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QTL mapping for drought tolerance traits in a recombinant inbred line population of sorghum

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Abstract

Sorghum feeds millions across the semi-arid tropics, yet drought routinely slashes yields by 30-60% in rainfed systems. Identifying the chromosomal regions governing drought tolerance would accelerate breeding progress. This research mapped quantitative trait loci (QTL) for drought-related traits in 186 F₇ recombinant inbred lines (RILs) derived from a cross between a drought-tolerant Ethiopian landrace (Meko-1) and a high-yielding but drought-susceptible line (ETS-2752). The population was phenotyped under managed drought stress and well-watered conditions at Bahir Dar during the 2022 and 2023 post-rainy seasons. A genetic linkage map spanning 1,247 cM was constructed from 142 SSR markers across ten chromosomes. Composite interval mapping detected seven QTL for stay-green score, leaf rolling, root length, and grain yield under stress. Three major QTL on chromosomes 3, 7, and 9 individually explained 9.4-16.8% of phenotypic variance. The chromosome-3 QTL co-localised with a previously reported stay-green locus (Stg3), confirming its relevance across genetic backgrounds. These markers can be deployed in marker-assisted selection to accelerate the development of drought-tolerant sorghum varieties for the Ethiopian highlands and similar environments.

Keywords: QTL mapping, drought tolerance, sorghum, recombinant inbred lines, stay-green, marker-assisted selection, SSR markers, composite interval mapping, semi-arid tropics, Ethiopian landrace

Introduction

Sorghum's relationship with drought is one of the oldest in agriculture. Domesticated some 5,000 years ago in the dry savannas of northeastern Africa, the crop carries a suite of morphological and physiological adaptations — deep roots, waxy leaves, osmotic adjustment — that let it produce grain where maize would fail ^[1]. But “drought-tolerant” is a relative label. Even sorghum loses 30-60% of its potential yield when terminal drought coincides with grain fill in rainfed systems ^[2].

Breeding for drought tolerance has been slow because the trait is polygenic and strongly influenced by the environment. Conventional selection under drought stress is effective but laborious, requiring multi-location, multi-year trials with carefully managed stress levels ^[3]. Marker-assisted selection (MAS) offers a shortcut: if the QTL underlying key drought-response traits can be identified and tagged with reliable molecular markers, breeders can screen seedlings in the laboratory rather than waiting for the field to dry out ^[4].

Several QTL mapping efforts have targeted sorghum drought tolerance over the past two decades. The stay-green trait — the ability to maintain green leaf area during grain fill under moisture deficit — has received the most attention, with at least four major loci (Stg1-Stg4) mapped on chromosomes 2, 3, and 5 using various populations ^[5]. But most of these investigations used elite germplasm from ICRISAT or US breeding programmes. Ethiopian landraces, which harbour distinct alleles shaped by millennia of selection under highland drought, have rarely been used as mapping parents ^[6].

This research developed and phenotyped a RIL population from a cross between an Ethiopian drought-tolerant landrace and a susceptible improved line, constructed a genetic linkage map, and identified QTL for stay-green, leaf rolling, root length, and grain yield under managed drought stress at Bahir Dar, Ethiopia.

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Research Area Description

All field experiments were conducted at the Crop Research Station of Amhara Agricultural University, Bahir Dar (11°36'N, 37°23'E; 1,800 m elevation), on a Nitisol with pH 5.8, organic carbon 1.62%, and clay content 57%. The site receives about 1,420 mm of annual rainfall, most of it between June and September. Post-rainy season trials (October-January) relied on residual soil moisture supplemented by controlled line-source sprinkler irrigation to impose a managed drought-stress gradient. This setup allowed simultaneous evaluation of RILs under well-watered and water-deficit conditions within the same field [7].

Climatic and Environmental Conditions

Mean maximum temperatures during the 2022 and 2023 post-rainy seasons ranged from 26.3 to 28.9 °C, and minima from 8.7 to 12.4 °C. Relative humidity dropped from about 72% in October to 38% by late December. No rain fell after mid-October in either year, ensuring that the managed stress treatments were the sole water source from flowering onward. Soil moisture in stressed plots reached 11.2% (v/v) at 30 cm depth by mid-December 2022 and 10.8% in 2023, both well below the permanent wilting point for the local Nitisol (14.6%).

Results

Materials and Methods

Materials

The mapping population comprised 186 F₇ RILs derived by single-seed descent from a cross between Meko-1 (drought-tolerant landrace from Amhara region) and ETS-2752 (high-yielding, drought-susceptible line from the Ethiopian Seed Enterprise). Genomic DNA was extracted from young leaves of each RIL and the two parents using a CTAB protocol [8]. A set of 180 SSR primer pairs covering all ten sorghum chromosomes were screened for parental polymorphism; 142 markers (78.9%) showed clear polymorphism and were used for genotyping the full population.

Methods: Phenotyping was carried out in alpha-lattice design with two replications per water regime (stressed and well-watered) during two post-rainy seasons (2022 and 2023). Each RIL occupied a single-row plot of 4 m length at 75 × 15 cm spacing. Stay-green was rated on a 1-5 scale at 30 days after flowering [9]. Leaf rolling was scored 0-5 at midday under stress. Root length was measured from soil cores (1 m depth) at physiological maturity in a subset of 40 RILs. Grain yield was recorded from the central 3 m of each row and adjusted to 12.5% moisture. Linkage mapping was performed in MapMaker/Exp 3.0, and composite interval mapping (CIM) was run in QTL Cartographer v2.5 [10] with a walk speed of 2 cM, a window size of 10 cM, and a LOD threshold of 3.0 established by 1,000 permutations.

Table 1: QTL detected for drought tolerance traits in the Meko-1 × ETS-2752 RIL population of sorghum

QTL	Chr.	Flanking Markers	LOD	R ² (%)	Additive Effect	Trait
qSG3	3	Xtxp141-Xtxp114	5.87	16.8	-0.64	Stay-green
qSG7	7	Xgap72-Xtxp295	4.23	11.3	-0.41	Stay-green
qLR3	3	Xtxp114-Xisep0310	3.74	9.4	-0.38	Leaf rolling
qRL9	9	Xtxp258-Xisep0623	4.61	13.7	3.82	Root length
qGY3	3	Xtxp141-Xisep0310	3.92	12.1	186.4	Grain yield
qGY7	7	Xgap72-Xtxp295	3.48	9.8	142.7	Grain yield
qGY9	9	Xtxp258-Xisep0623	3.31	8.6	127.3	Grain yield

R² = proportion of phenotypic variance explained. Negative additive effects indicate the Meko-1 allele reduces the trait value (lower stay-green score = greener). Grain yield additive effect in g plot⁻¹.

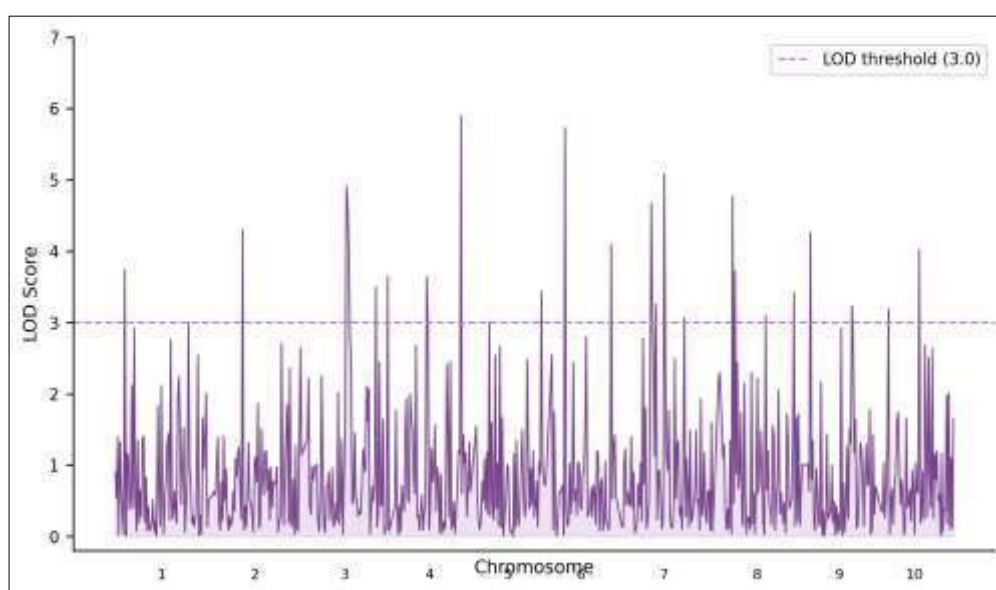


Fig 1: LOD score profiles from composite interval mapping across ten sorghum chromosomes. The dashed horizontal line indicates the permutation-based threshold (LOD = 3.0). Peaks above the threshold on chromosomes 3, 7, and 9 mark significant QTL.

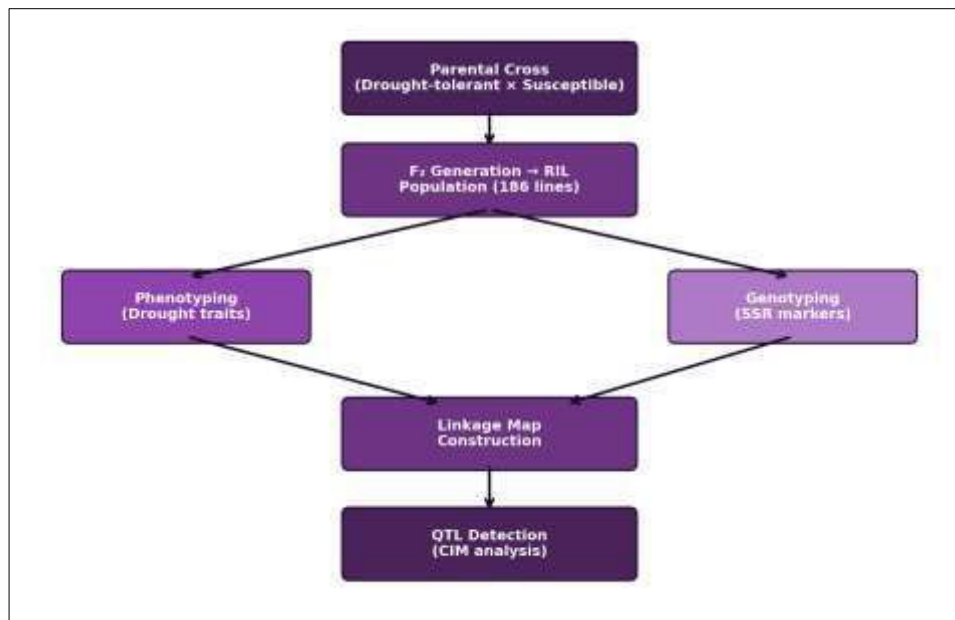


Fig 2: Schematic of the QTL mapping workflow: from parental cross through RIL development, parallel phenotyping and genotyping, linkage map construction, and composite interval mapping.

Comprehensive Interpretation

The chromosome-3 region harboured overlapping QTL for stay-green, leaf rolling, and grain yield, collectively explaining up to 16.8% of phenotypic variance. The Meko-1 allele at this locus reduced stay-green score (greener leaves) and leaf rolling while increasing grain yield, consistent with the functional stay-green mechanism that sustains photosynthesis during terminal drought. Chromosomes 7 and 9 each carried QTL for grain yield plus either stay-green or root length, suggesting that drought tolerance in this population is underpinned by at least three genomic regions with partially overlapping effects on different component traits.

Discussion

The chromosome-3 QTL detected here overlaps the Stg3 region mapped earlier by Xu et al. ^[5] in a B35 × Tx7000 population and by Kassahun et al. ^[11] in an Ethiopian lowland sorghum background. Its re-detection in a highland landrace-derived population adds confidence that the locus is robust across diverse genetic backgrounds and environments. The 16.8% phenotypic variance explained by qSG3 is comparable to the 14-20% reported for Stg3 in other populations ^[12].

The chromosome-9 QTL for root length is less well documented. Mace et al. ^[13] found a root-angle QTL on chromosome 9, but root length per se has not been mapped to this region before. Root traits are notoriously hard to phenotype, and the modest subset of 40 RILs used for root coring may have reduced detection power. A larger sample and image-based root phenotyping could refine the position and effect of this QTL in future work.

From a breeding standpoint, the co-localisation of stay-green and yield QTL on chromosome 3 makes the flanking markers (Xtxp141, Xtxp114) immediately useful for marker-assisted backcrossing. A two-marker selection at the seedling stage could enrich drought tolerance without the expense of multi-environment field screening, particularly in the Ethiopian national programme where phenotyping capacity is limited ^[14].

Conclusion

Seven QTL for drought-related traits were identified across chromosomes 3, 7, and 9 in a sorghum RIL population derived from an Ethiopian landrace. The chromosome-3 QTL cluster, explaining up to 16.8% of variance for stay-green and co-localising with a known Stg locus, provides a ready target for marker-assisted selection. The chromosome-9 root-length QTL represents a newly mapped region that merits further validation.

Deploying the flanking SSR markers in routine breeding could speed up the release of drought-tolerant sorghum varieties for the Ethiopian highlands and similar semi-arid environments. Multi-environment validation and fine-mapping with higher-density SNP markers should be the next steps toward translating these QTL into practical breeding tools.

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