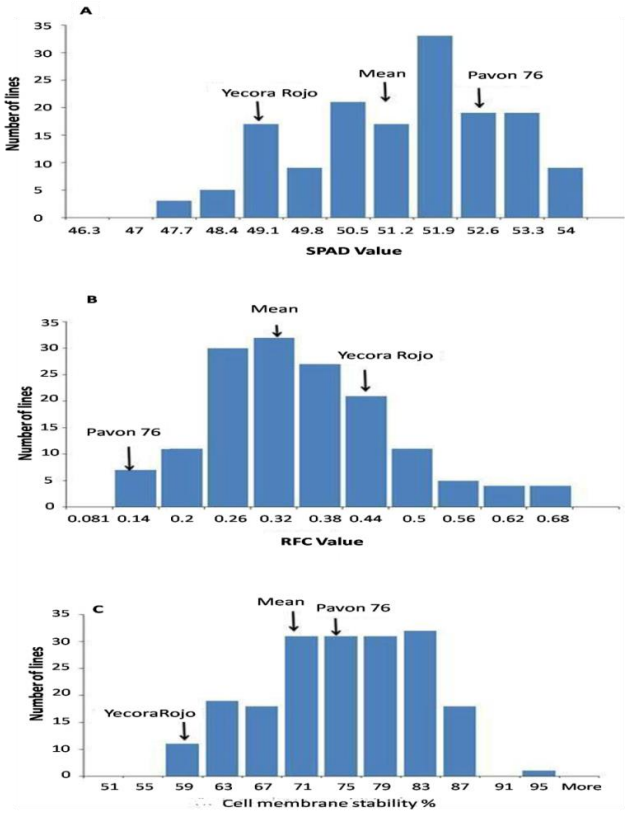

Fig 1. Frequency distribution of chlorophyll content (A), flag leaf senescence (B) and cell membrane stability (C) for 150 F_4 lines derived from a cross between Pavon76 and Yecora Rojo. The physiological traits values for two parents and the 150 F_4 lines are indicated.

The BSA was first reported by Michelmore et al. (1991) to identify RAPD markers tightly linked to genes for resistance to lettuce downy mildew. Identification of new microsatellite marker linked to the grain filling rate as indicator for heat tolerance genes in F_2 wheat population combined with bulked segregant analysis have been reported (Barakat et al., 2011). Several types of molecular markers associated with flag leaf senescence using bulked segregant analysis in wheat were identified under water-stressed conditions (Milad et al., 2011; Barakat et al., 2013). In the present study, we were able to identify several types of molecular markers associated with the three physiological traits in wheat under water-stress. We identified five markers for each physiological trait (SRAP11, SRAP14, SRAP15, SRAP54 and SRAP59), (SRAP20, SRAP24, SRAP29, SRAP80 and SRAP95) and (SRAP8, SRAP26, SRAP64, SRAP75 and SRAP85) linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits, respectively, as indicator for drought tolerance gene in wheat. These markers might be used for marker-assisted selection. The present results support the idea that BSA can provide fast detection of molecular markers linked to genes of interest.

QTL analysis

Multiple regression analysis was carried out to confirm the association between the detected SRAP markers and the physiological traits as an indicator for drought tolerance genes in all 150 F_4 families. The results revealed a highly significant regression between the SRAP markers SRAP11, SRAP14, SRAP15, SRAP54 and SRAP59 and leaf chlorophyll content of the phenotypes of F_4 families. The explained variances were 42%, 53%, 50%, 38% and 46% for SRAP11, SRAP14, SRAP15, SRAP54 and SRAP59, respectively. Also, the SRAP20, SRAP24, SRAP29, SRAP80 and SRAP95 markers were significantly ($P < 0.01$) associated with the flag leaf senescence and explained 45%, 40%, 35%, 40% and 40% of the variation, respectively. In addition, the SRAP8, SRAP26, SRAP64, SRAP75 and SRAP85 markers were significantly ($P < 0.01$) associated with the cell membrane stability and explained 44%, 31%, 38%, 41% and 25% of the variation, respectively (Table 2). This indicates that the SRAP markers were associated with the physiological traits as an indicator for drought tolerance genes. The linkage relationship between the fifteen SRAP markers (SRAP11, SRAP14, SRAP15, SRAP54, SRAP59, SRAP20, SRAP24, SRAP29, SRAP80, SRAP95, SRAP8, SRAP26, SRAP64, SRAP75 and SRAP85) and the physiological traits as an indicator for drought tolerance genes were estimated, using the F_4 families, deriving from the cross, Pavon76 × Yecora Rojo. The genetic distance between the above fifteen SRAP markers and drought tolerance genes were determined as 14.9, 12.6, 10.4, 20.4, 22.7, 22.6, 18.7, 23.5, 23.1, 10.7, 22.5, 22.9, 9.5, 20.7 and 16.5 cM, respectively, with LOD scores of 11.3, 11.9, 15.5, 7.8, 7.0, 5.8, 8.7, 6.4, 6.7, 14.7, 14.0, 7.3, 17.1, 8.1 and 10.3, respectively (Table 2). Therefore, these SRAP markers were

Table 2. Genetic characteristics of QTL related to chlorophyll content (CHC), flag leaf senescence (FLS) and cell membrane stability (CMS) traits as indicator of drought tolerance in the 150 F_4 plants population of Pavon76 × Yecora Rojo.

Trait	Primer name	Chromosome number	Sequence Nucleotide sequence (5'-3')	QTL (CM)	LOD	R^2 (%)	P value	Additive effect
CHC	Srap11	2A, 2B	F: TGAGTCCAAACCGGTAG R: GACTGCGTACGAATTGAC	14.9	11.3	42	0.0001	2.10
	Srap14	2A, 2B	F:TGAGTCCAAACCGGTAG R: GACTGCGTACGAATTTCG	12.6	11.9	53	0.0001	1.88
	Srap15	3B	F: TGAGTCCAAACCGGTCC R: GACTGCGTACGAATTCTG	10.4	15.5	50	0.0001	2.26
	Srap54	7A	F: TGAGTCCAAACCGGTTG R: GACTGCGTACGAATTTAG	20.4	7.8	38	0.0001	1.50
	Srap59	1D	F: TGAGTCCAAACCGGTGC R: GACTGCGTACGAATTAAT	22.7	7.0	46	0.0001	1.22
FLS	Srap20	3B	F: TGAGTCCAAACCGGTCC R: GACTGCGTACGAATTTGA	22.6	5.8	45	0.0001	- 0.13
	Srap24	3B	F: TGAGTCCAAACCGGTCC R: GACTGCGTACGAATTTGC	18.7	8.7	40	0.0001	-0.14
	Srap29	2A, 4A	F: TGAGTCCAAACCGGTCA R: GACTGCGTACGAATTCTG	23.5	6.4	35	0.0001	-0.12
	Srap80	1B	F: TGAGTCCAAACCGGACC R: GACTGCGTACGAATTTGC	23.1	6.7	40	0.0001	-0.12
	Srap95	5A	F: TGAGTCCAAACCGGAAG R: GACTGCGTACGAATTGAC	10.7	14.7	40	0.0001	-0.18
CMS	Srap8	2A, 2B	F: TGAGTCCAAACCGGTAG R: GACTGCGTACGAATTGGT	22.5	14.0	44	0.0001	0.08
	Srap26	3B	F: TGAGTCCAAACCGGTCC R: GACTGCGTACGAATTTAG	22.9	7.3	31	0.0001	0.06
	Srap64	1D	F: TGAGTCCAAACCGGTGC R: GACTGCGTACGAATTGGT	9.5	17.1	38	0.0001	0.12
	Srap75	1B	F: TGAGTCCAAACCGGACC R: GACTGCGTACGAATTCAG	20.7	8.1	41	0.0001	0.08
	Srap85	5A	F: TGAGTCCAAACCGGAAG R: GACTGCGTACGAATTCTG	16.5	10.3	25	0.0001	0.12

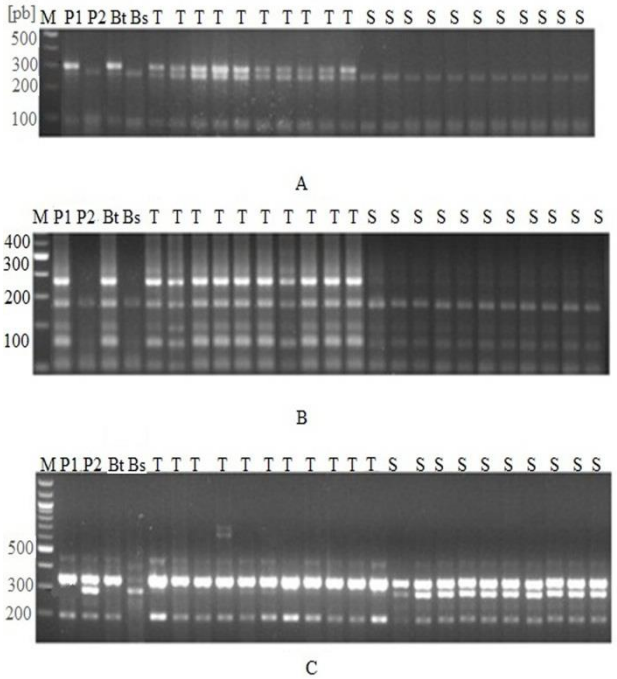

Fig 2. SRAP fragments produced by SRAP14 (A), SRAP20 (B) and SRAP64 (C) markers. M: Molecular weight, P1 and P2– parents Pavon76 and Yecora Rojo, respectively. BT- bulk tolerant, BS- bulk sensitive, T = F_4 tolerant lines, S = F_4 sensitive lines.

linked to the quantitative trait loci (QTL) for the physiological traits as an indicator for drought tolerance genes.

All of the QTLs for leaf chlorophyll content and cell membrane stability had a positive additive effect, indicating contribution of alleles increasing the leaf chlorophyll content and cell membrane stability by the tolerant parent 'Pavon76' (Table 3). Positive additive effect of the QTL on chromosomes 2A, 5A, 7A, 1B, 2B, 3B and 1D indicates contribution of QTL alleles in these loci from the tolerant parent, 'Pavon76'. In addition, the positive additive effects indicates the relative importance of additive gene effects in controlling the leaf chlorophyll content and cell membrane stability as an indicator for drought tolerance in F_4 families. The negative additive effects for flag leaf senescence indicate that the sensitive parent 'Yecora Rojo' alleles are in the direction of increasing the flag leaf senescence trait. In the present study, the fifteen SRAP markers were assigned to chromosomes 2A, 4A, 5A, 7A, 1B, 2B, 3B and 1D in agreement with previous report (Li et al., 2007). Homoeologous groups of chromosomes 2, 3, 5 and 7 of wheat contain a number of genes that are important for tolerance to abiotic stress (Dubcovsky et al., 1995; Golabadi et al., 2011). Previously, Cao et al. (2004) detected seven QTLs for chlorophyll content on chromosomes 2B, 4A, 5B, 6A, 7A, and 7D under nitrogen (N) sufficient environment, while nine QTLs were identified for chlorophyll content on chromosomes 2D, 3A, 4B, 5B, and 6A, when wheat seedlings are grown under N deficient environment. Yang et al. (2007) reported that four additive QTLs, controlling chlorophyll content under conditions of both rainfed and well watered conditions, mapped on chromosomes 1A, 5A, and 7A at grain filling stage. The QTL for flag leaf senescence were discovered on the chromosomes 2B and 2D and the QTLs identified on chromosome 2D associated with better performance under drought stress (Verma et al., 2004). Recently, Barakat et al. (2012) reported that quantitative trait locus for flag leaf senescence was associated with 1 RAPD marker, 4 ISSR markers, and 1 SSR marker and were located on chromosome 2D. Our results show that the allelic contribution to the physiological traits QTLs came from both parents. A negative additive effect indicates that the source of the allele for flag leaf senescence was Yecora Rojo. On the other hand, the positive additive effects indicates the relative importance of additive gene effects in controlling the leaf chlorophyll content and cell membrane stability as an indicator for drought tolerance in F_4 families.

Materials and methods

Plant materials and evaluation of drought stress

A set of 150 recombinant inbred lines (RILs, at F4) developed from the cross between Pavon76 cultivar (drought–tolerant) and Yecora Rojo (drought-sensitive) was used in this study. Pavon76 was introduced and characterized as drought–tolerant cultivar by International Maize and Wheat Improvement Center (CIMMYT). The Yecora Rojo was produced in USA and recommended for environment of Saudi Arabia since 1981. Yecora Rojo is a high yield, 2-gene dwarf cultivar but is very sensitive to drought stress, especially at the grain filling and maturity stages (Barakat et al., 2010).

The 150 recombinant inbred lines and the parents were tested for tolerance to drought under field stress condition. The water regimes were applied after germination based on free-surface evaporation which monitored by Weather Station at the Agricultural Research Station of King Saud University (Dierab, near Riyadh; 24° 42N, 44° 46E, 400Alt.). These regimes were applied as varying timing of flood irrigation with constant amount of water. Two different water regimes were applied, 250 mm (2500 m^3/ha) accumulated evaporation for drought stress and 750 mm (7500 m^3/ha) accumulated evaporation as control. The experiment was laid out in split-plot design with three replications. Water treatments were assigned to the main plots while the wheat genotypes distributed randomly over the sub-plots. Agronomic traits and three physiological traits were determined. The drought susceptibility index (DSI) was calculated from mean grain yield following the method of Fischer and Maurer (1978). They defined DSI as (1 -Yd/Yw) / D; where, Yd = mean yield of an entry under drought, Yw = mean yield of an entry under well-watered conditions, and D = environmental stress intensity = 1- (mean yield of all genotypes under drought/mean yield of all genotypes under well-watered conditions).

Measurement of physiological traits

Leaf chlorophyll content

Leaf chlorophyll was determined at the heading stage using a chlorophyll meter (SPAD-502, Konica sensing, INC., Japan) (Peng et al., 1993), using six flag leaves for each RILs and parents in well-watered and drought-stress conditions.

Flag leaf senescence

Leaf chlorophyll content representing the degree of leaf senescence in wheat was measured using chlorophyll meter (SPAD-502, Konica sensing, INC., Japan). Six flag-leaves for each RILs and parents were selected to evaluate the flag leaf chlorophyll content at heading (FCH). 35 days after heading, the same flag leaves were used to determine the chlorophyll content at maturity (FCM). The reduction speed of flag-leaf chlorophyll content (RFC) as indicator for flag leaf senescence was calculated as described by Dwyer et al. (1991): RFC = (FCH – FCM) / 35.

Cell membrane stability

Medium part of flag leaves (three plants/ replicate) was collected from field plots. Samples collected (2 cm segments) were washed three times in deionized water to remove electrolytes adhered on the surface according to the protocol of Blum and Ebercon (1981). The samples were then kept in a capped vial (20 ml) containing 10 ml of deionized water and incubated in the dark for 24 h at room temperature. The conductance was measured with a conductivity meter (HQ14d, Portable Meter, HACH Company, USA). After the first measurement the vials were autoclaved for 15 min to kill the leaf tissue and release the electrolytes. After cooling, the second conductivity reading was taken. These two measurements were carried out individually for all the samples from both the control and stress treatments. CMS was calculated as the reciprocal of cell-membrane injury following Blum and Ebercon (1981): $CMS\% = [(1-(T_1/T_2))/ (1-(C_1/C_2))]\acute{}100$, where T and C refer to the stress and control samples, respectively; the subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

DNA extraction

Frozen young leaves (500 mg) of 150 recombinant inbred lines (RILs, at F4) and their parents were individually ground to a powder in a mortar with liquid nitrogen. The DNA extraction was done using the CTAB method (Sagahai- Maroof et al., 1984).

PCR amplification

98 different SRAP primer combinations (Li and Quiros, 2001) were used in this study. The PCR reaction mixture consisted of 20 - 50 ng genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.5 µM primer and 1 U *Taq* polymerase in a 0.025 cm^3 volume. After 5 min at 94°C, 5 cycles were performed with 1 min at 94°C, 1 min at 35°C, 1 min 40 s at 72°C, then 35 cycles the same as previous except for the annealing temperature at 50°C and a final 7 min at 72°C. Amplification products were electrophor- etically resolved on 1.5% agarose gels containing 0.1 µg/ml ethidium bromides, and photographed on a UV transilluminator.

Bulked segregant analysis

Bulked – segregant analysis (BSA) was used in conjunction with SRAP analysis (Michelmore et al., 1991) to find markers linked to genes of physiological traits under drought stress. Tolerant and sensitive bulks were prepared from RILs (F_4 generation) individuals by pooling aliquots, containing equivalent amounts of total DNA, approximately, 50 ng/µl from each of ten sensitive and ten tolerant RILs plants selected, based on phenotypic assessments. SRAP primers were tested and screened on parents and two bulk DNA samples, based on polymorphic patterns of primer combinations, not only among parental genotypes, but also between the pair of the bulk DNA. Based on the evaluations of DNA bulks, individual RILs plants were analyzed with co- segregating primers to confirm SRAP markers linkage to the physiological traits as an indicator for drought tolerance genes.

Data and linkage analysis

Map Manager QTX Version 0.22 software (Meer et al., 2002) was used to perform composite interval mapping (CIM) (Zeng, 1994) to evaluate marker intervals putatively associated with trait phenotypes. Linkage was detected when a log of the likelihood ratio (LOD) threshold was 3.0 and maximum distance was 50 cM. The Kosambi's mapping function was used. Genetic loci with the most significant effect for each QTL were assembled into multiple regression models, using PROC REG of SAS version 9.1 software packages (SAS Institute, Cary, NC, 2007), to determine the total amount of the phenotypic variation explained (Nelson, 1997).

Conclusion

Our results indicated that SRAP markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to the chlorophyll content, flag leaf senescence and cell membrane stability traits as indicators for drought tolerance genes in wheat. The marker-assisted selection with SRAP markers might be useful for developing improved cultivars.

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