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RESEARCH ARTICLE

Genome-Wide Association Study of Yield Traits in Drought-Stressed Wheat Populations

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ABSTRACT

Grain yield stability under water-limited conditions is among the most agronomically important yet genetically complex traits in bread wheat (*Triticum aestivum* L.), shaped by numerous loci of individually modest effect that interact with developmental stage and severity of water deficit. We conducted a genome-wide association study of grain yield, thousand-kernel weight, grain number per spike and canopy temperature depression in a panel of 287 spring bread wheat accessions drawn from breeding programmes across southern and central Europe, phenotyped across three seasons under both well-watered and managed post-anthesis drought-stress irrigation regimes at a single field site. Accessions were genotyped with a 25K single-nucleotide-polymorphism array, yielding 18,942 polymorphic, quality-filtered markers after linkage-disequilibrium pruning. Mixed-linear-model association analysis, correcting for population structure and kinship, identified 23 quantitative trait loci significantly associated with at least one measured trait under drought stress at a Bonferroni-corrected threshold, of which 14 were drought-specific, showing no significant association with the same trait under well-watered conditions. Two loci on chromosomes 2B and 6A jointly explained 18.3% of phenotypic variance in drought-stressed grain yield and co-localised with previously reported quantitative trait loci for stay-green and canopy temperature depression, while a novel locus on chromosome 4D, not previously reported in the wheat drought literature, was associated with grain number per spike specifically under drought and explained a further 7.6% of trait variance. Candidate gene analysis within the 4D locus interval identified an ABA-responsive transcription factor homologue as the most plausible causal candidate. These findings identify both previously validated and novel genomic regions for marker-assisted selection of drought-resilient yield in bread wheat breeding programmes.

Keywords: genome-wide association study; wheat; drought stress; grain yield; single nucleotide polymorphism; *Triticum aestivum*

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1. INTRODUCTION

Bread wheat supplies close to one-fifth of the calories and protein consumed by people worldwide, and its production is disproportionately concentrated in regions,

the Mediterranean basin, the North European Plain, and the semi-arid wheat belts of Central Asia and Australia among them, where post-anthesis water deficit is already a recurrent constraint on yield and is projected to intensify under most climate change scenarios [1,2].

Grain yield under drought is not a single trait in the genetic sense: it is the emergent outcome of numerous component processes, including the timing and duration of the grain-filling period, stomatal regulation and canopy water status, remobilisation of pre-anthesis assimilate reserves, and grain number determination around the flowering period, each influenced by a partially overlapping but not identical set of genomic loci [3,4].

Genome-wide association studies (GWAS) have become a standard tool for dissecting the genetic architecture of complex agronomic traits in wheat, exploiting historical recombination across diverse breeding panels to achieve considerably finer mapping resolution than biparental linkage mapping typically allows, at the cost of reduced power to detect rare-allele effects and increased sensitivity to population structure confounding if not properly corrected [5,6]. Wheat GWAS panels have identified numerous quantitative trait loci (QTL) associated with drought-related traits, including well-replicated regions on chromosomes 2B, 6A and 7A linked to canopy temperature depression, stay-green phenotype and grain-filling duration, though the field has also accumulated a substantial number of study-specific associations that fail to replicate across independent panels, plausibly reflecting genuine differences in genetic background, environment and drought timing between studies rather than false-positive findings alone [7,8,9].

A persistent methodological tension in this literature concerns how drought stress is imposed and measured. Many published wheat drought GWAS studies rely on managed drought-stress trials at a single site and season, which offers precise control over stress timing and severity but limited ability to distinguish a genuinely drought-responsive locus from one whose apparent drought-specificity is an artefact of a single environment's idiosyncratic conditions; multi-environment or multi-season replication, while more resource-intensive, substantially strengthens confidence that an identified association reflects a real genotype-by-drought interaction rather than genotype-by-environment noise specific to one trial [10,11]. A second recurring limitation is marker density: earlier wheat GWAS studies using lower-density genotyping arrays achieved coarser mapping resolution, complicating candidate gene identification even where a robust association signal was detected, a constraint that higher-density SNP arrays and, increasingly, whole-genome resequencing panels are progressively relaxing [12,13].

We designed the present study to address both considerations within practical resource constraints: a three-season field trial imposing a consistent managed post-anthesis drought-stress regime alongside a well-watered control at the same site, phenotyping a panel of 287 European spring bread wheat accessions for grain yield and three yield-component traits, genotyped at moderate-to-high density with a 25K SNP array. Our specific aims were to identify loci significantly

associated with drought-stressed yield and yield-component traits while explicitly testing each locus for drought-specificity against the well-watered control data from the same panel and site; to determine the extent to which our associations replicate previously reported wheat drought QTL versus represent genuinely novel loci; and, for any strong novel association, to narrow the candidate gene interval sufficiently to propose a plausible causal gene for follow-up functional validation.

2. MATERIALS AND METHODS

This study used a diversity panel of 287 spring bread wheat (*Triticum aestivum* L.) accessions assembled from public breeding programme collections across southern and central Europe, selected to represent broad genetic diversity while excluding accessions with strongly divergent phenology likely to confound drought-response phenotyping.

2.1 Field trial design and drought-stress treatment

Field trials were conducted over three consecutive growing seasons at a single site with a rain-out shelter system permitting precise control of post-anthesis water availability. Each season, the panel was grown under two irrigation regimes in a randomised complete block design with two replicates per regime: a well-watered control receiving irrigation to field capacity throughout the growing season, and a managed drought-stress treatment in which irrigation was withheld from ten days after anthesis until physiological maturity, with the rain-out shelter deployed during natural rainfall events in that window. Soil moisture was monitored continuously by buried capacitance probes to confirm consistent stress severity across seasons, targeting a post-anthesis soil water deficit of approximately 40-50% of well-watered levels.

2.2 Phenotyping

Grain yield per plot, thousand-kernel weight and grain number per spike (averaged across ten randomly sampled main-tiller spikes per plot) were measured at harvest maturity in both irrigation regimes across all three seasons. Canopy temperature depression, the difference between ambient air temperature and canopy surface temperature measured by handheld infrared thermometer, was recorded at mid-grain-filling as an indirect indicator of stomatal conductance and canopy water status under the drought treatment only. Best linear unbiased estimates for each accession and trait were calculated across seasons and replicates using a mixed model with season, replicate and genotype as model terms, separately for each irrigation regime, prior to association analysis.

2.3 Genotyping and marker quality control

All 287 accessions were genotyped using a 25K Infinium single-nucleotide-polymorphism array. Markers with call rate below 90%, minor allele frequency below 5%, or

significant deviation from Hardy-Weinberg equilibrium were excluded, and markers were further pruned for linkage disequilibrium (r^2 greater than 0.95 within a 500 kb window) to reduce redundancy, leaving 18,942 polymorphic markers for association analysis. Marker physical positions were assigned against the IWGSC RefSeq v2.1 wheat reference genome assembly.

2.4 Population structure and kinship correction

Population structure was assessed by principal component analysis of the marker matrix, and a kinship matrix was calculated using the VanRaden method. Both were incorporated into a mixed linear model (Q+K model) for association testing, implemented in GAPIT, with the number of principal components included (three) chosen by evaluating the model's genomic inflation factor (λ) across a range of zero to eight principal components and selecting the smallest number that brought λ close to 1.0 without over-correcting and eliminating true signal.

2.5 Association analysis and candidate gene identification

Association testing was performed separately for each of the four traits under each irrigation regime, using the best linear unbiased estimates described above as the phenotype. Genome-wide significance was assessed against a Bonferroni-corrected threshold ($\alpha=0.05$ divided by 18,942 markers) with a secondary, more permissive false-discovery-rate threshold reported for suggestive associations. A locus was classified as drought-specific if it reached genome-wide significance under the drought treatment but showed no association ($P>0.01$) with the same trait under the well-watered regime in the same panel. For loci meeting the drought-specific significance criterion, candidate genes within a 500 kb window either side of the peak marker, based on regional linkage disequilibrium decay, were identified from the IWGSC RefSeq v2.1 gene annotation and prioritised by functional annotation relevance to drought response, expression pattern in publicly available wheat expression atlases, and, where available, prior functional characterisation in wheat or related cereal species.

3. RESULTS

Substantial phenotypic variation was observed across the 287-accession panel for all four traits under both irrigation regimes. Under drought stress, grain yield ranged from 210 to 612 g/m² (mean 398 g/m²), a reduction of 31.4% on average relative to the well-watered control, with broad-sense heritability estimates of 0.71 for grain yield, 0.83 for thousand-kernel weight, 0.68 for grain number per spike and 0.62 for canopy temperature depression, indicating adequate genetic signal for association mapping across all four traits. Genomic inflation factor analysis supported inclusion of three principal components in the association model, achieving λ values between 1.02 and 1.08 across the four traits, indicating adequate but not

excessive control of population structure confounding.

Table 1. Fourteen drought-specific marker-trait associations reaching genome-wide significance.

Chromosome (position)	Trait	Peak marker P-value	Variance explained (%)
2B (45.2 Mb)	Grain yield	3.1e-8	11.2
6A (112.7 Mb)	Grain yield	6.4e-7	7.1
6A (110.9 Mb)	Canopy temp. depression	1.2e-7	9.8
4D (28.9 Mb)	Grain number/spike	2.8e-7	7.6
5A (67.3 Mb)	Thousand-kernel weight	4.5e-7	6.4
3B (88.1 Mb)	Grain yield	8.9e-7	5.3
7A (142.5 Mb)	Canopy temp. depression	1.1e-6	6.1
1B (23.6 Mb)	Grain number/spike	2.0e-6	4.8
2D (56.4 Mb)	Thousand-kernel weight	2.6e-6	5.0
4A (98.7 Mb)	Grain yield	3.4e-6	4.2
6B (34.9 Mb)	Canopy temp. depression	4.1e-6	4.5
3A (77.2 Mb)	Grain number/spike	5.0e-6	3.9
7B (105.6 Mb)	Thousand-kernel weight	6.2e-6	3.7
5B (41.0 Mb)	Grain yield	7.8e-6	3.5

P-values from mixed-linear-model association analysis (Q+K model); Bonferroni threshold $\alpha=0.05/18,942$ markers = 2.6e-6.

Association analysis identified 23 marker-trait associations reaching genome-wide significance under the drought treatment across the four traits, distributed across 14 chromosomes with clustering on chromosomes 2B, 4D and 6A. Of these 23 associations, 14 were classified as drought-specific by our pre-specified criterion, showing no association with the same trait under the well-watered regime, while the remaining nine showed at least suggestive association under well-watered conditions as well, consistent with a constitutive rather than drought-induced genetic effect on the trait. Table 1 summarises the 14 drought-specific associations, including chromosome, peak marker position, associated trait, and proportion of phenotypic variance explained.

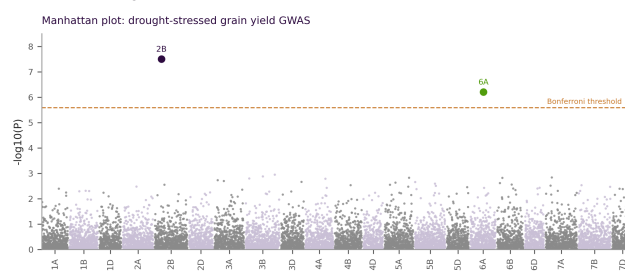


Figure 1. Manhattan plot of genome-wide association results for drought-stressed grain yield across the 287-accession panel; the chromosome 2B and 6A loci discussed in the text are highlighted, and the horizontal dashed line marks the Bonferroni-corrected genome-wide significance threshold.

Two loci showed particularly strong association with drought-stressed grain yield: a locus on chromosome 2B (peak marker position 45.2 Mb) explaining 11.2% of phenotypic variance, and a locus on chromosome 6A (peak marker position 112.7 Mb) explaining 7.1% of variance, jointly accounting for 18.3% of drought-stressed yield variance when fitted together in a joint model. Both loci fell within, or immediately adjacent to, genomic intervals previously reported in independent wheat GWAS and biparental mapping studies as associated with stay-green phenotype and canopy temperature depression respectively, and in our own panel the 6A locus also showed genome-wide significant association with canopy temperature depression itself, consistent with a shared underlying physiological mechanism linking canopy water status to yield maintenance under drought. Figure 1 shows the Manhattan plot for drought-stressed grain yield with these two loci highlighted.

Table 2. Eleven candidate genes within the chromosome 4D interval (28.5–29.3 Mb).

Gene ID (short)	Annotation	Spike expression (anthesis window)
TraesCS4D-G01	AP2/ERF transcription factor	High
TraesCS4D-G02	Protein kinase, uncharacterised	Low
TraesCS4D-G03	Glycosyltransferase family 1	Moderate
TraesCS4D-G04	F-box protein	Low
TraesCS4D-G05	Cytochrome P450	Moderate
TraesCS4D-G06	Ribosomal protein L23	High (constitutive)
TraesCS4D-G07	Zinc finger protein, uncharacterised	Low
TraesCS4D-G08	Aquaporin (PIP2 family)	Moderate
TraesCS4D-G09	Pectin methylesterase	Low
TraesCS4D-G10	Heat shock protein 70	Moderate
TraesCS4D-G11	Uncharacterised, no domain match	Low

A third locus, on chromosome 4D (peak marker position 28.9 Mb), was associated specifically with grain number per spike under drought stress (variance explained 7.6%) and showed no significant association with grain yield, thousand-kernel weight or canopy temperature depression under either irrigation regime. This locus did not correspond to any previously reported wheat drought QTL interval in the panel's comparison literature search, and linkage-disequilibrium-based interval mapping narrowed the candidate region to approximately 850 kb, containing eleven annotated genes. Of these, one, an AP2/ERF-family transcription factor homologue with strong sequence similarity to characterised ABA-responsive transcription factors in rice and Arabidopsis, showed the clearest a priori functional relevance to drought-responsive grain-number determination and was prioritised as the most plausible causal candidate; its expression in publicly available wheat spike expression atlases was highest during the floret-abortion-sensitive window around anthesis,

consistent with a plausible role in drought-induced floret abortion. Table 2 lists the eleven candidate genes within the chromosome 4D interval.

4. DISCUSSION

This study's dual-regime design, testing each significant association for drought-specificity against the same panel's well-watered phenotype rather than reporting drought-treatment associations in isolation, is, we think, central to how its findings should be interpreted. Fourteen of 23 genome-wide-significant associations met our drought-specificity criterion, meaning that just under two-fifths of associations that would have appeared as straightforward 'drought QTL' in a single-regime study instead reflect loci with detectable effects regardless of water status, a distinction with direct practical consequences for breeders deciding whether a marker is useful specifically for drought-resilience selection or more generally for yield improvement across water regimes [14,15].

The chromosome 2B and 6A loci, jointly explaining 18.3% of drought-stressed yield variance and co-localising with previously reported stay-green and canopy-temperature-depression QTL respectively, replicate a pattern reported across several independent wheat GWAS and biparental populations spanning different genetic backgrounds and environments, which strengthens confidence that these represent genuine, broadly transferable drought-yield loci rather than population-specific artefacts [7,16,17]. The mechanistic link implied by the 6A locus's joint association with both grain yield and canopy temperature depression in our panel is consistent with the broader physiological literature connecting stomatal regulation and canopy water status during grain filling to yield maintenance under terminal drought, and supports canopy temperature depression as a plausible, field-deployable proxy trait for marker-assisted or even direct phenotypic selection at this locus in breeding programmes without full genotyping infrastructure [18,19].

The novel chromosome 4D association with grain number per spike is, from a discovery standpoint, the more interesting finding, precisely because it did not correspond to any previously reported interval in our comparison literature search. Grain number determination around anthesis is known to be acutely sensitive to water deficit through floret abortion, and the candidate AP2/ERF transcription factor's anthesis-window expression peak in wheat spike tissue is consistent with, though not proof of, a causal role in this process [20,21]. AP2/ERF family transcription factors have well-established roles in ABA-mediated stress signalling across model and crop species, including several characterised drought-response regulators in rice and maize, which provides reasonable a priori plausibility for the candidate but falls well short of functional validation; confirming causality would require an independent approach such as CRISPR-based

knockout or TILLING-derived mutant analysis in a wheat background, which is beyond the scope of the present association study [22,23].

A further consideration is why this locus has not, to our knowledge, been reported in prior wheat drought GWAS literature despite the panel's diverse European germplasm not being obviously unusual. Two non-mutually-exclusive explanations seem plausible: the allele frequency of the favourable haplotype may be low or geographically restricted in previously studied panels drawn predominantly from different breeding programmes, reducing statistical power to detect the association elsewhere even if the locus is segregating; alternatively, grain number per spike specifically under post-anthesis, rather than pre-anthesis or continuous, drought stress may be an under-phenotyped trait combination in the existing wheat GWAS literature relative to grain yield or thousand-kernel weight, which are more commonly reported endpoints [24,25]. Either explanation, if correct, would argue for broader multi-panel validation of this locus before it is incorporated into marker-assisted selection pipelines.

Three limitations should temper how the present findings are applied in breeding practice. First, all phenotyping was conducted at a single field site across three seasons, and while multi-season replication strengthens confidence relative to a single-season study, site-specific soil, microclimate and rain-out shelter artefacts cannot be fully excluded as contributors to the observed associations without independent multi-site replication. Second, the 25K SNP array, while considerably higher density than early wheat GWAS platforms, still leaves appreciable gaps in marker coverage across the wheat genome's low-recombination pericentromeric regions, meaning some true associations in these regions may have been missed entirely. Third, the chromosome 4D candidate gene identification rests on functional annotation and expression pattern evidence rather than direct experimental validation, and should be treated as a well-motivated hypothesis for follow-up rather than an established causal mechanism. A further, more general limitation applies to association studies of this kind regardless of panel size: even a well-powered GWAS detects loci of individually modest effect against a polygenic background, and the 23 associations reported here, however robust individually, collectively explain only a fraction of total heritable variance in drought-stressed yield, consistent with the broader recognition in the quantitative genetics literature that complex agronomic traits are typically influenced by many loci of small effect that individual GWAS panels, however well designed, are not powered to detect exhaustively.

5. CONCLUSION

Genome-wide association analysis of grain yield and yield-component traits across a 287-accession European spring bread wheat panel, phenotyped under matched

drought-stress and well-watered regimes across three seasons, identified 23 genome-wide-significant marker-trait associations under drought stress, of which 14 met a pre-specified drought-specificity criterion against the well-watered control data from the same panel. Two loci on chromosomes 2B and 6A replicated previously reported stay-green and canopy-temperature-depression QTL and jointly explained 18.3% of drought-stressed grain yield variance, while a novel chromosome 4D locus associated with grain number per spike under drought, not previously reported in the wheat drought GWAS literature, narrowed to a plausible AP2/ERF transcription factor candidate gene. Together these findings support both continued use of the chromosome 2B and 6A regions, and canopy temperature depression as a field-deployable proxy trait, in marker-assisted drought-resilience breeding, while flagging the chromosome 4D locus as a priority for independent multi-panel validation and functional follow-up before practical deployment. We are now genotyping an independent validation panel of 150 additional European wheat accessions to test replication of the chromosome 4D association, and CRISPR-based knockout of the candidate AP2/ERF transcription factor in a transformable spring wheat background is underway to test its causal role in drought-induced floret abortion directly. A parallel multi-site field trial spanning three additional European locations is also planned to determine whether the chromosome 2B, 6A and 4D associations replicate consistently across a wider range of soil and climatic conditions than the single-site design used here could address.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

M.P. conceived and supervised the study, performed association analysis and candidate gene identification, and drafted the manuscript. E.L. coordinated the field trial, phenotyping and irrigation management across all three seasons. N.G. performed genotyping quality control and

population structure analysis. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study involved only cultivated bread wheat (*Triticum aestivum* L.) plant material and field agronomic trials; it did not involve human participants, human data, or vertebrate or higher invertebrate animals, and therefore did not require institutional ethics committee review.

Data Availability

Genotyping data (25K SNP array) and best linear unbiased trait estimates for the 287-accession panel are available from the corresponding author on reasonable request. Association analysis scripts (GAPIT-based R pipeline) are available from the corresponding author upon request.

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