

Genome-wide association studies identify putative pleiotropic locus mediating drought tolerance in sorghum

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Abstract

Drought is a key constraint on plant productivity and threat to food security. Sorghum (*Sorghum bicolor* L. Moench), a global staple food and forage crop, is among the most drought-adapted cereal crops, but its adaptation is not yet well understood. This study aims to better understand the genetic basis of preflowering drought in sorghum and identify loci underlying variation in water use and yield components under drought. A panel of 219 diverse sorghum from West Africa was phenotyped for yield components and water use in an outdoor large-tube lysimeter system under well-watered (WW) versus a preflowering drought water-stressed (WS) treatment. The experimental system was validated based on characteristic drought response in international drought tolerant check genotypes and genome-wide association studies (GWAS) that mapped the major height locus at *QHT7.1* and *Dw3*. GWAS further identified marker trait associations (MTAs) for drought-related traits (plant height, flowering time, forage biomass, grain weight, water use) that each explained 7–70% of phenotypic variance. Most MTAs for drought-related traits correspond to loci not previously reported, but some MTA for forage biomass and grain weight under WS co-localized with staygreen post-flowering drought tolerance loci (*Stg3a* and *Stg4*). A globally common allele at S7_50055849 is associated with several yield components under drought, suggesting that it tags a major pleiotropic variant controlling assimilate partitioning to grain versus vegetative biomass. The GWAS revealed oligogenic variants for drought tolerance in sorghum landraces, which could be used as trait predictive markers for improved drought adaptation.

KEY WORDS

abiotic stress, adaptation, crop, QTL mapping, transpiration

1 | INTRODUCTION

Drought is the most common abiotic stressor limiting plant productivity globally (Barb   & Lebel, 1997). Plants species may use a variety of drought adaptive mechanisms such as escape mechanisms (e.g., early flowering to the life cycle), avoidance mechanisms (e.g., reduced plant size or transpiration), or tolerance mechanisms (e.g., reactive oxygen

detoxification or osmotic adjustment) (Blum, 2004; Juenger, 2013). Still, for most plant species, the genetic mechanisms underlying variation for drought adaptation in the field is not known—deciphering the mechanisms of drought adaptation remains a major challenge in plant biology and breeding (Mickelbart et al., 2015; Tardieu et al., 2018; Tuberrosa, 2012). The challenge is due in part to the fundamental complexity of drought adaptation, due to pleiotropy, epistasis, and

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genotype-environment interactions (Juenger, 2013; Tardieu et al., 2018), as well as the practical difficulty of studying drought stress in field conditions given year-to-year variation in rainfall (Atlin et al., 2017; Cooper et al., 2014). Genome-wide association studies in controlled environment or managed drought stress have recently been used to address this challenge and investigate the genetic variation in drought tolerance across diverse plant germplasm (Faye, Akata, et al., 2021; Guo et al., 2018; Spindel et al., 2018; Varshney et al., 2014; Wang et al., 2016).

Semiarid regions have experienced prolonged periods of drought that have led to severe crop yield losses in smallholder farming systems while shifts in precipitation patterns were observed in recent years (Burke et al., 2009). In future climates, the frequency of extreme events such as drought and floods are expected to increase in many regions (Biasutti, 2019). This is especially true in the Sahelian and Soudanian zones of Africa, large bands of semiarid grassland and savannah, respectively, where severe drought is common (Barbé & Lebel, 1997). In smallholder farming systems in the Sahel, recurrent droughts can occur throughout the growing season and limit crop production, with 10–50% yield reductions observed (Sultan et al., 2013; Traoré et al., 2011). Drought stress in the Sahel could be moderate or severe (short/intermittent and long periods of water deficit, respectively) throughout the growing season. Given such climatic variability, developing plant varieties with drought tolerance, particularly in terms of yield stability, becomes among the major priorities in breeding programs (Rapsomanikis, 2015; Walker & Alwang, 2015).

Sorghum (*Sorghum bicolor* L. Moench) is a C4 cereal crop adapted to different environmental conditions, including drought-prone areas where many other staple crops fail (National Research Council, 1996; Smith & Frederiksen, 2000). In West Africa, where dual-purpose sorghum (grain and forage) landraces are a key food security crop, the species exhibits abundant diversity across agroclimatic zones and botanical types (caudatum, durra, guinea, bicolor, and intermediates) (Bhosale et al., 2011; Faye, Maina, et al., 2021). One physiological mechanism that could contribute to drought adaptation in African landraces is staygreen post-flowering drought tolerance. During post-flowering drought, staygreen genotypes maintain their photosynthetic activity more than senescent lines due to water-saving growth and development dynamics at earlier stages (Borrell, Mullet, et al., 2014; Borrell, Oosterom, et al., 2014). Four major stay-green quantitative trait loci (QTLs) were reported (Stg1–4), which were obtained from an Ethiopian sorghum but may have a broader distribution in Africa (Borrell, Oosterom, et al., 2014; Faye, Akata, et al., 2021; Harris et al., 2007). For preflowering drought tolerance, a few loci have been mapped, but the underlying mechanisms have not been characterized (Tuinstra et al., 1996; Upadhyaya et al., 2017). Despite sorghum's reputation for exceptional drought tolerance among staple crops, mechanisms underlying drought adaptation in sorghum landraces are not well understood (Upadhyaya et al., 2017).

To better understand the genetic basis of drought adaptation in sorghum landraces, we conducted a GWAS of the drought response

of diverse germplasm under managed stress in an outdoor lysimetry system. Given the differential adaptation of sorghum to semiarid versus subhumid zones in West Africa (Craufurd et al., 1999), we hypothesized that this germplasm harbors major variants (oligogenic variation) for drought resilience traits. Based on studies of drought tolerance mechanisms in sorghum and other plants (Barnabás et al., 2008; Borrell, Oosterom, et al., 2014), we also hypothesized that variation in plant development and water-use dynamics underlies variation in drought tolerance. Our goals in this study were (i) to test these overarching hypotheses on the genetic basis of drought adaptation in sorghum landraces and (ii) to generate specific hypotheses on the genetic loci involved. Our findings suggest that major variants for drought tolerance exist in African sorghum landraces and that some affect multiple drought-related traits pleiotropically.

2 | MATERIAL AND METHODS

2.1 | Plant materials

The West African sorghum association panel (WASAP), consisting of landraces and breeding lines, was previously assembled from sorghum breeding programs in four countries (Mali, Niger, Senegal, and Togo), genotyped, and phenotyped for agro-morphological traits (Faye, Maina, et al., 2021). In this study, we used a subset of 219 genotypes from the WASAP, hereafter referred to as WASAP_Lysi (Table S1; Data S1). WASAP_Lysi is a curated subset of the WASAP, selected based on the genotypic data to capture most of the genetic diversity existing in West Africa (Figures 1a and S1) and based on phenotype data (Faye, Maina, et al., 2021) to remove highly photoperiod sensitive genotypes that would confound drought phenotyping under common-garden conditions (Tuberosa, 2012). In addition, two breeding lines that have been widely used for drought tolerance studies in the United States and Australia were included as checks for preflowering drought tolerance (Tx7000) and postflowering drought tolerance (BTx642) (Borrell, Mullet, et al., 2014; Borrell, Oosterom, et al., 2014; Harris et al., 2007; Tuinstra et al., 1996). Along with the WASAP_Lysi, genotypic information of BTx642 and Tx7000 were used (Figure S1C).

2.2 | Genomic diversity analyses

Genotypic data used were generated using the genotyping-by-sequencing (GBS) method with the *ApeKI* restriction enzyme (Faye, Maina, et al., 2021; Hu et al., 2019). To characterize the genetic diversity of WASAP_Lysi relative to the global sorghum diversity (sorghum association panel; SAP) (Figure 1a) and the West African sorghum diversity (as represented in USDA-GRIN genebank; WAGRIN) (Figure S1), we analyzed sequence reads obtained from previous studies (Faye et al., 2019; Lasky et al., 2015; Maina et al., 2018; Morris et al., 2013; Olatoye et al., 2018). The reference genome BTx623 version 3.1 (McCormick et al., 2018; Paterson et al., 2009)

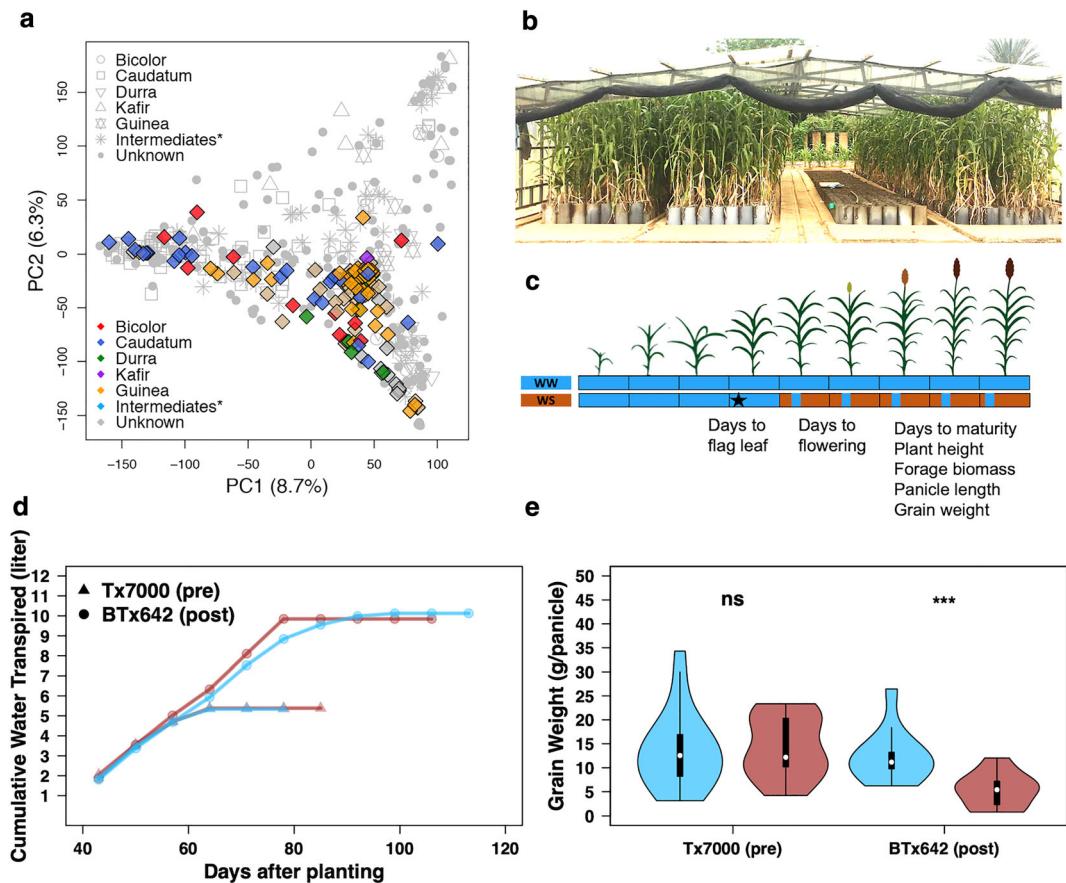


FIGURE 1 Experimental system for managed drought phenotyping of diverse sorghum. (a) Principal coordinate analysis (PCA) of WASAP_Lysi compared with a global sorghum association panel (SAP) with botanical type noted. (b) Lysimeter facility at ICRISAT Sadoré-Niger, with plants grown in large tubes ($1.30\text{ m} \times 0.25\text{ m}$) during the normal cropping season under a retractable rain-out shelter. (c) Schematic representation of well-watered (WW, blue) and intermittent preflowering water-stressed (WS, brown) treatments, with phenotype collected listed below. To account for phenology differences among the genotypes, WS was imposed from flag leaf appearance for each individual at different times (represented by the star). (d) Cumulative water use for Tx7000 and BTx642 and (e) grain weight (g/panicle) in WW and WS treatments for international drought tolerance check lines, Tx7000 (preflowering drought tolerance check; “pre”) and BTx642 (postflowering drought tolerance; “post”)

was used to align sequencing data using the Burrows–Wheeler alignment (Li & Durbin, 2010), and the SNPs were discovered using TASSEL 5 GBS pipeline (Glaubitz et al., 2014). Missing SNPs were imputed in Beagle v1.4 (Browning & Browning, 2009). SNPs were filtered at MAF > .01 and only biallelic sites were kept. A total of 219 WASAP_Lysi genotypes, 1527 West African genotypes, and 342 SAP genotypes were further analyzed (Figure 1a). To characterize the WASAP_Lysi genetic diversity relative to the SAP and the WAGRIN, principal component analysis (PCA) was performed in R using the SNPRelate package (Zheng et al., 2012). First, the variance components of each genotype in the WASAP_Lysi using the training set from SAP with ~90,000 biallelic SNPs present in both collections (WASAP_Lysi and SAP) was predicted. Next, the variance components of WASAP_Lysi genotypes were predicted using WAGRIN as a training set with ~138,000 biallelic SNPs present in both collections (WASAP_Lysi and WAGRIN).

2.3 | Lysimeter phenotyping system

Experiments were conducted in a lysimeter system at the ICRISAT Sahelian Center, Niger (Sadoré, 13.15°N , 2.18°E) during the normal cropping season from June to November, in 2017 and 2018. The rainy season at this location is June to October. The lysimeter system, equipped with a rainout shelter, consists of evaluating the physiological characteristics of crops under managed experiments in large PVC tubes (Falalou et al., 2018; Vadez et al., 2008, 2013). Tubes of 1.30 m in height and 0.25 m in diameter were placed in trenches (Halilou et al., 2015) and filled with field soil from a farm in Sadoré station (Figure 1b). Each tube was fertilized with 6 g of $15-15-15$ ($\text{N-P}_2\text{O}_5-\text{K}_2\text{O}$) following a recommended standard fertilization (Ministère de l’agriculture du Niger, 2012). The soil surface was covered with transparent plastic bags and 350 g of polyethylene beads in each lysimeter tube to limit soil evaporation.

2.4 | Water treatments

To control water treatments for differences in plant size and phenology, watering was applied to each plant individually based on their water use and flag leaf appearance (Figure 1c). The experiment was conducted in a split-unit design with two treatments, well-watered (WW) and water-stressed (WS), and with three replicates. The experimental unit was each lysimeter tube. Three seeds were planted in each tube, and two plants were left in the tube a week after emergence. Two weeks after planting, one individual plant in each tube was kept for phenotyping. The two genotypes with known drought stress response, Tx7000 and BTx642 were highly replicated in both treatments ($n = 14$) during the second year of the experiment (Borrell, Mullet, et al., 2014). In the well-watered treatment (WW), genotypes were irrigated at 80% field capacity until physiological maturity of the genotype. In WS treatment, genotypes were irrigated at 80% field capacity, as in the WW, until fully flag leaf appearance for each genotype, then intermittent drought stress was applied (Figure 1c). WS treatment consisted of skipping irrigation upon the observation of a wilting point on the last three leaves before relieving the water stress with 1 L of water.

2.5 | Trait phenotyping

Plant height was measured from the base of the stem to the tip of the last fully emerged leaf each week. Days to flowering was defined as the number of days until anther appearance on each plant. Yield components include final dry forage biomass, on a per plant basis (vegetative biomass in g/plant), and grain weight (manual threshing) on per panicle basis (g/panicle). The weights are dry weights based on air drying during the dry season in the arid climate. Beginning 6 weeks after planting, lysimetric tubes (one individual plant in each tube) were weighed weekly using a hanging scale (Mettler Toledo; 20 g accuracy) to estimate weekly water use on a per plant basis. Total water transpired after weighing was estimated weekly as the total water added until physiological maturity of each lysimeter tube. Cumulative water use was calculated as the total water used at each time point starting from the first weighing.

2.6 | Statistical analysis of phenotypes

Statistical analyses were performed in the R program (R Core Team, 2018). To estimate the variance components across years within a treatment, the *lme4* package was used (Bates et al., 2015):

$$\text{lmer}(\text{Trait} = 1 + Y + (1|G) + (1|G:Y:B) + (1|G:B) + (1|G:Y)),$$

where G is genotype, Y is year, and B is replicate block. Year is treated as a fixed effect.

Best linear unbiased predictions (BLUPs) were then estimated for genome-wide association studies. Broad-sense heritability across years within the same treatment was estimated as

$$H^2 = \frac{\sigma^2_G}{\sigma^2_G + \left(\frac{\sigma^2_{G:Y}}{n_R} \right) + \left(\frac{\sigma^2_e}{n_R \times n_Y} \right)},$$

where σ^2_G is the genotypic variance, σ^2_e is the residual variance, n_Y is the number of years, n_R is the number of replicate blocks, and $\sigma^2_{G:Y}$ is the genotype-by-year interaction.

Analysis of variance (ANOVA) was performed to assess whether the sources of variation were significant for the evaluated traits between treatments for each country and botanical type on a mean basis.

Drought response for all traits was calculated as the difference between well-watered and water-stressed treatments on a mean basis for the 2 years. The broad-sense heritability was estimated using the estimate of genetic variance (σ^2_G) and the residual variance (σ^2_e).

2.7 | Genome-wide association studies

To identify marker-trait associations, we used 73,730 genome-wide SNP markers in WASAP_Lysi after filtering for minor allele frequency (MAF = .05). Next, we conducted GWAS for yield components in both treatments separately for WW, WS, and the difference between WW and WS. BLUPs were estimated with the linear model above mentioned and were carried out GWAS using the general linear model (GLM), mixed linear model (MLM), and multilocus mixed linear model (MLMM) (Lipka et al., 2012; Segura et al., 2012) for plant height, days to flowering, forage biomass, and grain weight for WW and WS treatment across 2 years. We used the Bonferroni correction threshold (conservative threshold, $\alpha = .05$, p value $< 10^{-7}$) to identify significant markers associated with the phenotypes. To further identify outlier SNPs (marker-trait associations, MTAs) associated with each trait, the top 0.01% lowest p values in the MLMM model were selected.

To determine whether MTAs co-localized with previously identified and putative genes, a list of *a priori* candidate genes was generated from a literature review (File S2). This list ($n = 67$) of candidate genes includes their genomic positions in sorghum and orthologs of rice and maize for plant height, maturity, grain yield, biomass, and drought tolerance. The sorghum QTL Atlas was also used to determine the co-localization of MTAs with previously identified QTLs from the literature (Mace et al., 2018). Putative orthologs were identified based on the highest similarity in the “Protein Homologs” in Phytozome 13 and expression data was from GeneAtlas v1 Tissue Sample in Phytozome 13 (Goodstein et al., 2012). Geographic distribution of alleles was based on existing GBS resources for georeferenced sorghum landraces (Hu et al., 2019; Lasky et al., 2015).



3 | RESULTS

3.1 | Genetic structure of the diversity panel

To first characterize the genetic structure of our experimental panel (WASAP_Lysi) with respect to global sorghum diversity, we analyzed 90,148 genome-wide SNPs. Taking the SAP as representative of the global diversity, the first principal and second principal component explained 8.7% and 6.3% of the total variation, respectively (Figure 1a). When the WASAP_Lysi was projected on the principal components (PCs) of SAP (Figure 1a), the WASAP_Lysi genotypes clustered with the caudatum, durra, and guinea types in SAP on the first two PCs. To characterize the genetic structure of WASAP_Lysi with respect to West African sorghum diversity, we conducted PCA on 138,000 genome-wide SNPs. Consistent with our observations in the SAP, PC1, PC2, and PC3 distinguish major groups of caudatum, durra-caudatum, and guinea respectively (Figure S1). Since the majority of WASAP_Lysi have not been classified into botanical types morphologically ($n = 72$), we used genotyping data to classify accessions into botanical types. We inferred botanical types from admixture ($K = 3$, cross-validation error = .62) using membership probability $>.6$ to assign WASAP_Lysi genotype to a botanical type for further analyses (Figure 1a, Data S1). After the botanical type assignment combining major groups on PCA and admixture, 12, 53, and seven genotypes were assigned into the caudatum, durra-caudatum, guinea botanical types, respectively (Data S1).

3.2 | Phenotypic variation and drought response

To validate the quality of the managed drought lysimeter phenotyping, we first characterized drought response phenotypes for two international drought tolerance checks, Tx7000, the preflowering drought tolerance check, and BTx642, the preflowering drought susceptible check. Tx7000 had less water transpired compared with BTx642 in both treatments (Figure 1d). There were no significant differences in total water transpired for either BTx642 and Tx7000 between treatments ($p = .6$ and $p = .9$, respectively; Figures 1d and S2B). There was a substantial reduction in both average grain weight

(57%, $p < .001$) and harvest index (55%, $p < .001$) under WS versus WW for BTx642, but not Tx7000 (Figures 1e and S2G–H; Table S2). The maximum water use in both genotypes was reached 2 weeks after flowering. In both genotypes, the drought response on flowering time, plant height, and forage biomass was negligible (Figure S2). Days to flowering was slightly delayed in the WS treatment for Tx7000 (+3 days; $p < .08$) but not for BTx642 ($p < .2$) (Figure S2D). The performance of the checks was similar in both years (Figures S3 and S4).

Next, we characterized phenotypic variation under managed stress across the WASAP_Lysi diversity panel. Overall, a significant genotype effect was observed for all morphological and phenological traits, in both WW and WS treatments ($p < .05$; Table 1; Figures 2 and S4). The phenotypes (plant height, days to flowering, and forage biomass) followed approximately a normal distribution in both treatments (Figure S4). Under both WW and WS, broad-sense heritability estimates were high (.75–.97), with the highest heritability for days to flowering in WW (Table 1). The heritabilities of the drought response (WW-WS) of forage biomass and grain weight were high (.86 and .50, respectively), while the heritabilities of drought response of plant height and days to flowering were low (.05 and .09, respectively) (Table 1). Overall, there was 35% reduction in grain yield under WS versus WW treatment (8.1 g/plant vs. 12.6 g/plant, respectively), demonstrating that substantial stress was experienced under WS treatment. Further, within each botanical type and country of origin, a significant reduction of grain weight was observed in WS compared with WW, as would be expected (Figure 2j; Tables S3 and S4). Across three major botanical types, yield reduction under WS versus WW ranged from 21% to 27%, while across four countries of origin the yield reduction ranged from 19% to 31%.

Water use in the WASAP_Lysi showed significant genotypic variation ($p < 10^{-15}$). In all of the botanical types, cumulative water use was reduced significantly ($p < .01$) under WS versus WW treatment (Figure 3a,b). On average, caudatum types had less cumulative water use compared with durra-caudatum and guinea types in both treatments (Figure 3a,b). The majority (80%) of the genotypes showed a reduction of cumulative water use in WS compared with WW, as much as 15 L in some genotypes (Figure 3c). Heritability of water use was moderate (.4–.6) in the first 5 weeks and high thereafter (up to ~.8 by physiological maturity) under both WW and WS treatments (Figure 3d).

TABLE 1 Means, variance components, and heritability for BLUPs of five traits under well-watered and water-stressed treatment and their drought response

Trait ^a	Well-watered (WW)					Water-stressed (WS)					Response (WW-WS)			
	Mean	SD	G	GxY	H ²	Mean	SD	G	GxY	H ²	Mean	SD	G	H ²
PH	255	46	48***	25	.96	252	45	49***	8***	.96	2	22.5	0	.05
DF	91	23	24***	1	.96	92	4	25***	3	.97	-1	9	40	.09
FB	105	22	27***	16***	.77	92	23	26***	14***	.83	12	14.5	12***	.86
GW	12.6	6	55***	17*	.75	8	5	40***	7*	.79	4.5	3	6.7***	.50

Note: SD: standard deviation; G: Genotype; GxY: Genotype-by-Year interaction; H²: Broad-sense heritability. Significance at $p < .05$, .01, and .001 for two-way ANOVA is noted with *, **, and ***, respectively. G and GxY terms without asterisk did not pass the significance threshold.

^aTraits: PH: Plant height (cm); FB: Forage biomass (g); DF: Days to flowering (days); GW: Grain weight per panicle (g).

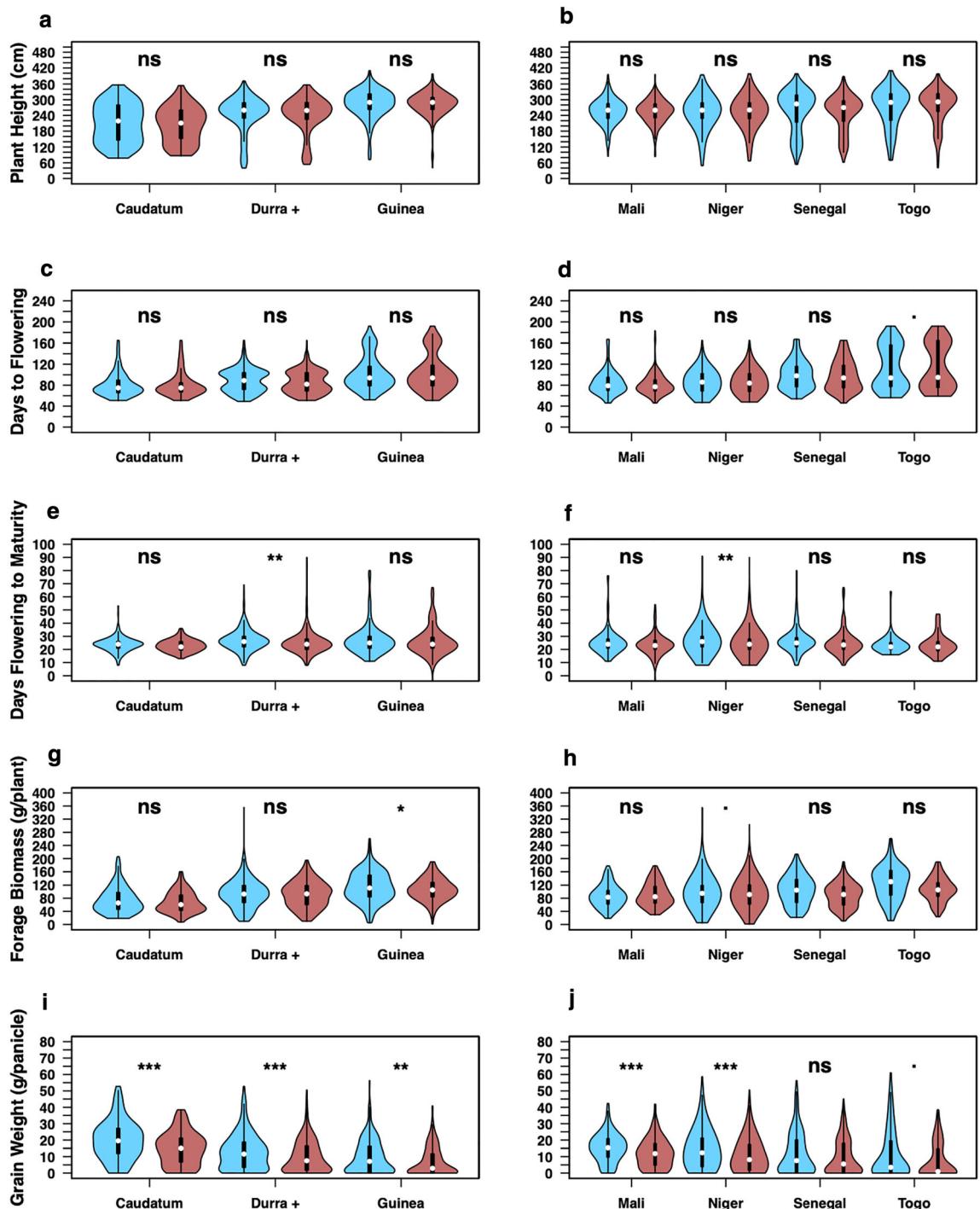


FIGURE 2 Drought response phenotypes by botanical types and country of origin. Plant height (a,b); days to flowering (c,d); days flowering to maturity (e,f); forage biomass (g,h); grain weight (i,j). Botanical types according to genotypic classification: Caudatum ($n = 28$), durra+ (durra and durra-caudatum) ($n = 74$), Guinea ($n = 50$). Country of origin: Mali ($n = 31$), Niger ($n = 116$), Senegal ($n = 37$), Togo ($n = 28$). Asterix *, **, and *** represent significance at $p < .05$, $.01$, and $.001$, respectively; ns: not significant

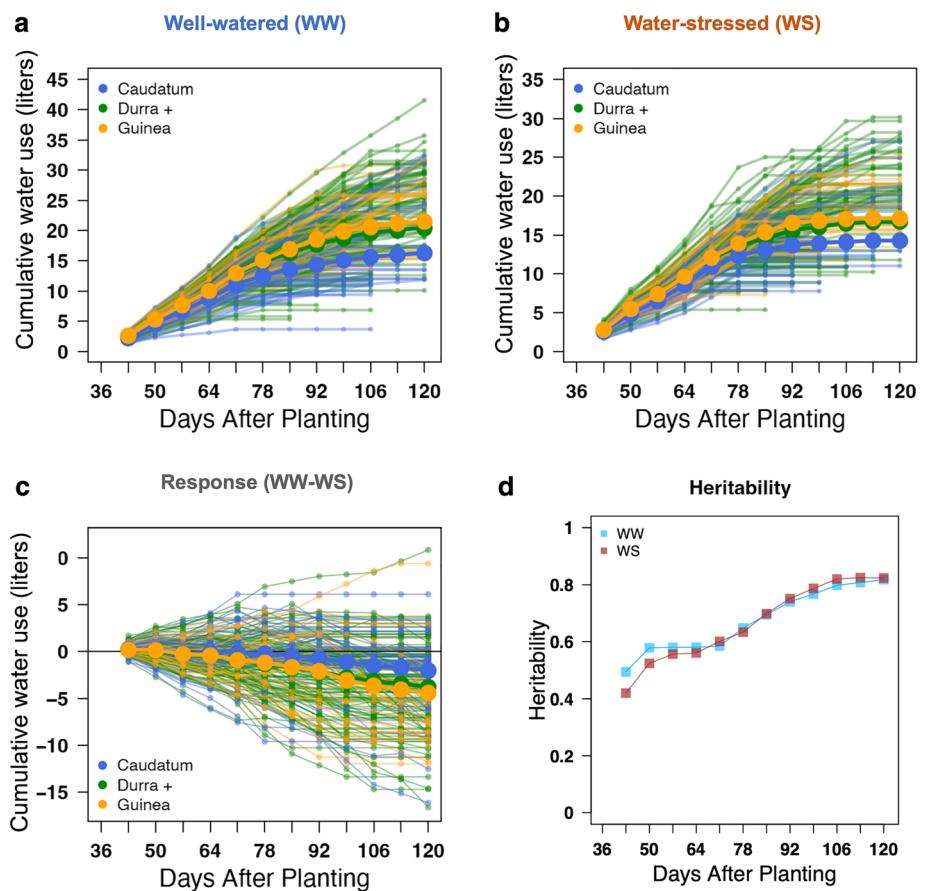
3.3 | Marker-trait associations for plant height and flowering time and their drought response

To validate our experimental system for GWAS, we first identified MTAs for plant height and flowering time for each year individually

using a standard approach of GLM and MLM GWAS (Figures S5 and S6). MTAs for plant height on chromosomes 4, 6, 7, and 8 explained between 11% and 25% of the total phenotypic variance for the 2017 data. Among the associations, S7_59412395 (MAF = .05) explained 19% of the variation and co-localized with the classical dwarfing gene



FIGURE 3 Genetic variation for water use under drought, by botanical type and genotype. Cumulative water use in well-watered (WW; a) and water-stressed (WS; b) treatments, and drought response for water use (WS-WW) (c). Genotypes were color-coded by botanical type. Averages of each botanical type are represented in thicker lines color-coded by type, with large solid circles representing the mean for each botanical type. (d) Broad-sense heritability estimates for cumulative water use by time point under WW (blue) and WS (brown) treatments



Dw3 (~0.4 Mb) (Figure S5). In 2018 data, 19 MTAs were identified on chromosomes 6, 7, and 10 (Figure S5). The phenotypic variation explained by the MTAs ranged from 12% to 23%. With MLM, 10 MTAs on chromosomes 7 and 10 in both years explained 12–19% of variation. For flowering time, three MTAs were identified via GLM on chromosome 6 (S6_50696803; MAF = .13, S6_50716126; MAF = .16, and S6_50716244; MAF = .16) for 2017 data, whereas no significant MTA were identified in 2018 data (Figure S6). These flowering time MTAs did not co-localize with the a priori candidate genes for maturity on chromosome 6 (*Ma6*, *Ma1*, *SbCN4*, *abph1*, *zf1*; Data S2).

To further validate the experiment and gain insights on these drought-related traits, we conducted MLMM GWAS on plant height and flowering time using across-year BLUPs, for each treatment (Figure 4a–f). Note, MLMM are designed to identify individual MTA at a QTL, rather than broad association peaks of multiple MTA typical of GLM and MLM. Under WW treatment, a few significant associations were identified, two for plant height and one for days to flowering, respectively (Figure 4a,d). For plant height, the top association was S7_59412395 ($p < 10^{-20}$; MAF = .05), roughly co-localized with the classical dwarfing gene Dw3 (~0.4 Mb) and closely co-localized with the top MLMM MTA from a field-based GWAS of the WASAP in Senegal (S7_59400476; 11 kb away) (Faye, Maina, et al., 2021). The second most significant MTA was S6_2900751 ($p < 10^{-7}$; MAF = .09,

which is within the QTL interval QHGBT6.4 (2.37–3.6 Mb) (Takai et al., 2012). The proportion of the phenotypic variance explained (PVE) by the MTAs is 17% (Table 2). For flowering time, S4_64398335 (MAF = .07; $p < 10^{-9}$; 46% PVE) was found within the QTL region QDTFL4.25 (61.02–65.08 Mb) (Mocoeur et al., 2015). Under WS treatment, there were eight MTAs across all traits (Figure 4b,e). Three MTAs for plant height were observed, with the top association at S7_59459123 near Dw3 (MAF = .06), along with associations at S7_50055849 (MAF = .14) and S9_56534065 (MAF = .07). The latter MTA overlaps with QHGBT9.45 (56.1–57.8 Mb) for plant height (Figure 4b) (Felderhoff et al., 2012). There were two MTAs for days to flowering under WS (Figure 4e), on chromosome 3 (S3_19589652; MAF = .13) and chromosome 4 (S4_14172212; MAF = .11). The majority of Malian and Togolese genotypes are later flowering, associated with the minor allele (C) at S4_64398335. Togolese genotypes ($n = 18$) with the G allele at S4_14172212 flower later under WS treatment.

To identify loci that may underlie variation in drought response for flowering time and height, we conducted MLMM on the difference between WW and WS (WW-WS). One MTA for drought response of plant height was identified above the conservative threshold at S4_149131 (MAF = .04) (Figure 4c). Another flowering time MTA was found above the conservative threshold on chromosome 5 (S5_52090779; MAF = .07; $p < 10^{-7}$; 70% of the

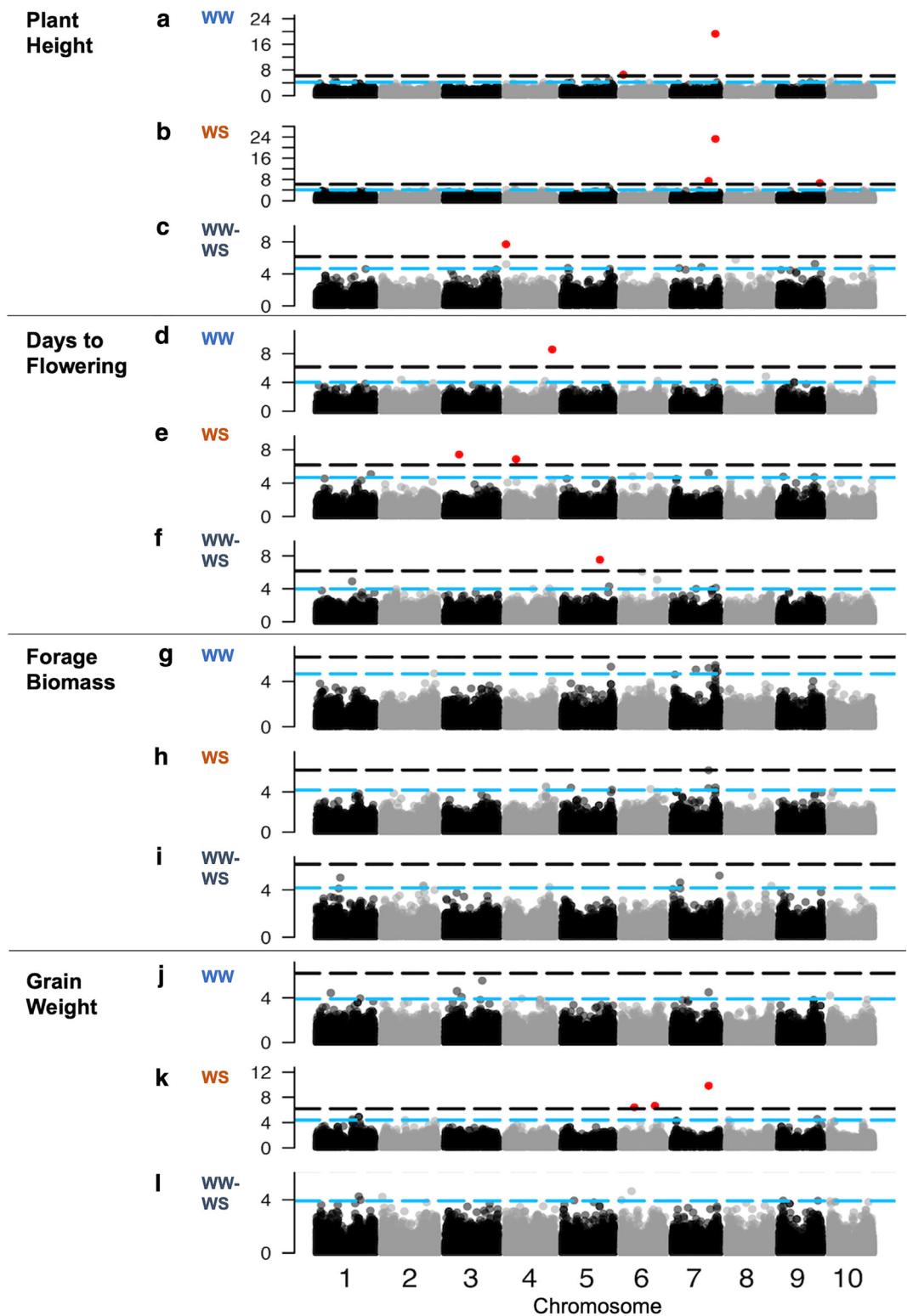


FIGURE 4 Genome-wide association study for plant traits and their drought response. Multilocus mixed model associations for traits in well-watered treatment (WW; a,d,g,j), water-stressed treatment (WS; b,e,h,k), and the drought response of the traits in WW versus WS treatments (WW-WS; c,f,i,l). Black dashed lines represent the conservative threshold of $\alpha = .05$ with a Bonferroni correction (with red points indicating marker-trait associations above the threshold), while the blue line represents 0.01% lowest p values

phenotypic variance explained) (Figure 4f, Table 2), which co-localized with QDTFL5.8 (9 Mb region) for days to flowering (Mace et al., 2013). Among the top 0.01%, MTAs for drought response

of flowering time (Data S3), S5_64932515 (MAF = .05, Figure 4f) co-localized with flowering time locus QDTFL5.10 (0.8 Mb) (Srinivas et al., 2009).

**TABLE 2** Marker-trait associations from MLMM above the Bonferroni threshold ($\alpha = .05$)

Trait	Treatment ^a	SNP	−log (p)	MAF ^b	Allele (mean phenotype)	PVE ^c (%)
Flowering	WW	S4_64398335	8.6	.07	G (88), C (145), S (77)	46
Flowering	WW-WS	S5_52090779	7.5	.07	T (−1), C (46), Y (0)	70
Flowering	WS	S3_19589652	7.4	.13	C (93), Y (106), T (94)	7
Flowering	WS	S4_14172212	6.9	.08	A (89), G (138), R (126)	39
Grain weight	WS	S7_50055849	9.8	.14	T (7), A (14), W (5)	27
Grain weight	WS	S6_46923493	6.7	.05	C (7.4), A (13), M (5.8)	12
Grain weight	WS	S6_18075344	6.4	.18	G (6.7), A (11), R (8)	33
Plant height	WS	S7_59459123	23	.06	T (263), A (140), W (292)	12
Plant height	WW	S7_59412395	19	.05	G (267), A (125), R (237)	48
Plant height	WW-WS	S4_149131	7.7	.04	G (3), C (−35), S (19)	23
Plant height	WS	S7_50055849	7.4	.14	T (269), A (209), W (261)	39
Plant height	WS	S9_56534065	6.6	.07	T (259), G (251), K (231)	6
Plant height	WW	S6_2900751	6.5	.09	G (265), A (206), R (234)	5

^aWW: well-watered; WS: water-stressed; WW-WS: drought response.

^bMinor allele frequency.

^cPercentage of phenotypic variance explained based on linear model.

3.4 | Marker-trait associations for forage and grain yield components and their drought response

Next, to identify loci that may underlie variation for yield under preflowering drought, we conducted MLMM for forage and grain yield components under WW and WS, and their drought response (Figure 4g–l). Under WW treatment, no associations for forage biomass and grain weight per panicle were above the conservative threshold (Figure 4g,j). To identify MTAs that fall below the conservative threshold but may still be of interest, we investigated MTAs with the slowest 0.01% *p* values for each trait (Data S3) and noted cases where they are co-localized with previously identified QTL. Under WW treatment, the top MTA for forage biomass was S7_59459123 (MAF = .06; *p* < 10⁻⁵), which was also a plant height MTA and co-localized with the Dw3 gene (290 kb away). Another MTA for forage biomass, S2_72712959 (MAF = .19; *p* < 10⁻⁵), co-localized with QTDBM2.4 (total dry biomass, 70–76 Mbp) (Mocoeur et al., 2015). For forage biomass under WS treatment (Figure 4h), the top MTA was at S7_50055849 and the fourth-highest was the same MTA (S7_59459123) observed under WW treatment (Data S3).

For the drought response of forage biomass, the top MTA is on chromosome 7 (S7_64933026; *p* < 10⁻⁵; Figure 4i). Note, this MTA is not co-localized with the plant height MTAs near Dw3 and QHT7.1. In addition, three MTAs were observed (S2_57620549, S2_57664163, and S2_57663973) spanning a 43 kb region within staygreen locus Stg3a (57.1–61.8 Mbp) (Figures 4i and S7; Data S3). The minor alleles associated with lower forage biomass under drought were at high frequency in the panel (MAF = .42, .38, and .39, respectively).

For grain weight per panicle under WS, three associations were found, two on chromosome 6 (S6_18075344, MAF = .18; S6_46923493, MAF = .05) and one on chromosome 7 (S7_50055849; MAF = .14) (Figure 4k). For the drought response of grain weight, an

MTA was identified S6_18075344, which was also identified under WS treatment (Figure 4l; Table 2). The average values for the genotypes with the G allele (mean = 4.9 g) at S6_18075344 (which included BTx642) were greater than the ones with the A allele (mean = 4.6 g) (which included Tx7000) (Table 2). Genotypes originated from Mali (*n* = 7), Niger (*n* = 21), and Senegal (*n* = 3) have the same preflowering drought tolerance-associated alleles as Tx7000. By contrast, at the second most associated marker (S6_46923493) (Figure 4l), the minor allele (A) associated with moderate reduction in grain weight under WS, is carried only by Nigerian genotypes (*n* = 12). One MTA for drought response of grain weight (S5_16199662; MAF = .09, *p* > 10⁻⁴) co-localized with Stg4 (Figure 4l). Genotypes with the non-reference allele at this marker (A, *n* = 31) had less of a reduction in grain weight under drought than genotypes with the reference allele (G, *n* = 153).

3.5 | Marker-trait associations for water use and its drought response

To identify loci that may underlie variation in water use dynamic and play a role in drought avoidance, the water use of each genotype under both WW and WS treatment was measured weekly over 12 weeks and MTAs were identified. Using MLMM for water use at each time point, a total of 62 MTAs were identified. Several MTAs appear at two or three consecutive time points (e.g., S2_63494925, S8_51257440, S8_570367, and S9_32820789) among the top 0.01% of the lowest *p*-values (Figure 5a). At 85 days after planting, a MTA was observed at S2_63675212 (MAF = .23), which co-localized with Stg3b (62.7–64.6 Mb).

For water use under WS treatment, 12 MTA were identified. While some of the MTAs were observed at a single time point, the

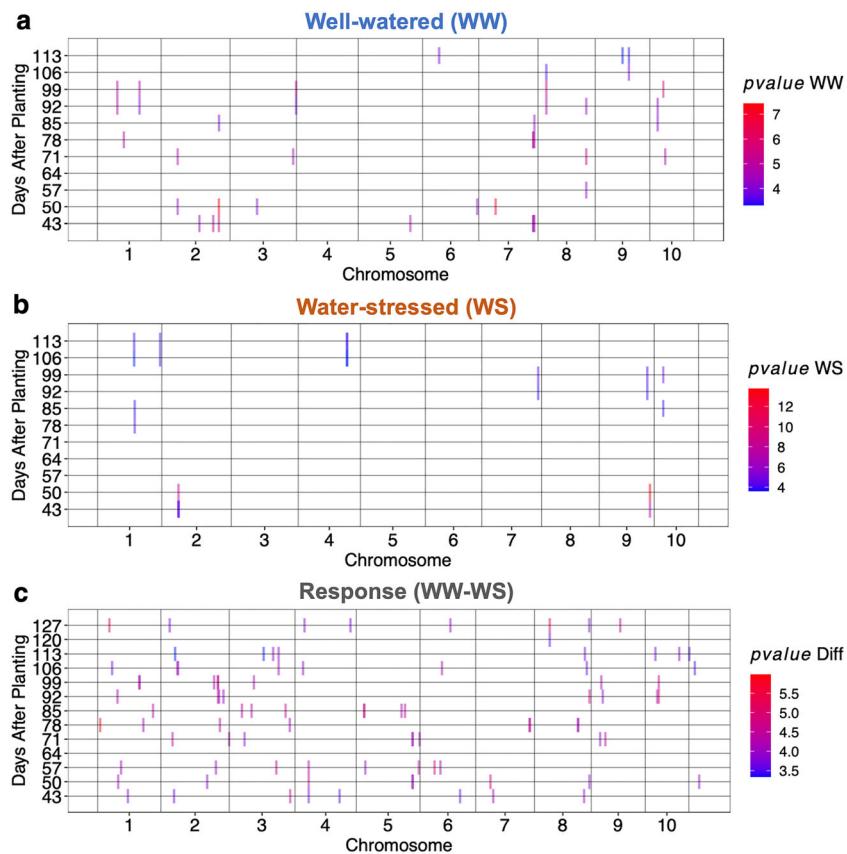


FIGURE 5 Genome-wide association study of water use and its drought response. Multilocus mixed linear model genome-wide association studies for weekly water use under (a) well-watered treatment, (b) water-stressed treatment, and (c) for difference between well-watered and water-stressed treatment. The x-axis represents the genomic position of each marker, and the y-axis represents the days after planting. Vertical bars (color-coded by $-\log$ of the p values) represent the genomic position of marker-trait associations at each of the 12 time points

majority of MTAs (84%) were shared across two or more consecutive time points (Figure 5b). The strongest association for water use under WS was on chromosome 9 ($S9_54114067$, $p < 10^{-14}$, MAF = .36) at 50 days after planting. For the drought response, a total of 89 MTAs among the top 0.01% of the lowest p -values were observed across time points (Figure 5c). Some drought response MTAs appear at two or three consecutive time points. For instance, the MTA at $S4_7506983$ is observed at consecutive time points 43, 50, and 57 days after planting. Another MTA at $S2_63345899$ ($p < 10^{-5}$) was found within the staygreen region $Stg3b$ (62.7–64.6 Mb) at two consecutive time points after 90 days.

3.6 | Putative pleiotropic QTL and their geographic distribution

To identify loci that may be pleiotropically influencing multiple drought-related traits, we cataloged associations that were shared across multiple traits (Figure 6a). Shared associations ($n = 5$) on chromosomes 4, 5, and 7 were observed for multiple traits. For instance, variation at $S7_59459123$ (MAF = .06) is associated with variation in plant height in both water treatments (Figure 6a). Most notably, allelic variation at $S7_50055849$ (MAF = .14) is associated with variation of several traits we characterized: grain weight and forage biomass under WS, plant height under WS, days to flowering under WS, forage biomass (WW, WS), and grain weight (WW, WS) (Figure 6b). The minor

allele (A) is associated with shorter stature, earlier flowering, less forage biomass, and greater grain weight per panicle ($p = .005$).

Within the WASAP_Lysi, accessions carrying the minor allele at $S7_50055849$ (A; MAF = .14) originated from Niger ($n = 21$), Mali ($n = 4$), and Senegal ($n = 2$), while none of the Togolese germplasm (mostly from the Guinean agroclimatic zone) carries the minor allele. To characterize the global origin and prevalence of the putative variant, we mapped the global distribution of alleles at $S7_50055849$ across georeferenced landraces (Figure 6c,d). The minor allele is common (21%) across Africa and in India (Figure 6c). Within West Africa, the minor allele is distributed at high frequency in southern Senegal and Sierra Leone compared with Niger, Nigeria, Mali, and Burkina Faso (Figure 6d).

4 | DISCUSSION

4.1 | Quantitative trait genomics to understand drought adaptation in sorghum

Quantitative trait genomic analyses of plants under managed drought stress could contribute to a better understanding of the genetic basis of drought tolerance (Lovell et al., 2016). Sorghum landraces harbor extensive diversity of drought response phenotypes (Haussmann et al., 1998; Lasky et al., 2015; Upadhyaya et al., 2017), but it is not known what aspects of the phenotypic response contribute to

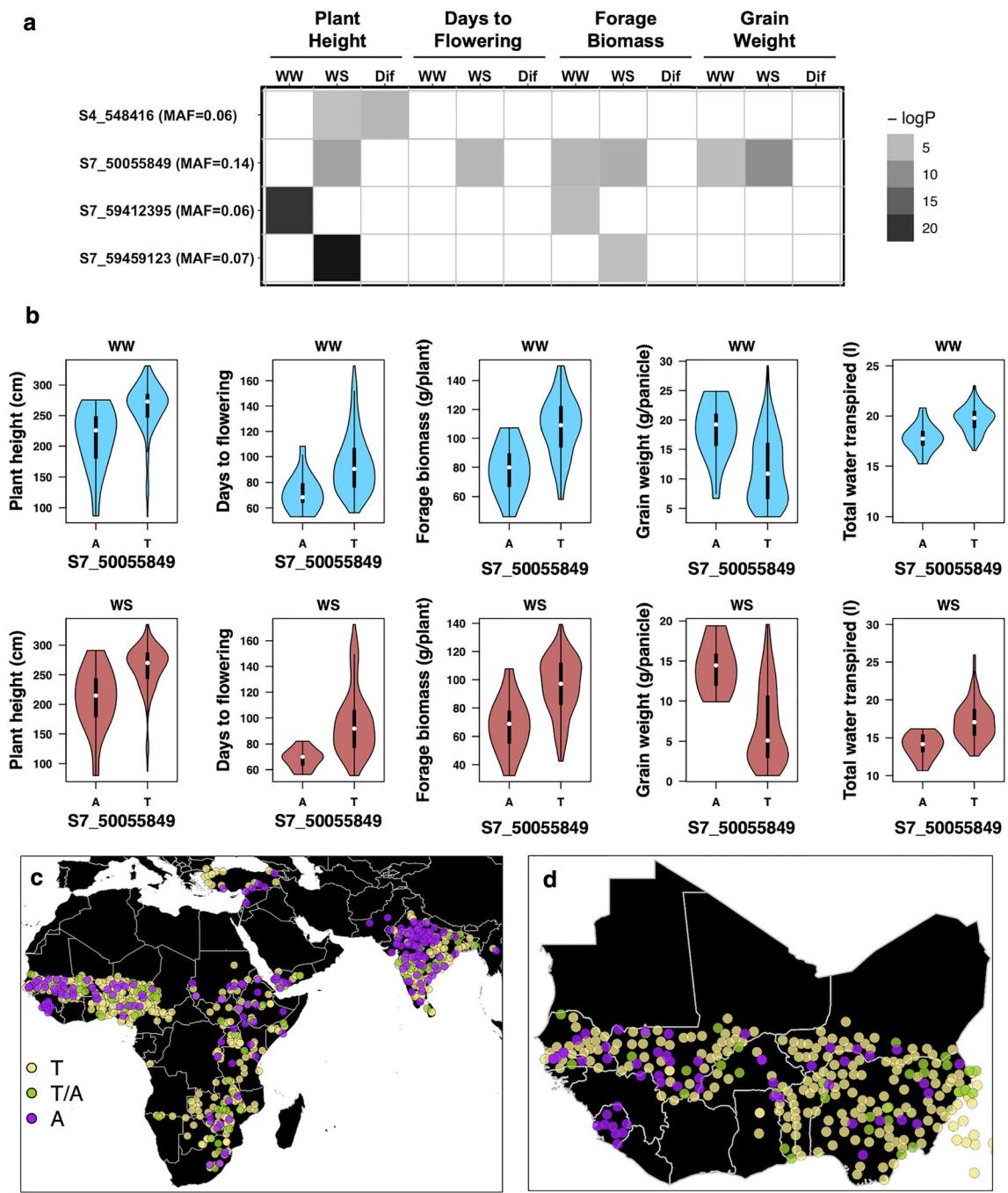


FIGURE 6 Shared marker-trait associations across traits identify putative pleiotropic loci. (a) Heatmap for $-\log(p$ values) of shared marker-trait associations for plant height, days to flowering, forage biomass, grain weight, and total water use in well-watered (WW) and water-stressed (WS) treatments. (b) Phenotypes for accessions carrying the A (minor allele) or T (major allele) at SNP S7_50055849, a putative pleiotropic locus. Distribution of alleles at S7_50055849 in georeferenced accessions (c) across Africa and Asia, and (d) in West Africa

drought resilience. The goals of this study were to test some hypotheses on the genetic architecture of drought adaptation in African sorghum landraces and to generate specific hypotheses on loci that could underlie variation in drought adaptive traits. In sub-Saharan Africa, drought can occur throughout the growing season, from germination to grain filling stages (Biasutti, 2019). We used managed stress experiments to repeatedly investigate stress scenarios that are important in the target population of the environment (TPE) but are difficult to

study due to unpredictable occurrence (Cooper et al., 2014). We focused on preflowering drought because (1) it is less well-studied and understood than postflowering drought (Borrell, Oosterom, et al., 2014) and (2) it is tractable to precisely apply water deficit at the same phenological stage, even for diverse germplasm with flowering time variation. We studied a large regional landrace diversity panel that captures much of the global diversity of sorghum (Figure 1a).



High-quality phenotypic data are essential for dissecting complex traits, such as those underlying drought adaptation (Cobb et al., 2013; Mackay et al., 2009). For stress response traits particularly, quality phenotyping requires extensive controls using genotypes with known stress responses to validate the experimental system (Tuberosa, 2012). Several lines of evidence suggest that high-quality managed stress phenotyping was obtained in this study, such that resulting findings are relevant to phenotypic response of sorghum to natural field-based drought. First, treatment-by-year effects were minor for both WW and WS ($p = .99$ and $.94$, respectively), suggesting that unintended sources of variation were minimized (Figures S3 and S4). Second, genotype-by-environment interaction was observed between international drought tolerance check genotypes, in the expected direction based on their known preflowering drought reaction. Finally, the lower reduction in grain weight for Tx7000 than BTx642 (Figures 1e, S2, and S3) confirmed that the stress imposed in our study is comparable with natural preflowering drought stress (Kebede et al., 2001; Tuinstra et al., 1996). Still, further research will be needed to test the hypothesis that the lysimeter managed drought stress (Figure 1b,c) is a good proxy for drought in the TPE, for instance, by confirming that managed stress phenotypes (lysimeter system) are positively correlated to those in the TPE (multienvironmental trials) (Cooper et al., 1995).

4.2 | Evidence of oligogenic variation for drought tolerance

A key hypothesis we aimed to test in this study is that sorghum landraces harbor a few major variants for preflowering drought tolerance (oligogenic hypothesis). If this hypothesis is true, then the effect of individual drought tolerance variants can be characterized, and these variants could be deployed in marker-assisted selection (Bernardo, 2008). However, if variation for preflowering drought tolerance is due to many variants of small effect (polygenic hypothesis), then characterizing individual variants will be unnecessary and infeasible, and phenotypic or genomic selection would be required. As would be predicted under the oligogenic hypothesis, a modest number of MTAs ($n = 13$) were identified across all traits and treatments (Table 2), a few co-localized with previously identified QTLs and others not previously described (Data S3). However, given that this is an association study, it is not possible to eliminate the hypothesis that the novel MTAs include spurious associations (Vilhjálmsson & Nordborg, 2013). To confirm the hypotheses on genetic variants, it would be necessary to confirm phenotypic differences in drought-related traits in controlled genetic backgrounds, such as recombinant or near-isogenic lines (Bergelson & Roux, 2010).

Flowering time is a drought-related trait because early flowering can contribute to drought escape, whereas plant height is a drought-related trait because reduced biomass can contribute to drought avoidance (water saving) (Blum et al., 1989; Tuberosa, 2012). Substantial flowering time variation exists in West African sorghum

(Bhosale et al., 2011; Upadhyaya et al., 2017) that could contribute to drought escape, and we observed strong MTAs that could be contributors (Figure 4d–f; Data S3). The flowering time MTAs did not co-localize with a priori candidate genes for flowering time, suggesting that variation at known genes (e.g., *Maturity* loci, orthologs of maize or rice flowering genes; Data S3) does not underlie the variation we observed, and that previously unreported genes may be involved. This contrasts previous field-based studies of the WASAP that observed flowering time associations near known genes such as *Maturity6/SbGHD7*, *SbCN8*, *SbCN12*, and *SbZFL1* (Faye, Akata, et al., 2021; Faye, Maina, et al., 2021). However, in this study, we selected genotypes with little to no photoperiod response (based on the previous common-garden experiments) to reduce confounding effects of flowering time variation on drought phenotyping (Tuberosa, 2012). Thus, the lack of MTA at known genes could be explained by the panel design, which excluded major photoperiodic flowering time variants. For plant height, by contrast, several MTAs were found between the two major genes controlling plant height in sorghum *QHT7.1* and *Dw3* (Figure S5), which matches the field-based WASAP study (Faye, Maina, et al., 2021). Plant height is a major trait that drives adoption in smallholder multipurpose cropping systems; however, studies have shown that plant height is associated with lodging under drought stress (Wang et al., 2020). In the future studies, it might be useful to test the hypothesis that selection for lodging resistance in West African germplasm could improve yield under water-deficit environments.

Among the components of drought tolerance, yield stability is particularly important in smallholder cropping systems given the severe consequences of crop failure in this context (Rapsomanikis, 2015). MTAs for the drought response of yield components (grain weight and forage biomass) are candidate loci for preflowering drought tolerance since they reflect yield stability under drought. Several MTAs with forage biomass (S2_72712959, S2_57620549, S2_57664163, and S2_57663973) and grain weight (S7_64933026, S6_46923493, S5_16199662) co-localized with previously identified QTLs for the same traits (Data S3) (Mace et al., 2018). Some of the top MTAs for the drought response of forage biomass (S2_57620549, S2_57664163, and S2_57663973) and grain weight (S5_16199662) (Figures 4i–l and S7) co-localized with *Stg3a* and *Stg4*, respectively. This finding is consistent with the hypothesis that staygreen alleles, which delay senescence under postflowering drought in semi-dwarf grain sorghums (Borrell, Oosterom, et al., 2014), also contribute to drought tolerance in West African landraces (Faye, Akata, et al., 2021). MTAs associated with water use in both treatments (Figure 5) might suggest that loci with small effect size could mediate water-use efficiency under drought conditions (Ferguson et al., 2021).

4.3 | A major pleiotropic variant mediating water use and drought tolerance?

The most notable association in the study is the putatively pleiotropic MTA at S7_50055849 (Figure 6a), which is the top association for

both grain weight and forage biomass under drought (Figures 4h–k and 6a) as well as a leading association for flowering time (third highest) and plant height (second highest) under drought (Data S3). This SNP is also associated with grain and forage yield under WW, but in these cases, the association is marginal ($p \approx 10^{-5}$) and it is not the top association. One hypothesis to explain these findings is that a variant exists near S7_50055849 that mediates partitioning of assimilates to grain versus vegetative biomass under drought, along with other aspects of plant growth and development (Blum, 1998; Ongom et al., 2016; Tuberrosa, 2012; Wang et al., 2020). Under this assimilate partitioning hypothesis, the variant would pleiotropically affect multiple yield and drought-related traits as downstream effects.

Given that S7_50055849 is associated with flowering time and height, an alternate hypothesis would be that a nearby maturity variant is delaying flowering, thus increasing forage biomass and decreasing grain yield. However, under this delayed maturity hypothesis, one would expect that the top associations for grain weight would be the top flowering time associations (e.g., S4_64398335 and S3_19589652), which was not the case (Figure 4; Data S2). Therefore, the shared associations at S7_50055849 for yield components and flowering time are unlikely to be due to delayed maturity, but more likely to be due to a variant mediating assimilate partitioning across the plant that in turn affects maturity.

Consistent with the hypothesis that the MTA at S7_50055849 represents a novel loci mediating assimilate partitioning, the MTA did not co-localize with any known maturity or height genes (Data S2). One interesting post hoc candidate gene near S7_50055849 is Sobic.007G115400 (second-nearest gene model, 43 kb away), an uncharacterized gene that is moderately expressed (.6–5.5 FPKM) in stem tissues and lowly expressed in other tissues. While the putative ortholog of this gene in maize (GRMZM2G136353) and rice (Os08g28870) have not been functionally characterized, the putative ortholog in Arabidopsis (AT1G28440) is *HAESA-LIKE 1* (*HSL1*). In Arabidopsis, *HSL1* is a peptide hormone receptor that negatively regulates stomatal development (Qian et al., 2018). Thus, one gene-level hypothesis for the MTA at S7_50055849 is that a variant at Sobic.007G115400 affects stomatal development, and in turn grain and forage yield under drought. Validation and fine mapping of the QTL using near-isogenic lines would be necessary to test this mechanistic hypothesis.

If there is a major pleiotropic variant near S7_50055849 as suggested by the multitrait GWAS (Figures 4 and 6a,b), the geographic analysis of alleles at this SNP (Figure 6c,d) suggests that the variant is widespread in landraces across both East and West Africa and South Asia. If this hypothesis is true, future genome-wide mapping studies of drought-related traits in diverse East African and South Asian germplasm should identify MTA in the same genomic region. A recent GWAS for field-based managed drought stress using the full WASAP did not identify a preflowering drought tolerance MTA at S7_50055849 but did identify one at S7_50507851, 450 kb upstream (Faye, Akata, et al., 2021).

4.4 | Conclusion

In this study, we used a quantitative trait genomics approach, with managed drought stress phenotyping and GWAS, to investigate the genetics of preflowering drought adaptation in diverse sorghum landraces. We found abundant phenotypic variation for drought-related traits, including drought response of yield components and water use, and evidence of oligogenic trait variation, with several strong MTAs, including a putative pleiotropic drought tolerance variant near S7_50055849. If confirmed in field environments and controlled genetic backgrounds, the alleles associated with higher yield under drought or favorable drought response of yield components could be the basis for trait-predictive markers used in breeding for climate-resilience and future genetic dissection of drought adaptation (Tuberrosa, 2012; Varshney et al., 2014).

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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