Drought adaptation attributes and associated molecular markers via BSA in the Seri/Babax hexaploid wheat (*Triticum aestivum*, L.) population

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ABSTRACT

Agronomic and physiological traits associated with drought adaptation were assessed in Mexico and South Australia within the Seri/Babax recombinant inbred line (RIL) population, the progeny of which are similar in height and maturity but divergent in their sensitivity to drought. Under drought, canopy temperature (CT) was the trait best associated with yield (averaged, r= –0.84, P<0.0001), with a relative high heritability (h^2= 0.65, P<0.0001). CT reflects a mechanism of dehydration avoidance expressed throughout the growing season and across latitudes, which can be used as a selection criteria to identify performance under drought. The importance of early vigour under drought, suggested by a high association of CT with estimates of biomass at booting (r= –0.44, P<0.0001) and plant height (r= –0.64, P<0.0001), contrasted with the small relationships with anthesis, maturity and osmotic potential. Hence, the results suggest that the ability to extract water from the soil under increasing soil water deficit is a major attribute of drought adaptation.

Bulked segregant analysis (BSA) of CT and associated secondary traits under drought was performed using 127 PCR-based and AFLP markers, with the associated loci ($F \geq 5.00$, P<0.05) explaining the phenotypic variance under drought in Mexico (19-46%) and Australia (19-42%). While the value of such associations needs to be investigated in other genetic backgrounds and environments, the results suggest a viability in the efficient tailoring of markers to improve yields in regions and latitudes with different rainfall patterns and drought environments. Further genomic and transcriptomic studies should be conducted in the Seri/Babax RILs to dissect the basis of the dehydration avoidance mechanism epitomised by CT and to unravel the complex relationship between drought adaptation and performance under drought.

INTRODUCTION

Globally, drought stress is a limiting factor in the yield of wheat (*Triticum aestivum* L.), likely to increase in its severity as the availability of reliable sources of high quality irrigation water decline. Breeding for drought adaptation in wheat has focussed on: drought escape; selection for traits indirectly contributing to water use efficiency; and, drought-adaptive (physiological) traits consistently associated with yield. Within the latter approach, a number of physiological mechanisms have been identified contributing to maintenance of plant water status under stress. As there is an increase in both the understanding of their relative contribution and in the number and reliability of indirect assessment methods, they are progressively being used as selection criteria in physiology breeding programmes. However, there is a limited systematic evaluation of these traits in stable wheat genetic backgrounds across drought environments.

The present study aimed at the: (*i*) evaluation of genetic diversity in traits associated to drought adaptation across different environments in the Seri/Babax RIL population, specifically developed for low variation in phenology and height; (*ii*) elucidation of the drought-adaptation strategy revealed by the traits best associated with yield under drought; and, (*iii*) identification of genomic loci associated to drought adaptive traits that could be used as markers for advancing wheat physiological breeding.

METHODOLOGY

A hexaploid wheat population (Seri/Babax RILs) was developed for assessing the expression of agronomic and physiological traits under drought stress in Mexico (DRT, at Obregon in 1999/2000, 2000/2001 & 2001/2002 cycles) and rainfed conditions at two sites in South Australia (AUS, Charlick and Roseworthy, in 2001) [1].

BSA was conducted on transgressively segregating traits most strongly associated with performance under drought [1]: CT at booting and at grain filling both in the morning and the afternoon (CTAM-boot and CTPM-boot, and CTAM-gf and CTPM-gf, respectively), flag leaf chlorophyll content at booting (CHLO-boot) and at grain filling (CHLO-gf), normalised difference in vegetative index (red spectrum) at booting (RNDVI-boot) and at grain filling (RNDVI-gf), leaf rolling at grain filling (LROLL-gf), height at maturity (HEIGHT-gf), kernel number (KNO), thousand kernel weight (ATKW) and grain yield (YLD). For the BSA, a 127 RILs subset was used, with individual bulks for each trait of interest outlined separately for the drought (DRT, averaging all trait data collected across cycles) and rainfed environments (AUS, averaging data from the two sites), to avoid conclusions being confounded by the large environmental interactions derived from an overall data averaging. Two different set of bulks were considered for each individual trait, each consisting of ten genotypes sampled from the two extremes of the distribution. In preparing the bulks, equal volumes of DNA from each genotype selected (diluted at equal concentrations from the original 1 µg DNA stock) were evenly and carefully

mixed. The molecular assessment of the bulks was performed with an assortment of 107 polymorphic markers (97 PCR-based and 10 AFLPs), covering all seven chromosomic groups in each of the three hexaploid wheat genomes [For DNA extraction details, and marker, PCR and electrophoresis experimental details, see [2]].

Phenotypic data were analysed as described in [1]. For the genotypic data, linkage association was performed using MAPMAKER v2.0 [3]; two-point linkage mapping was declared when –log(*P* value) (LOD ≥ 3.0 and θ≤ 0.40). Recombination frequencies and the related genetic distances between linked markers, were defined by means of the Haldane mapping function [4]. Resulting linkage groups and loci chromosomic position were verified with published maps [5, 6, 7, 8, 9, 10]. Association analyses between genotypic and phenotypic datasets were performed with Q-Gene software [11]. Simple linear regression at every locus for every trait was used to calculate the coefficient of determination (R^2), as a proportion of the phenotypic variation explained by the markers, with the threshold declared at $P<0.05$ and $F\geq 5.00$.

RESULTS AND DISCUSSION

Under DRT, CT was highly associated with yield, both phenotypically and genotypically ($r= –0.75$, and R(g)= –0.95, $P<0.0001$) [1], similarly to previous reports in other backgrounds and conditions [12, 13, 14]. Highly heritable ($h^2= 0.65$, $P<0.0001$), CT explained most of the genetic variation in yield under DRT ($R^2= 0.71$, $P<0.0001$), regardless of phenological stage or time of day. At booting, CT was significantly ($P<0.0001$) correlated with increased kernel set under DRT (averaged, $r= –0.73$) –effecting a strong association ($r= 0.97$) between kernel number and yield– and at grain filling with kernel weight ($r= –0.40$). As CT reflects the rates of transpirational cooling of the canopy, dependent on the ability to extract soil moisture, it suggests that differences in performance under drought stress are associated with dehydration avoidance.

The evapotranspiration strategy of the high yielding Seri/Babax RILs on maintaining a low CT, is exerted early in the crop cycle and maintained until physiological maturity. A number of significant ($P<0.0001$) associations of CT with other physiological and agronomic parameters support the strategy [1]: a) it was inversely related to plant height (averaged, $r= –0.64$), suggesting a lower CT in those RILs able to access more water earlier in the cycle; b) it was correlated with RNDVI during booting (averaged, $r= –0.46$), suggesting a rapid early biomass production under cooler canopies; and, c) it was less associated with RNDVI during grain filling (averaged, $r= –0.43$) because leaf rolling increased as stress intensified –itself associated with warmer canopies and inversely with RNDVI during grain filling ($r= –0.62$).

Transgressive segregation was observed in a number of agronomic and secondary traits under contrasting conditions, suggesting the complexity of the genetics underlying drought response and yield –as yield potential *per se* only explained *ca.*50% performance under drought [1]. The transgressively segregated response in both DRT and AUS, contrasted with a low polymorphism (*ca.*27%) between the parental genotypes with the >300 available markers (at CIMMYT), preventing complete mapping [2]. Although BSA has been conventionally used to dissect qualitative traits involved in biotic stresses [16, 17, 18, 19, 20, 21], an increased marker dataset (in collaboration with CSIRO), was explored as an alternative to dissect the quantitative traits expressed under drought in Mexico and Australia.

Linkage for all the marker/genotypic combinations were congruous with published hexaploid wheat chromosomic locations; none of the observed chimerical associations were considered for association analyses. The phenotypic variation for CT in all latitudes was significantly ($P<0.05$) explained by loci specific for phenological stages and latitudes: 12 and 13 loci under DRT and AUS, respectively (Table 1). Loci significantly ($P<0.05$) explaining the phenotypic variation in the tailored genetic bulks for the two different agroecosystems were convergent (in 20%: in 1A, 3B, 4A and 6A; and, notably, in 5A, 5B, 5D and 6B), although their corresponding phenotypic correlations were not. The role and importance of these genomic regions in the expression of CT is supported by recent evidence (G.J.Rebetzke, *pers.comm.*) on significant ($P<0.05$) chromosomic loci identified for the trait in Australian germplasm via QTL mapping.

For all the complex traits assessed via BSA, a 19-46% of the phenotypic variation was explained in DRT by 88 loci (62 assigned, 26 unassigned), whereas a 19-42% variation in AUS was described by 69 loci (63 assigned, 6 unassigned) (Table 1). The verification with either a full Seri/Babax QTL map [22], in other linkage maps or different genetic backgrounds would allow to elucidate: a) the Seri/Babax genetic architecture and the influence of regions explaining the phenotypic variation reflected by putative loci co-location phenomena, or indicating epistatic and pleiotropic genetic effects in the variation observed; b) the relative importance of using a BSA platform prior to full QTL mapping, as a cost-efficient marker development strategy in physiological breeding; and, c) the role of regulo-transcriptomic systems in the expression of CT and drought-adaptive traits in the Babax parental genotype and in those high-performing RILs when subjected to drought.

CONCLUSIONS

The Seri/Babax population was phenotypically and genetically evaluated for the expression of complex traits, of which CT was remarkably and consistently associated with high agronomic performance under drought stress in Mexico and South Australia. Marker loci significantly explaining the phenotypic variation for CT were developed via BSA for both latitudes, some of which have been reported to be of relevance in other

genetic backgrounds. Further genetic and transcriptomic characterisations of the drought-adapted Babax parental genotype and of those high-yielding RILs could allow to elucidate the basis of the dehydration avoidance strategy exerted under drought.

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Table 1. Significant (P<0.05) loci identified via BSA explaining complex traits variation in a subset of the *Seri/Babax* population in Mexico and Australia under drought stress

CHROMO-SOME	TRAIT (%) UNDER DROUGHT STRESS[1]	
	MEXICO[2]	AUSTRALIA[3]
1A		CT-gf (29), HEIGHT-gf (20)
1B	CT-boot (19), CT-gf (19), LROLL-gf (23), KNO (19), ATKW (30)	CHLO-boot (20)
1D	CHLO-boot (25), CHLO-gf (40)	CHLO-boot (36), LROLL-gf (36), HEIGHT-gf (24), YLD (44)
2A	RNDVI-gf (24)	CHLO-boot (43)
2B	RNDVI-boot (30), KNO (24), ATKW (30), YLD (24)	HEIGHT-gf (24)
2D		CT-gf (26), CT-gf (20)
2A, 2B, 2D	CT-gf (22)	
3A		CT-boot (19)
3B	CT-boot (23), CT-gf (19), ATKW (20), YLD (20)	CT-gf (22), CHLO-gf (20), YLD (24)
3A,4B	LROLL-gf (32), ATKW (26), YLD (26)	CHLO-boot (26)
4A	CT-gf (21), RNDVI-boot (28), ATKW (29)	CT-boot (36), HEIGHT-gf (34), YLD (20)
4B	CHLO-boot (42), CHLO-gf (46), HEIGHT-gf (22)	LROLL-gf (34)
4B,4D		CT-gf (28), LROLL-gf (28)
5A	CT-gf (23), KNO (19)	CT-boot (23)
5B	CT-gf (26)	CT-boot (22), LROLL-gf (38), YLD (30)
5D	CT-boot (32), CT-gf (19), HEIGHT-gf (20)	CT-gf (31)
5A,6A		CT-gf (26), CHLO-boot (32), CHLO-gf (36)
6A	CT-boot (25), YLD (19)	CHLO-boot (25)
6B	CT-gf (19), KNO (22)	CT-gf (27), CHLO-boot (23)
7A	CHLO-boot (25)	CT-boot (36), CHLO-gf (19), LROLL-gf (21)
7B	KNO (27), YLD (26)	LROLL-gf (20)
7D	KNO (20)	LROLL-gf (35), HEIGHT-gf (23)

NOTE: [1]Percentage of variation explained by marker/trait association at the identified loci; [2]Averaged data of Mexican cycles (at Obregon, Sonora): 1999/2000, 2000/2001 & 2001/2002; [3]Averaged data of South Australian trials at Charlick and Roseworthy in 2001.

For traits codes, see METHODOLOGY section.

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