

Identification of TRAP and SRAP markers linked with yield components under drought stress in wheat (*Triticum aestivum* L.)

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Abstract

In order to identify TRAP and SRAP markers linked to yield components under drought stress in wheat (*Triticum aestivum* L.), a segregating F₄ population from the cross between drought-sensitive (Yecora Rojo) and drought-tolerant (Pavon 76) genotypes was generated. The parents and 150 F₄ families were evaluated phenotypically for drought tolerance using two irrigation treatments [2.5 and 7.5 m³(H₂O) m⁻²(soil)]. The polymorphism among parental genotypes and F₄ families were tested using 40 and 98 different TRAP and SRAP primer combinations, respectively. The results revealed that ten of fourteen TRAP and nine of nineteen SRAP markers are linked to the agronomic traits and can be introduced as new markers. The 14 TRAP markers were assigned to chromosomes 2A, 4A, 5A, 1B, 3B, 6B and 2D. The 19 SRAP markers were assigned to chromosomes 2A, 4A, 5A, 7A, 1B, 2B, 3B, 1D and 3D. Results also showed that all of the QTLs had a positive additive effect on agronomic traits, indicating contribution of alleles by the tolerant parent 'Pavon76'. QTLs for the six agronomic traits was associated with above mentioned markers and explained from 5 to 42 % of the phenotypic variation for all the agronomic traits. The genetic distance ranged from 10.1 to 38.1 cM. Therefore, the TRAP and SRAP markers linked to the QTL for the drought tolerance can be further used in breeding for drought tolerance in wheat.

Keywords: agronomic traits, QTL analysis, water stress.

Abbreviations: SRAP_Sequence Related Amplified Polymorphism; TRAP_Target Region Amplification Polymorphism; QTL_Quantitative Trait Loci.

Introduction

Drought is a common abiotic stress that seriously affects wheat (*Triticum aestivum* L.) production in many parts of the world, particularly in arid and semi-arid regions. Therefore, maintaining a sufficient yield under drought conditions has become a priority, particularly considering global environmental changes and increase in world population (Takeda and Matsuoka, 2008). However, improving yield or yield components under drought stress is difficult because they are complex traits controlled by polygenes and get greatly affected by the environments (McWilliam, 1989). Therefore, understanding the genetic and physiological bases of drought tolerance in crop plants are a prerequisite for developing superior genotypes through conventional breeding.

The genomics based approaches provide access to agronomically desirable alleles exist at quantitative trait loci (QTLs). This would enable us to improve the drought tolerance and yield of crops under water limited conditions more effectively. Identifying QTLs that control important traits such as physiological traits and yield components in spring wheat under drought stress may help to develop cultivars that are improved for those traits (Tuberosa and Saliva, 2006). There are reports of QTL controlling grain yield and its components under drought stress conditions in

bread wheat (Kirigwi et al., 2007), durum wheat (Maccafferri et al., 2008), rice (Bernier et al., 2007) and sunflower (Abdi et al., 2012). In addition, selection for field performance is based on the selection for physiological traits related to drought tolerance. In recent years, some QTLs for physiological traits under drought stress have been detected in wheat (Barakat et al., 2013; Elshafei et al., 2013; Saleh et al., 2014).

The molecular markers provide tools to study quantitative traits such as drought tolerance through QTLs analysis and are crucial in projects aiming to increase selection efficiency. Marker-assisted selection for improving drought responses in wheat was reported a few years ago (Quarrie et al., 2003). The application of QTLs analysis to study the yield components traits will improve our understanding of genetic factors that influence these complex traits. Grain yield in wheat can be dissected into several components, which are also under QTL control but have higher heritabilities than grain yield itself (Hai et al., 2008). Furthermore, individual traits correlated with wheat grain yield are often controlled by analogous genomic regions (Li et al., 2007; Hai et al., 2008; Cuthbert et al., 2008). McIntyre et al. (2010) reported four putative QTLs for yield under irrigated and rainfed conditions, which all co-localized with QTLs of yield

components. Ramya et al. (2010) reported 10 QTLs for grain weight and 15 QTLs for kernel dimensions. The pleiotropic QTLs were found on chromosomes 2B, 2D, 4B, and 5B. Recently, a total of 165 putative additive QTLs were identified, 22 of which showed significant additive-by-environment interaction effects. A total of 65 QTLs (51.5 %) were stable across environments, and 23 of these (35.4 %) were stable QTLs that were identified in at least two populations (Cui et al., 2014). Marker assisted selection may reduce problems associated with genotype \times environment interactions, improve the selection efficiency and facilitate combining different tolerance traits into a single efficient genotype. Recently, identification of new SRAP and TRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat under water-stressed condition have been reported (Elshafei et al., 2013; Saleh et al., 2013). However, very few studies have been carried out to detect TRAP and SRAP markers associated with QTLs in wheat for agronomic traits under different environments (Li et al., 2007; Wang et al., 2011; Yong-lu et al., 2011). The objective of this study was to identify TRAP and SRAP markers associated with QTL conditioning yield components under water-stressed conditions in F₄ wheat families using bulked segregant analysis (BSA).

Results and Discussion

Phenotypic analysis of yield and yield components

Analysis of variance revealed that the days to heading (days), plant height (cm), spike number/m², kernel number/spike, 1000-kernel weight (gm) and grain yield (t/ha) were significantly influenced by differences in water treatment, wheat genotypes and their interaction, except for the water treatment \times wheat genotypes interaction for days to heading which was not significant. In general, the well irrigated treatment had higher mean values ($P \leq 0.05$) for all agronomic traits than the limited irrigation treatment. There were no significant differences between Pavon76 and Yecora Rojo for spike number/m² and kernel number/spike under well-water treatment (Suppl. Fig. 1). But under both water treatments, Pavon76 was significantly higher for days to heading, plant height, 1000-kernel weight and grain yield than Yecora Rojo. In addition, Pavon76 was significantly higher for spike number/m² and kernel number/spike only under drought stress than Yecora Rojo (Suppl. Fig. 1).

All agronomic traits under each of the irrigation regimes exhibited normal distribution. Most variables were distributed across a similar range under both irrigation regimes, except for spike number and grain yield (Suppl. Fig. 1), which exhibited wider distributions under the well water treatment than drought stress treatment. The F₄ families exhibited transgressive segregation for most traits. Our results from the agronomic traits showed approximately normal distributions. This showed clear continuous and transgressive segregation. Frequency distribution of the traits showed transgressive segregation in both directions (Suppl. Fig. 1). The existence of individuals with higher and lower values compared to the parents indicated polygenic inheritance with partial gene association (Kearsey and Pooni, 1996). Continuous variation and transgressive segregation are the two obvious characters of multiple genes inheritance (Poehlman and Sleper, 1995). However, the continuous distribution of a quantitative trait does not exclude the possibility that only one gene is involved in any particular instance but simply implies the fact that, if it is so controlled, then the phenotypic differences among the

genotypes at that locus are small relative to variation caused by environmental influences (Kearsey and Pooni, 1996). Wide or continuous frequency distributions with transgressive segregants were observed, as one would expect for QTLs. Our results also are in conformity with the results of several earlier studies, where many loci located on several chromosomes were reported to control both traits (Ahmed et al., 2000; Börner et al., 2002; Huang et al., 2003). They reported that the presence of transgressive segregants for all the agronomic traits had alleles associated with low and high values of these traits with the parents selected for this purpose. The continuous distributions indicated that the characters are polygenic in nature and quantitatively inherited. Polygenic inheritance helps us to understand the way, in which grain yield are inherited and focused. Also, polygenic inheritance explains the phenotypic expression of a trait involving the interaction of many genes that influence on agronomic traits. This would give us the additive effect value for genes that influence these traits.

The spike number per square meter, the kernel number per spike and 1000-kernel weight were the components that had the greatest impact upon grain yield under drought stress conditions. The reduction in grain yield under water stress treatment was closely associated with a reduction in the spike number, kernel spike number and 1000-grain weight of the main tillers. Our results showed that drought stress caused significant decline for spike number per m² in all the F₄ families and two parents. Pavon76 showed less reduce than Yecora Rojo in spike number per m², indicating that Pavon76 genotype copes with stress. The number of tillers per plant has direct contribution towards grain yield. It means that there will be simultaneous increase in yield due to increases in number of productive tillers. Number of spikes per plant is related to yield; thus, it will be affected by drought stress. When growth resources are limited by drought stress, the size of plant organs such as leaves, tillers and spikes are reduced (Fisher, 1984). Blum and Pnuel, (1990) found that water stress, occurring during the later stages of development, caused a greater reduction in grains per spike and in the total number of tillers per plant. Garcia-del Moral et al., (2003) found significant decrease in number of wheat spikes under drought stress as compared to non-stressed conditions. Recently, Gevrek and Atasoy, (2012) reported that tiller number, spike number, spikelet and kernel number, single plant yield, spike and stem weight are decreased as a result of drought stress after post anthesis. Average spike number of plants was 2.5 under the control condition, whereas 2.0 under the drought stress.

Reduction in kernel number per spike has been noticed in F₄ families under water stress as compared to non-stress condition, where the stress reduced the kernel number per spike. An interaction between wheat genotypes and water treatments was found to be significant during current work, which means the F₄ families behaved differently under well-water and drought stress. Similarity, results reported that the number of kernels per spike has been influenced by different irrigation regimes in wheat cultivars at post anthesis, which is the most sensitive stage for number of grains per spike (Khan et al., 2004). Recently, Khan and Naqvi, (2011) reported that there is significant decrease in number of wheat grains under water stress.

The results of the present study are also agreement with the findings of (Chen et al., 2012) who reported that the moisture deficit induced reduction in yield primarily due to reduction in kernel weight, while the effects of moisture deficit on the yields of specific cultivars were largely attributed to the effects of drought on the number of kernels per spike.

Table 1. Genetic characteristics of QTLs related to days to heading (DH, day), plant height (PH, cm), spike number/m² (SN/ m²), kernel number/spike (KN/ S), thousand kernel weight (TKW) and grain yield (GY) traits as indicator of drought tolerance in the 150 F₄ families derived from Pavon76 × Yecora Rojo.

Trait	Primer name	Locus	QTL (CM)	LOD	R ² (%)	P Value	Additive effect
DH	TRAP37	3B	34.7	4.0	6	0.001	0.64
	SRAP2	3D	11.0	13.9	10	0.0001	1.31
	SRAP65	1D	11.8	14.6	12	0.0001	1.25
	SRAP77	1D	34.7	3.0	5	0.004	0.80
	TRAP2	2A	18.4	8.4	19	0.0001	7.66
	TRAP9	5A	33.4	3.6	15	0.0001	5.67
PH	TRAP15	2D	13.9	13.3	28	0.0001	7.93
	TRAP16	3B	15.3	9.6	22	0.0001	9.35
	TRAP21	6B	38.1	7.2	24	0.0001	102.40
	SRAP11	7B	20.6	8.7	35	0.001	8.81
	SRAP75	5A	16.5	11.9	32	0.001	4.98
	SRAP85	1B	24.7	6.9	25	0.001	7.30
	TRAP12	2D	21.6	2.4	16	0.0001	5.98
	TRAP13	1B	24.3	8.6	21	0.0001	96.0
SN/ m ²	TRAP15	2D	24.4	7.5	17	0.0001	83.89
	SRAP14	2B	27.6	6.0	17	0.0001	84.9
	SRAP15	3B	23.0	8.5	27	0.0001	103.9
	SRAP97	5A	24.0	8.2	30	0.0001	106.1
	TRAP4	5D	20.2	9.7	13	0.0001	15.80
	TRAP9	5A	16.6	13.6	32	0.0001	18.90
KN/ S	TRAP15	2D	34.6	4.3	11	0.001	17.30
	SRAP4	3B	15.6	14.8	23	0.001	1.10
	SRAP8	3B	10.1	20.8	38	0.001	1.39
	SRAP29	4A	15.4	14.2	31	0.001	1.28
	TRAP1	4A	23.2	7.0	37	0.0001	4.26
	TRAP8	4A	24.2	6.9	42	0.0001	4.30
TKW	TRAP10	3B	23.9	7.6	32	0.0001	3.63
	TRAP14	5A	22.2	8.0	28	0.0001	3.58
	SRAP5	4A	21.9	8.3	7	0.004	1.88
	SRAP24	3B	27.6	5.7	25	0.001	3.27
	SRAP26	3B	16.0	12.0	40	0.001	4.40
	TRAP15	2D	16.8	13.2	25	0.0001	0.90
	TRAP21	6B	18.1	12.3	27	0.0001	0.93
	TRAP35	3B	14.9	14.3	38	0.0001	1.14
GY	TRAP37	3B	14.6	14.1	36	0.0001	1.02
	SRAP5	4A	17.9	12.6	33	0.001	1.00
	SRAP20	3B	27.8	6.8	15	0.001	0.67
	SRAP27	3B	25.7	8.1	17	0.001	0.71
	SRAP54	7A	23.4	8.5	29	0.001	1.00
	SRAP89	5A	19.5	12.0	37	0.0001	1.04

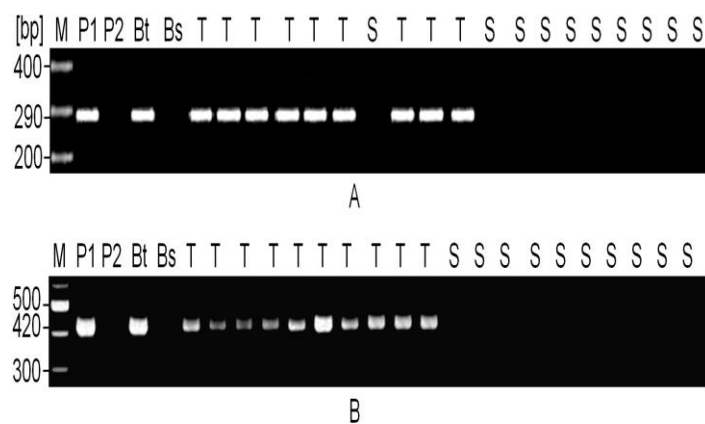


Fig 1. Selective genotyping of F₄ families of Pavon76 × Yecora Rojo wheat hybrids with TRAP15 (A) and SRAP89 (B) markers for plant height, grain yield traits, respectively. M- molecular weight, P1- Pavon76, P2- Yecora Rojo, BT- tolerant bulk; BS- sensitive bulk T- F₄ tolerant lines, S- F₄ sensitive lines.

Drought directly affects the development of grain which naturally leads to the lower grain weight and yield.

All agronomic traits in our study showed evidence of transgressive segregation, indicating that alleles from both parents influenced on traits (Suppl Fig. 1). The continuous segregation in RIL population and the transgressive segregation observed in all traits suggest that the traits approximately followed normal distributions and the experimental data of this study were suitable for QTL analysis.

QTL analysis

Out of 40 different TRAP primers and 98 different SRAP primers used in this study, only 17 and 25 primer pairs, respectively, generated polymorphisms between the two parents, Pavon76 and Yecora Rojo. Each of these markers was used to screen DNA bulks of the ten tolerant and the ten sensitive F_4 families according to the agronomic traits. The TRAP markers generated one polymorphic fragment and ranged from 270 to 570 bp, existed only in the tolerant bulk and Pavon76 (tolerant parent) which missing in the sensitive bulk and Yecora Rojo (sensitive parent) (Fig. 1). The SRAP markers generated one polymorphic fragment which ranged from 150 to 620 bp. The identified SRAP markers were present in the tolerant bulk and Pavon76 (tolerant parent) and were missing in the sensitive bulk and Yecora Rojo (sensitive parent) (Fig. 1). QTLs detected by CIM are listed in Table 1. A total of 33 QTLs were identified, ranging from 4 to 9 QTLs for each trait (Table 1).

Days to heading (DH)

Four QTLs, distributed on three chromosomes, were significantly associated with days to heading. The largest portion of the total phenotypic variation ($R^2 = 12\%$) was explained by SRAP65- 1D with an additive effect of up to a 1.25 day increase from the Pavon76 allele (Table 1).

Plant height (PH)

Eight QTLs significantly influenced plant height and each of them explained from 15 to 35% of phenotypic variance with a LOD value of 3.6 to 13.3 (Table 1). One QTL was mapped on each chromosome of 2A, 1B, 3B, 6B, 7B, and 2D. Two QTLs were mapped on chromosome of 5A. For all the QTLs, the Pavon76 alleles increased the plant height (Table 1).

Spike number per square meter (SN/ m^2)

Six QTLs for spike number per square meter were detected and each of them explained 16 to 30% of phenotypic variance with a LOD value of 2.4 to 8.6 (Table 1). One QTL was mapped on each chromosome of 5A, 1B, 2B and 3B. Two QTLs were mapped on chromosome of 2D. For all the QTLs, the Pavon76 alleles increased the spike number per square meter (Table 1).

Kernel number per spike (KN/ S):

Six QTLs were detected for kernel number per spike, explaining 11 to 38% of the phenotypic variance with a corresponding LOD of 4.3–20.8 (Table 1). Six QTLs were distributed on four chromosomes; one QTL on each of chromosome 4A, 5A, 2D and 5D, and two QTLs on chromosome 3B. For all the QTLs, the Pavon76 alleles increased the kernel number per spike (Table 1).

Thousand-kernel weight (TKW)

A total of seven QTLs associated with thousand-kernel weight; 3, 1 and 3 QTLs on chromosomes 4A, 5A and 3B, respectively, were identified in individual, explaining 7 to 42% of the phenotypic variance (Table 1). All QTLs identified showed additive effects positive with an additive effect of up to a 4.4g increase from the Pavon76 alleles (Table 1).

Grain yield (GY)

Nine QTLs for grain yield were detected and each of them explained from 15 to 38% of the phenotypic variance, with LOD scores of 6.8 to 14.3 (Table 1). One QTL was mapped on each chromosome of chromosomes 4A, 5A, 7A, 6B and 2D. Four QTLs were mapped on chromosome of 3B. The TRAP35- 3B had the largest additive effect with LOD= 14.3 and explained 38% of the phenotypic variance (Table 1). For all the QTLs, the Pavon76 alleles increased the grain yield (Table 1).

In earlier studies, QTLs for SN/ m^2 , KN/S and TKW were reported on chromosomes 4A, 1B and 3B using TRAP markers (Li et al., 2007; Wang et al., 2011). In the present study, similar two QTLs for TKW were detected on chromosomes 4A and 3B and one QTL for SN/ m^2 was detected on chromosome 1B. However, the other QTLs obtained in the present study have been not reported before (using TRAP markers). Using SRAP markers in earlier studies, QTLs for KN/S were reported on chromosomes 4A, 5A, 7B, 1D and 5D (Li et al., 2007; Wang et al., 2011). In the present study, similar QTLs were detected on chromosomes 4A for TKW, 5A and 7B for PH, 1D for DH and 5D for KN/ m^2 . One QTL for GY was detected on chromosome 3B in the similar region to Xsrp14-3B, in which Young-lu et al. (2011) found a QTL for the same trait. However, the other QTLs obtained in the present study using SRAP markers are novel and have been not reported before. The results showed also that the regression analysis for the relationship between the TRAP and SRAP markers and the phenotypes of F_4 families for the six agronomic traits was highly significant. This indicates that the TRAP and SRAP markers were associated with the agronomic traits as an indicator for drought tolerance genes. The genetic distance between the fourteen TRAP markers and drought tolerance genes were determined and ranged from 13.9 to 38.1 cM. The genetic distance between the nineteen SRAP markers and drought tolerance genes were determined and ranged from 10.1 to 34.7 cM. Therefore, these TRAP and SRAP markers were linked to the quantitative trait loci (QTL) for the agronomic traits as an indicator for drought tolerance genes.

Identification of associated molecular markers at a major locus contributing to water-stress tolerance would be useful for the indirect selection of wheat plants for water-stress tolerance (Visser, 1994). However, identifying molecular markers associated with important genes or traits in most instances requires screening of a relatively large number of individuals in the population (Lawson et al., 1994). Bulk segregant analysis (BSA) was originally developed to overcome this difficulty (Michelmore et al., 1991), because comparing bulk samples is easier than evaluating many individuals in different populations (Altinkut et al., 2003; Barakat et al., 2011). In the present study, we identified 14 TRAP and 19 SRAP markers linked to the six agronomic traits 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.

Results in the present investigation indicated that all of the QTLs using TRAP and SRAP markers for agronomic traits had a positive additive effect, indicating contribution of alleles increasing the agronomic traits by the tolerant parent 'Pavon76' (Table 1). Positive additive effect of the QTL, on chromosomes 2A, 4A, 5A, 1B, 3B, 6B and 2D using TRAP markers and on chromosomes 2A, 4A, 5A, 7A, 1B, 2B, 3B, 1D and 3D using SRAP markers, indicates contribution of QTL alleles in these loci from the tolerant parent, 'Pavon76'. In addition, the positive additive effect indicates the relative importance of additive gene effects in controlling the agronomic traits as an indicator for drought tolerance in F₄ families. In the present study, the 14 TRAP markers were assigned to chromosomes 2A, 4A, 5A, 1B, 3B, 6B and 2D in agreement with previous report (Li et al., 2007). Also, the 19 SRAP markers were assigned to chromosomes 2A, 4A, 5A, 7A, 1B, 2B, 3B, 1D and 3D in agreement with previous report (Li et al., 2007). Maccaferri et al. (2008) reported a total of 16 QTL for grain yield, among them two major QTLs were located on chromosomes 2BL and 3BS with R² values of 21.5 and 13.8%, respectively. Peleg et al. (2009) reported that a total of 110 QTLs were mapped for 11 agronomic traits, with LOD score range of 3.0– 35.4. Molecular detection of genomic regions associated with grain yield and yield-related components in elite bread wheat cross evaluated under irrigated and rainfed conditions have been reported (McIntyre et al., 2010). Recently, Wu et al. (2012) reported a total of 241 QTLs controlling yield-associated traits. The number of QTLs for individual traits ranged from 12 to 33.

Materials and Methods

Plant materials

A set of 150 recombinant wheat (*Triticum aestivum* L.) inbred lines (RILs, at F₄) developed from a cross between Pavon76 (drought tolerant cultivar introduced from CIMMYT) and Yecora Rojo (drought sensitive cultivar developed in USA and recommended for environment of Saudi Arabia since 1981) was used in this study. Yecora Rojo is a high yield, 2-gene dwarf cultivar but is very sensitive to environmental factors, such as drought stress, especially during the grain filling period (Barakat et al., 2010).

Field evaluation

The 150 recombinant inbred lines and the two parents (Pavon76 and Yecora Rojo) were tested for tolerance to drought under field condition. The water regimes were established after germination on the basis of free-surface evaporation, monitored at a weather station located at the Agricultural Research Station of King Saud University (Dierab, near Riyadh; 24° 42' N, 44° 46' E, 400 m above sea level). Two irrigation regimes [0.25 and 0.75 m³(H₂O) m⁻²(soil)] were applied two weeks after sowing. The seeding rate was 140 kg ha⁻¹. Fertilizers were applied at the rate of 120 kg N and 80 kg P₂O₅ ha⁻¹. The cultural practices were carried out according to the recommended practices adopted in Riyadh area, Saudi Arabia. Six agronomic traits were determined. Grain yield was determined as the weight of grain harvested per unit area and converted to grain yield per hectare (t/ha). Thousand kernel weight was calculated as the weight of a 1000-grain sample. Number of spikes per m² was recorded as the number of spike per m². KNS was measured at maturity on 10 random main stems. Days to heading (days) was determined as the number of days from date of sowing to the date of the first anthers exertion of 50% of the ears of

each genotype. Plant height (cm) was measured from the soil surface to the tip of the main ear at maturity, excluding awns.

DNA extraction

Frozen young leaves (500 mg) were ground to a powder in a mortar and a pestle with liquid nitrogen. The DNA extraction was done using the CTAB method (Saghai-Maroofof et al., 1984).

PCR amplification

Forty different TRAP primer combinations (Hu and Vick, 2003) and 98 different SRAP primer combinations (Li and Quiros, 2001) were used in this study (Suppl. Table 1 and Suppl. Table 2). The PCR reaction mixture consisted of 20 to 50 ng genomic DNA, 1× PCR buffer, 1.5 mM MgCl₂, 0.1 mM of each dNTP, 0.5 μM primer, and 1 U *Taq* polymerase in a volume of 0.025 cm³. After incubation at 94 °C for 5 min, 5 cycles were performed with 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min 40 s. Further, the similar 35 cycles were performed with exception for the annealing temperature at 50°C and a final extension at 72°C for 7 min. Amplification products were electrophoretically resolved on 2- 3 % (m/ v) agarose gels containing 0.1 μg/ ml ethidium bromide and photo-graphed on a UV trans-illuminator.

Bulked segregant analysis

Bulked- segregant analysis (BSA) was used in conjunction with TRAP and SRAP analysis (Michelmore et al., 1991) to find markers linked to genes of the yield component traits under drought stress. Tolerant and sensitive bulks were prepared from F₄ family individuals by pooling aliquots containing equivalent amounts of total DNA, approximately 50 ng/ml from each of ten sensitive and ten tolerant F₄ families, selected according to on phenotypic assessments. Then, TRAP and SRAP primers were screened on the parents and two bulk DNA samples, from which some primer combinations revealed polymorphic bands, not only among parental genotypes but also between the pair of the bulk DNA. The name and sequence of all polymorphic polymorphic markers are listed in supplementary Tables (Suppl. Table 1 and Suppl. Table 2). Based on the evaluations of DNA bulks, individual F₄ families were analyzed with co-segregating primers to confirm TRAP and SRAP markers linkage to the yield component traits as an indicator for drought tolerance genes.

Data and linkage analysis

Analysis of variance was performed using the SAS 9.1 program. The ANOVA was estimated for all traits according to Steel and Torrie, (1980). Map manager QTX v. 0.22 (<http://manager.roswellpark.org/mmQTX.html>) was used to analyze the linkage relationship of TRAP and SRAP markers detected from BSA. Linkage was detected when a log of the likelihood ratio (LOD) threshold of 3.0 and maximum distance was 50 cM. 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 v. 9.1 software packages (SAS Institute, Cary, NC, USA) to determine the total amount of the phenotypic variation explained (Nelson, 1997).

Conclusion

Our results from the agronomic traits showed approximately normal distributions. This showed clear continuous and transgressive segregation. Frequency distribution of the traits showed transgressive segregation in both directions. Our results also indicated that TRAP and SRAP markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to six agronomic traits; (days to heading (days), plant height (cm), spike number/m², kernel number/spike, 1000-kernel weight (gm) and grain yield (t/ha)), as indicators for drought tolerance genes in wheat. The marker-assisted selection with TRAP and SRAP markers might be useful for developing improved cultivars.

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Supplementary data

Suppl. Fig. 1: Frequency distributions of the yield- related trait means in the 150 F₄ population under well- water (A) and drought stress (B). Phenotypic values of the parents of Pavon76, Yecora Rojo and mean of F₄ lines are indicated by arrows

Suppl. Table 1: The available TRAP primer pairs.

Suppl. Table 2: The available SRAP primer pairs.

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