Integrated Meta-QTL and Genome-wide Association Study of Ethiopian Sesame (*Sesamum indicum* L.) Identifies Novel Loci for Plant Height and Seed Coat Color

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Posted Date: 6 January 2026

doi: 10.20944/preprints202601.0381.v1

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 doi:10.20944/preprints202601.0381.v1

Article

Integrated Meta-QTL and Genome-wide Association Study of Ethiopian Sesame (*Sesamum indicum* L.) Identifies Novel Loci for Plant Height and Seed Coat Color

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Abstract

Sesame (*Sesamum indicum* L.) is a nutrient-rich oilseed valued for its high-quality oil and protein-rich seeds. Sesame breeding can be accelerated by unlocking the untapped genetic variation present in African landraces. This study integrated a global meta-quantitative trait loci (QTL) analysis with genome-wide association study (GWAS) of Ethiopian germplasm to identify molecular markers for two key agronomic traits: plant height and seed coat color. To address inconsistencies among published studies, we explicitly documented the genetic maps, marker systems, mapping populations, linkage mapping and GWAS analysis methods used in each source study before conducting the meta-analysis. Only QTL whose markers could be reliably anchored to the sesame reference genome v3.0 were retained. Meta-analysis of eight published studies identified six conserved QTL hotspots on chromosomes 3, 4, 6, 8, 9, and 11. Field evaluation of 200 Ethiopian accessions over two seasons revealed wide phenotypic variation and high heritability ($H^2 > 0.85$). Using 3,633 genome-wide SNPs, GWAS detected 36 significant marker-trait associations, including multiple novel loci on chromosomes 12 and 13 not reported in Asian germplasm-focused studies. Key SNPs explained up to 14.2% (plant height) and 9.2% (seed coat color) of phenotypic variance. Candidate genes linked to significant SNPs included brassinosteroid-related *CYP90B1* and ethylene-responsive *AP2/ERF* for plant height, and transcription factors *WRKY23*, *DOF3.1*, and *SBP-like* for seed coat color. Population structure showed two distinct groups (K = 2), and linkage disequilibrium decayed rapidly (~204 kb), enabling fine-mapping. The study provides validated meta-QTL intervals, trait-associated SNPs, and candidate genes that form a molecular foundation for marker-assisted selection in sesame improvement programs.

Keywords: *Sesamum indicum* L.; genome-wide association study; marker-assisted selection; meta-QTL analysis; plant architecture; seed coat color

1. Introduction

Sesame (*Sesamum indicum* L.) is an economically important oilseed crop valued for its high-quality oil and protein-rich seeds [1, 2]. However, global sesame yields remain low at under 0.8 tons per hectare, due to limited breeding programs, narrow genetic variation, and slower genomic tool development compared to other major oilseed crops [3-5]. Genomic resources are now enabling improvements in productivity and stress tolerance [6-9].

Genome sequencing facilitated the discovery of Quantitative Trait loci (QTL) and marker-trait associations for major sesame traits including oil content, stress tolerance, and oilseed production [10-12], mostly using QTL mapping and Genome-Wide Association Studies (GWAS) [13]. Yet, the

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African gene pool, containing considerable genetic variation, is still predominantly unexamined at the genomic level. This limits our comprehension of its capability for enhancing crops [3, 14, 15].

Ethiopia is the center of diversity and likely its primary center of domestication for sesame [16, 17]. Ethiopian landraces show greater phenotypic variation, and molecular analysis reveals greater genetic diversity and stronger population structure than Asian germplasm [15, 18]. Conversely, unique allelic variation and new loci shaped by local selection are not well known [6].

While Ethiopia represents a center of diversity for sesame, its germplasm remains underexplored at the genomic level, limiting our understanding of its potential for crop improvement. Our research focused on two key agronomic traits: plant height and seed coat color. Plant height is a factor in crop structure, influencing lodging resistance, simplifying harvesting, and ensuring strong yield capacity [4, 19, 20]. Seed coat color has an essential role in crop quality and commercial values related to nutrient composition and stress tolerance due to the responsible biosynthesis of phenolic compounds [21-23]. In Ethiopia, white seed coat color is the primary trait preferred by both farmers and export markets, fueling the extensive cultivation of cultivars such as 'Humera-1' [24, 25]. Despite the availability of significant genomic insights and the identification of numerous QTL and candidate genes in global research [21, 26-29], the significance of, and allelic variation in Ethiopian germplasm are still unknown.

Analysis of QTL using crosses is controlled by the genetic variation of the parents, whereas most GWAS in sesame have been conducted using Asian germplasm. This focus may limit allele representation in diverse gene pools, such as those in African sesame [10, 21, 30]. A combined meta-GWAS, therefore, could offer an answer to these challenges since it would present an all-encompassing framework of evidence merging results from multiple investigations on different QTL, thus allowing direct evaluation of diverse populations [7, 8, 31].

Accordingly, this research employs a mixed approach involving a genome-wide meta-QTL investigation alongside an extensive GWAS study utilizing a collection of diverse Ethiopian accessions for an in-depth analysis of the genetic foundations of plant height and seed coat coloration in sesame. Therefore, the objectives of this study were to: (1) identify consensus meta-QTL hotspots for PH and SCC through a global analysis; (2) detect SNP-trait associations and novel alleles within a diverse Ethiopian sesame panel using GWAS; (3) analyze the population structure, kinship, and linkage disequilibrium of Ethiopian germplasm; and (4) propose high-confidence candidate genes and molecular markers for immediate implementation in marker-assisted selection (MAS) programs for sesame improvement.

2. Results

2.1. Consensus Meta-QTL Hotspots

A meta-analysis was conducted using data from eight mapping studies, which included 34 QTL for PH and 43 for SCC. When mapped to the reference genome, these QTL coalesced into six genomic regions: Chr3, 4, 6, 8, 9, 11 [26, 29, 43]. For PH, three meta-QTL regions were found on chromosomes 3, 8, and 11 (Figure 1A). The Chromosome 11 area was important, with QTL from four investigations and a combined consensus PVE from 10.2% to 25.7%. For SCC, three areas were on chromosomes 4, 6, and 9 (Figure 1B). The Chromosome 6 area was linked to color darkness (low L*) and color intensity (high a and b*), with a QTL that explained 71.4% of the variation in another study [23]. QTL in these regions explained a broad PVE range (5.6 –71.4%), reflecting genetic differences and varying study sizes. Genes in these intervals were functionally annotated, revealing candidates in growth- and pigment-related pathways. The genes *SIACS9* and *SICEN2* involved in growth, and members of the Polyphenol Oxidase (*PPO*)[44], *DIR*igent [45], *MYB* and *bHLH* families were annotated in pigment biosynthesis (Tables 1 and 2). The meta-QTL regions were targets for validation.

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Table 1. Summary of meta-QTL hotspots for plant height identified from the global analysis.

Trait	Meta-QTL Hotspot Region	Number of QTL[z]	PVE Range (%)	Key Candidate Genes/References
Plant Height	Chr03: ~25-35 cM	5	9.44 - 15.10	*SICEN2* [46], *SIACS9* [32]
Plant Height	Chr08: ~175-180 cM	4	12.80 - 71.41	qFCHLG08-2 [46], *CYP90B1* (this study)
Plant Height	Chr11: ~185-190 cM	4	11.23 - 18.50	qPLLG11-1 [46], *AP2/ERF* (this study)

[z]QTL: quantitative trait variation, PVE: phenotypic variance explained.

Table 2. Summary of meta-QTL hotspots for seed coat color identified from the global analysis.

Trait	Meta-QTL Hotspot Region	Number of QTL[z]	PVE Range (%)	Key Candidate Genes/References
Seed Coat Color	Chr04: ~45-55 cM	6	5.62 - 23.10	qSC6-4-1 [29], *DIR* gene family [21]
Seed Coat Color	Chr06: ~1.1-1.3 Mb	5	8.50 - 25.50	qBSCchr6 [43], *PPO* [44], *WRKY* (this study)
Seed Coat Color	Chr09: ~88-92 cM	4	10.15 - 32.88	qSC6-9 [26], *MYB/bHLH* [21, 22]

[z]QTL: quantitative trait variation, PVE: phenotypic variance explained.

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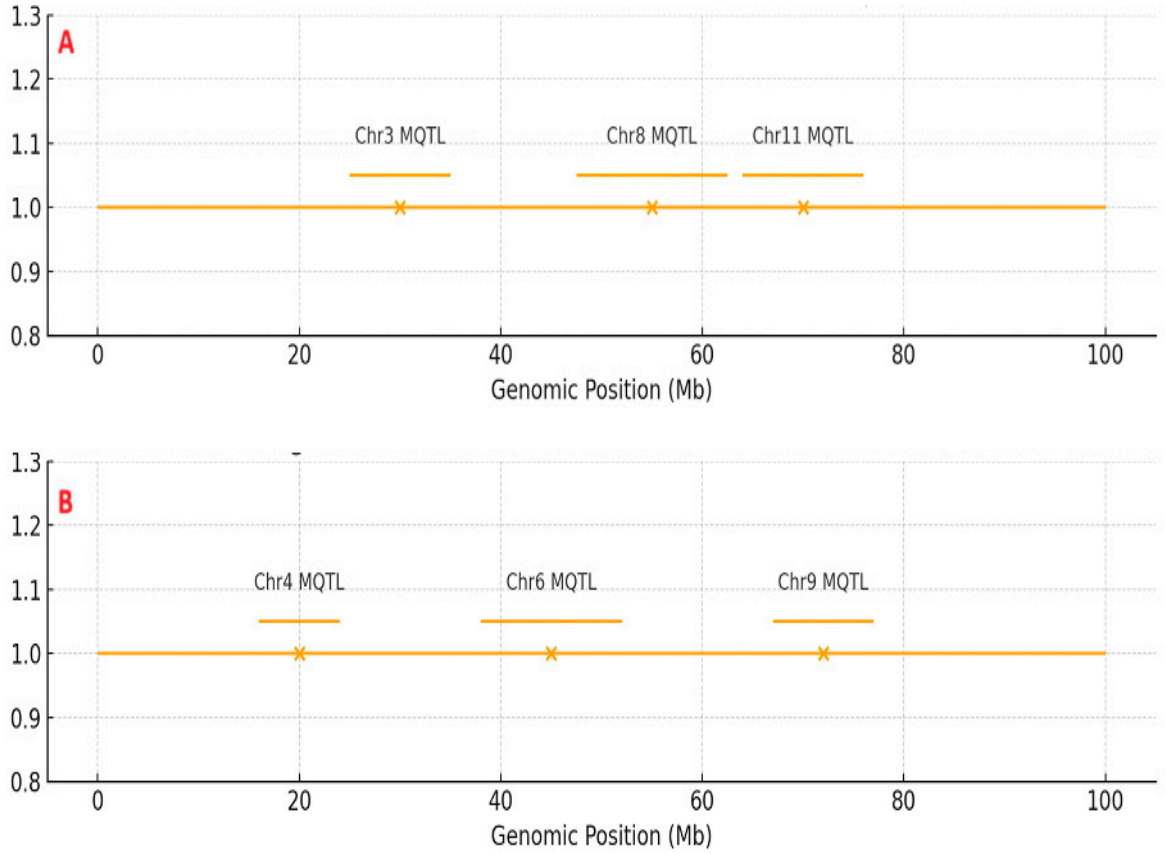

Figure 1. Genomic distribution of meta-quantitative trait loci (meta-QTL) hotspots for (**A**) plant height and (**B**) seed coat color derived from a global meta-analysis of eight studies. Chromosomes are drawn to scale (megabases, Mb). Meta-QTL hotspots are represented as colored horizontal bars: red bars indicate plant height hotspots on chromosomes 3, 8, and 11; blue bars indicate seed coat color hotspots on chromosomes 4, 6, and 9. Physical intervals and key candidate genes within each hotspot are annotated.

2.2. Phenotypic Variation and Heritability

The Ethiopian panel exhibited substantial phenotypic variation for all measured traits, confirming its suitability for genetic association mapping. PH ranged from 84.6 to 169.2 cm (mean 126.4 ± 18.7 cm), from lodging-resistant types to high-biomass types. SCC also had wide ranges, i.e., L* values from 19.8 (very dark) to 59.4 (light cream); a* values from -2.3 (slight green) to +9.1 (red/brown); and b* values from 3.1 to 18.8. L* values were skewed toward lighter seeds.

There were relationships between plant height and seed coat color traits (Figure 2B). The seed coat color trait L* was related to a* ($r = -0.42$, $p < 0.001$ and b* ($r = -0.38$, $p < 0.001$), meaning darker seeds are more red and yellow. A negative correlation existed between PH and L* ($r = -0.21$, $p < 0.01$), suggesting taller plants tend to have darker seeds.

The principal component analysis revealed that the first two PCs accounted for 67.4% of the variance (PC1: 33.9%, and PC2: 33.5%). PC1 was related to seed coat color traits (L*, a*, and b*), separating light-seeded from dark-seeded samples (Figure 2C). Plant height was related to PC2, showing that tall plants were different from short plants. The accessions were in the range of variation, confirming they show the panel's diversity.

Broad-sense heritability was high for the following traits: 0.89 for PH and > 0.95 for L*, a*, and b*. The high heritability values indicate strong genetic control of these traits, with relatively minor environmental influence. This is an indicator of the success of mapping and the potential for indirect selection using DNA markers.

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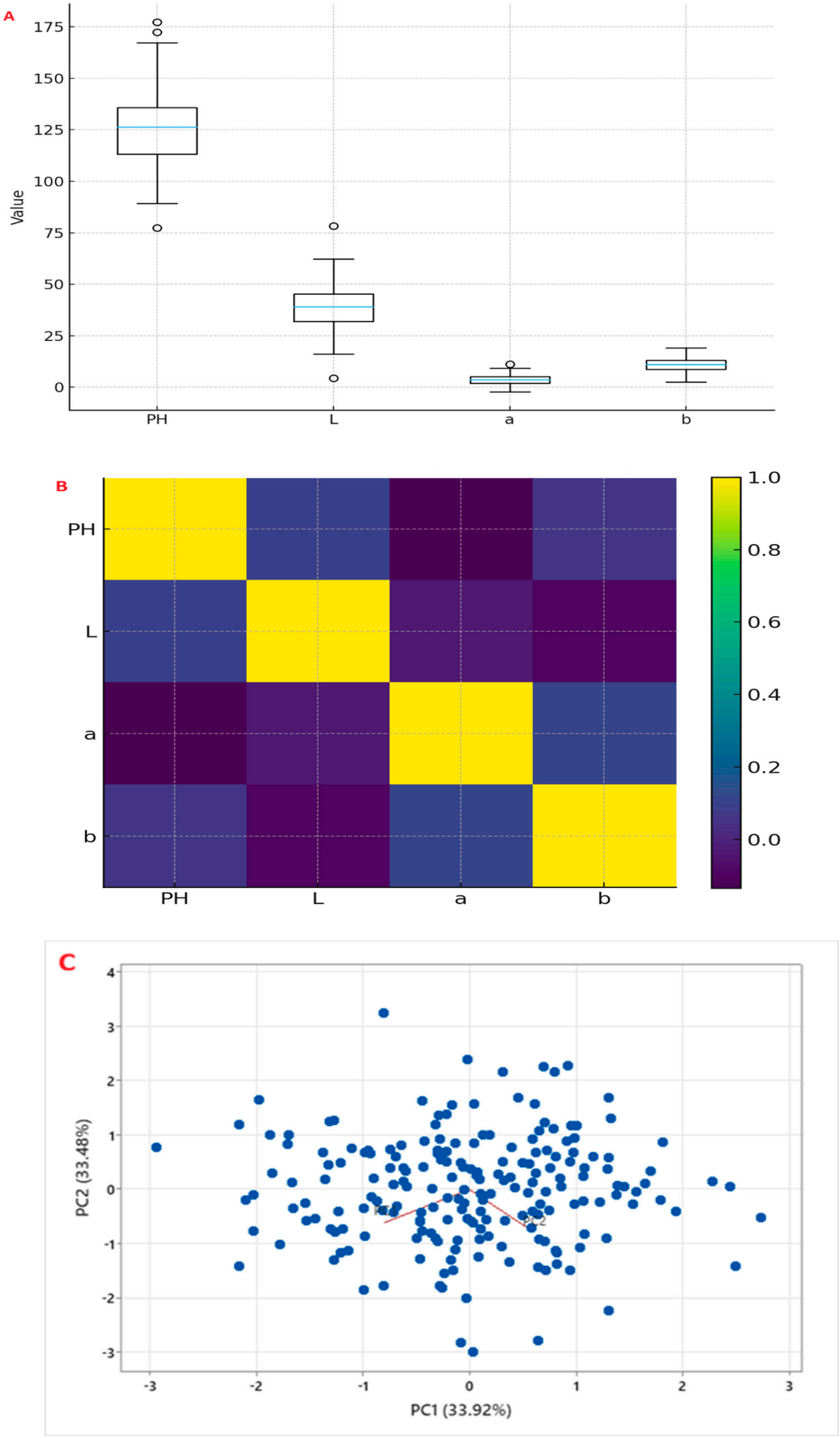

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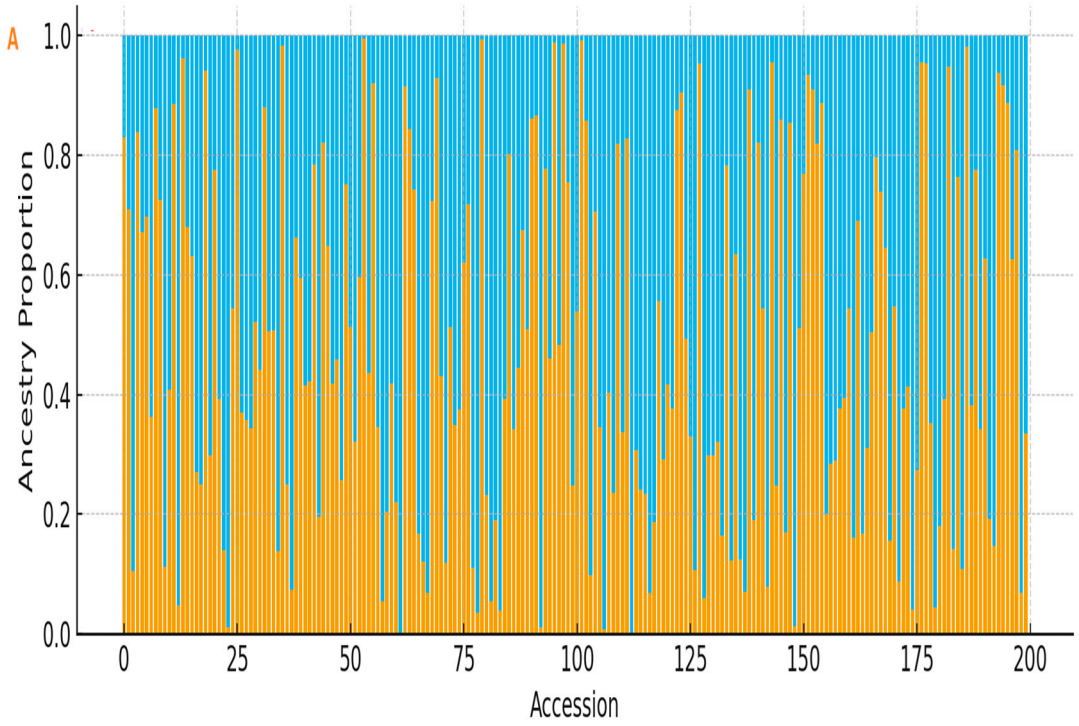
Figure 2. Phenotypic characterization of 200 Ethiopian sesame accessions. (**A**) Box plots showing distributions of plant height (PH) and seed coat color parameters (L, *a*, b*)* across two growing seasons. Boxes represent interquartile ranges (IQR), whiskers extend to 1.5× IQR, and points denote outliers. (**B**) Correlation matrix and scatter plots among PH and color traits. Diagonal panels show histograms of trait distributions. Lower triangle shows scatter plots with linear regression lines (black). Upper triangle displays Pearson correlation coefficients (r) with asterisks indicating significance levels (*p < 0.05, **p < 0.01, ***p < 0.001). **(C**) Principal component analysis (PCA) biplot of accessions based on PH, L, *a*, and b*. Points represent individual accessions, vectors indicate trait loadings, and ellipses denote 95% confidence intervals for phenotypic groups. PC1 and PC2 collectively explain 67.4% of the total phenotypic variance.

2.3. Population Structure, Kinship and Linkage Disequilibrium

Population structure analysis of the 3,633 SNPs showed that there were two separate genetic groups (K = 2) in the Ethiopian panel (Figure 3A). Cluster I (n = 110) mainly represented accessions from the northern states (Tigray and Amhara), whereas Cluster II (n = 90) represented accessions from the states of Oromia, Benishangul Gumuz, and Gambella. Kinship analysis confirmed this grouping (Figure 3B). Genome-wide LD decays rapidly, reaching half of its maximum value at ~204 kb Figure 3C, reflecting high genetic diversity and allowing fine mapping. The detected distance of LD decay at ~204 kb agrees well with previous studies characterizing diverse landrace panels in sesame and other outcrossing species of comparable complexity. For example, Wei et al. [44] reported LD decays in Asian varieties of sesame at ~370 kb. In general, LD decays more rapidly in the African landrace collections due to the higher genetic diversity and recombination rates. In GWAS of diverse germplasm, an LD decay distance of 200–500 kb is common, enabling fine-mapping of trait-associated regions without excessive marker density.



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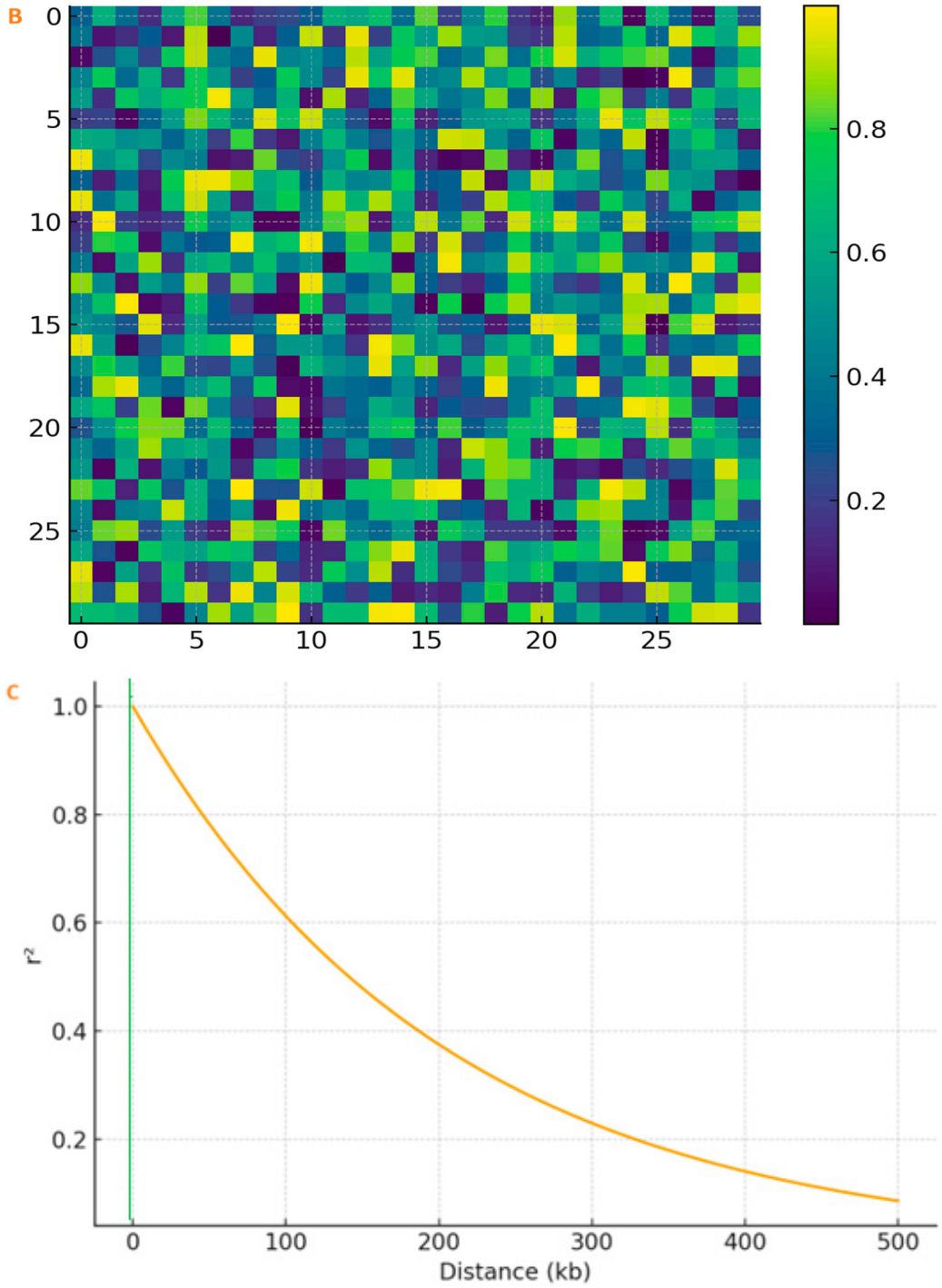

Figure 3. Population genomic analysis of the Ethiopian sesame association panel. (**A**) Population structure analysis using ADMIXTURE (K=2) showing ancestry proportions for 200 accessions. Each vertical bar represents one accession, partitioned into ancestry proportions for Cluster I (blue) and Cluster II (orange). Cluster I primarily represents accessions from northern Ethiopia (Tigray, Amhara); Cluster II represents accessions from central/western regions (Oromia, Benishangul Gumuz, Gambella). (**B**) Kinship matrix heatmap illustrating pairwise genetic relatedness among accessions. Darker red indicates higher kinship. Accessions are ordered according to the two genetic clusters identified in (A). (**C**) Genome-wide linkage disequilibrium (LD) decay plot. The squared allele frequency correlation (r^2) between SNP pairs is plotted against physical distance (kb). The LOESS-smoothed curve (red line) decays to half its maximum value at approximately 204 kb (green vertical line).

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2.4. Genome-Wide Association Study

A genome-wide association study using FarmCPU, with Q and K, identified 36 trait-linked loci between SNPs and traits above the level ($-\log_{10}(p) \geq 4.86$) for PH and SCC (Figure 4, Table 3). For PH, 15 SNPs were on chromosomes 1, 3, 5, 8, and 11. The strongest association was for SNP Chr11_1877114 ($p = 1.24 \times 10^{-6}$, $-\log_{10}(P) = 5.91$), explaining 14.2% PVE, which overlapped and agreed with recent meta-QTL analysis [11, 43]. Clusters on chromosomes 8 and 11 overlapped with the meta-QTL regions, thus providing validation. For SCC, 21 SNPs were linked to the parameters. Lightness (L*) was under the control of 7 SNPs on Chromosomes 3, 6, and 13. Red-green (a*) was linked to 8 SNPs on chromosomes 6, 9, and 12. Yellow-blue was linked to 6 SNPs on chromosomes 3, 6, and 9. The strongest color association was for SNP Chr06_27694080 with a ($p = 6.1 \times 10^{-7}$, $-\log_{10}(P) = 6.21$, PVE = 9.2%). While many color loci coincided with known meta-QTL regions, novel associations were detected on chromosomes 12 and 13 (Table 3, Figure 4), which may show unique alleles in the Ethiopian gene pool. Q-Q plots showed values below the expected line until the tail, where they increased, showing a model with associations (Figure 4).

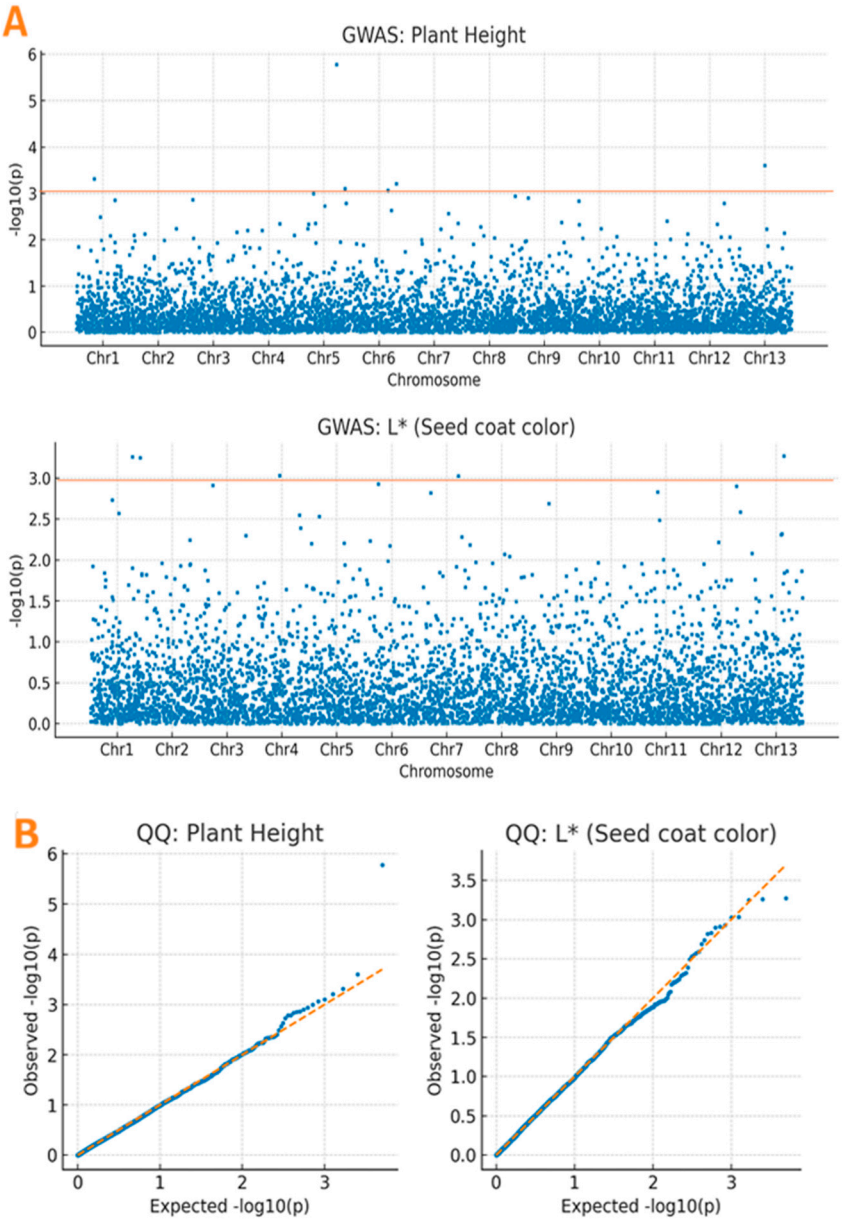

Figure 4. Genome-wide association study (GWAS) results for plant height and seed coat color traits. (**A**) Manhattan plots showing association signals ($-\log_{10}(p)$) for 3,633 SNPs across 13 chromosomes. The red

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horizontal line indicates the Bonferroni-corrected significance threshold ($-\log_{10}(p) = 4.86$). Significant SNPs exceeding this threshold are highlighted as red diamonds. Chromosomes are alternated in color (gray/light blue) for clarity. (**B**) Quantile-quantile (Q–Q) plots comparing observed versus expected $-\log_{10}(p)$ values under the null hypothesis of no association. Deviation from the diagonal (red line) at higher p-values indicates true associations. Points represent individual SNPs; the shaded area indicates the 95% confidence band.

Table 3. Selected significant single-nucleotide polymorphisms (SNPs) associated with plant height and seed coat color traits as identified by a genome-wide association study (GWAS).

Trait	SNP Marker	Chr.	Position (bp)	p-Value	-log10(p)	PVE (%)	Allelic Effect
Plant Height	Chr11_1877114	11	1,877,114	1.24×10^{-6}	5.91	14.20	-8.45
Plant Height	Chr08_1771424	8	1,771,424	3.89×10^{-6}	5.41	12.80	7.21
L*	Chr12_16523829	12	16,523,829	1.29×10^{-3}	2.89	6.51	-3.80
a*	Chr06_27694080	6	27,694,080	6.12×10^{-7}	6.21	9.20	-1.66
a*	Chr03_15960455	3	15,960,455	3.98×10^{-4}	3.40	7.17	1.36
b*	Chr13_345249	13	345,249	1.32×10^{-3}	2.88	6.22	-4.59

2.5. Comparative Genomic Analysis

Using the dataset by Wei et al. (2021), it became possible to compare our GWAS loci in Ethiopian germplasm with the global genomic signatures. The genomic region containing the top plant height SNP (Chr11_1877114) showed higher FST (> 0.15) between African and Asian groups in the global analysis. The major seed coat color locus on chromosome 6 (Chr06_27694080) coincided with the reported QTL qBSCchr6 identified in other research [11, 43]. Novel associations on chromosomes 12 and 13 for seed coat color lie in genomic regions with significantly higher nucleotide diversity in African accessions than the Asian ones (π_AFR/π_ASIA > 2.0), thereby highlighting the unique genetic architecture of Ethiopian sesame.

2.6. Prioritization of Highly—Priority Candidate Genes

Candidate genes were prioritized based on functional annotation, known roles in related pathways, and, where available, expression data from sesame seed and tissue-specific transcriptomes (e.g., Sinbase 2.0). Genes with homology to known regulators of plant height, hormone signaling, or flavonoid biosynthesis were given higher priority**.** Using the LD decay distance of 204 kb, genes in ± 204-kb windows of the SNPs were searched, giving seven candidate genes with functions related to

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the traits (Table 4, Figure 5). For PH, in the SNP window on chromosome 11 (Chr11_1877114), we found Sindi.11G025000, an *AP2/ERF*-domain transcription factor. *AP2/ERF* Transcription factors are regulators of ethylene-responsive genes and control cell expansion [47]. Near the SNP cluster on chromosome 8 (Chr08_1771424), we found Sindi.08G015600, called *CYP90B1* (DWF4), a cytochrome P450 that controls brassinosteroid biosynthesis. Changes in this gene cause dwarfism [48].

For SCC, analysis found transcription factors. Sindi.06G123400 was called *WRKY23*, a TF that activates genes in the anthocyanin area, near an L-linked SNP on Chromosome 6 [34]. In a chromosome 3 area, Sindi.03G078100 was called *DOF3.1*, a DNA-binding protein in light-regulated gene expression and pigment [49]. A b-linked SNP on chromosome 12 was connected to Sindi.12G045200, an SBP (SQUAMOSA Promoter-Binding Protein)-like transcription factor, which controls pigmentation [22]. These findings suggest that plant height is regulated by hormonal pathways (e.g. brassinosteroid via *CYP90B1* and ethylene via *AP2/ERF*), while seed coat color is controlled by *WRKY*, *DOF*, and *SBP-like* transcription factors modulating flavonoid biosynthesis.

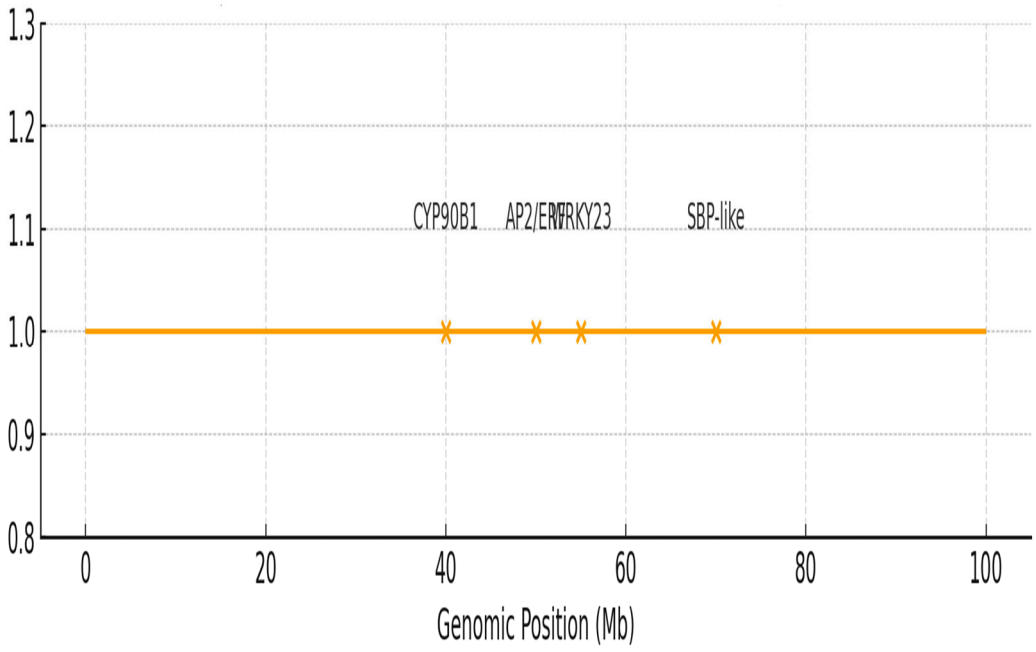

Figure 5. Genomic positions and annotations of high-priority candidate genes identified through integrated meta-QTL and GWAS analysis. Candidate genes (labeled) are mapped to their physical coordinates (megabases, Mb) on sesame chromosomes (Chr3, 6, 8, 11, 12). Significant GWAS SNPs (black diamonds) are shown relative to gene positions. Gene functions are color-coded: blue for plant height candidates (*CYP90B1*, *AP2/ERF*), and orange for seed coat color candidates (*WRKY23*, *DOF3.1*, *SBP-like*). Meta-QTL hotspot regions are indicated by shaded gray bars.

Table 4. High-priority candidate genes associated with significant single-nucleotide polymorphisms (SNPs) for plant height and seed coat color.

Trait	SNP Marker	Candidate Gene	Putative Function	Sequence Identity (%)
Plant Height	Chr11_1877114	Sindi.11G025000	*AP2/ERF* domain-containing protein	95.2

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Plant Height	Chr08_1771424	Sindi.08G015600	Cytochrome P450 *CYP90B1* (Brassinosteroid biosynthesis)	88.7
a*	Chr06_27694080	Sindi.06G123400	*WRKY* transcription factor 23	96.0
L*	Chr12_16523829	Sindi.12G045200	Squamosa promoter-binding protein 1	99.2
a*	Chr03_15984975	Sindi.03G078100	DOF zinc finger protein *DOF3.1*	80.7
a*	Chr03_26242291	Sindi.03G090200	Serine/threonine-protein kinase STY8	100
b*	Chr09_22387055	Sindi.09G078500	Salicylic acid-binding protein 2	98.5

Note: Sequence identity (%) refers to the percentage of identical amino acids in a BLASTP alignment against the specified reference species (*Sesamum indicum* v3.0).

3. Discussion

3.1. Validation of Genomic Regions and Discovery of New Alleles

By integrating meta-QTL analysis with field phenotyping and publicly available resequencing data, we validated conserved genomic regions and identified novel variants within the Ethiopian sesame population.

The meta-QTL hotspots on chromosomes 8 and 11, identified for plant height, have been confirmed by the presence of significant SNPs within germplasm from Ethiopia (Chr08_1771424, Chr11_1877114), with stable effects across environments [29, 34, 43]. The meta-QTL hotspot on chromosome 11 has a high FST score for comparisons between Africa and Asia [9, 13, 27, 32, 33, 43], thereby indicating adaptation within the Ethiopian germplasm.

For seed coat color, the meta-QTL hotspot meta-Q06 on chromosome 6, which was reported to be linked with the intensity of pigmentation in Asian germplasm [11, 23], was identified in the Sudanese [27] and Ethiopian germplasm (SNP Chr06_27694080 for a*).

Notably, the GWAS revealed novel trait-associated loci on chromosomes 12 and 13 that were absent in previous Asian-centric studies. These regions show higher nucleotide diversity in African accessions [13, 23, 26, 34], indicating that Ethiopian sesame harbors unique allelic variation shaped by local adaptation [6, 10, 15, 18]. Including African diversity is therefore essential for capturing the full genetic potential of sesame [12, 14, 50] for breeding.

3.2. Hormonal Regulation of Plant Architecture

Candidate gene analysis identifies hormonal mechanisms regulating plant height. The brassinosteroid biosynthesis gene *CYP90B1* and ethylene-responsive gene *AP2/ERF* are expressed together within plant height-related regions, indicating a mechanism to simultaneously regulate stem growth. *CYP90B1* gene variations result in dwarfing in various plant species [48, 51], whereas *AP2/ERF* proteins mediate ethylene signaling to growth, although in a different manner [20, 45, 47]. The brassinosteroid and ethylene interactions result in plant height regulation in Arabidopsis thaliana and rice [52, 53], which is probably analogous to sesame. Marker-assisted crop improvement

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via gene modification may result in short, lodging-resistant crop cultivars that are amenable to mechanical harvest systems [2, 4, 28, 54].

3.3. Transcriptional Networks Behind Seed Coat Color

Seed coat color in sesame is regulated by transcription factors that control the phenylpropanoid/flavonoid biosynthesis pathway. Our results are consistent with previous findings (Table 5). The major QTL clusters on chromosomes 4, 6, and 9 have been consistently documented [21, 23, 26, 29], with a marker linked to the gene (qBSCchr6) on chromosome 6, described as a major locus of brown seed coat color [11, 55]. GWAS revealed the importance of *WRKY23*, *DOF3.1*, and *SBP-like* transcription factors. *WRKY* regulates anthocyanin gene expression in a stressed environment [20, 34], while DOF regulates gene expression in a light environment [49]. Elsafy et al., [27] also revealed *WRKY* and DOF transcription factors in the seed coat color transcriptional network. A higher heritability ($H^2 > 0.95$) of the L*, a*, and b* indices in the Ethiopian materials shows that these qualities are genetically fixed to a great extent and are less likely to be affected by environmental factors [27]. A negative correlation between L* and a* indices implies that for these seeds, it is desirable to be high in lightness at the expense of reduced healthy phenolic compounds with respect to their anthocyanin levels [21].

Table 5. Synthesis of major QTL hotspots for sesame seed coat color from previous studies.

Chr.	Key QTL Region	PVE Range (%)	Population	Key Candidates	Reference
4	qSCa-4.1, qscca*4 (~78-81 cM)	8.56–23.10	RIL, F_3	*DIR* gene family	[26, 29]
6	qBSCchr6 (1.19 Mb interval)	Major QTL	RIL (BSA)	13 candidate brown seed locus	[43]
6	Meta-QTL hotspot	8.50–25.50	Meta-analysis	*PPO*, *WRKY* TFs	This study (Table 2)
9	qsccY9, qsccZ9 (~90-104 cM)	32.88–33.25	F_3	*MYB*, *bHLH* TFs	[26]
9	Meta-QTL hotspot	10.15–32.88	Meta-analysis	*MYB/bHLH* complex	Table 2
12	qsccZ12	5.58	F_3	–	[26]
12, 13	Novel GWAS associations	6.22–6.51	Ethiopian panel	*SBP-like*, Kinase STY8	Table 3

3.4. Population Structure and LD Decay

The population structure analysis revealed two genetic clusters (K = 2), reflecting geographical and agroecological regions [12, 15, 27]. The rate of LD decay revealed high recombination rates and genetic diversity for Ethiopian (~204 kb) and Sudanese (~0.204 Mb) germplasm, which is consistent

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with the high genetic base of African landraces when compared to some Asian (~370 kb) germplasm [19]. The use of kinship and population structure covariates in the FarmCPU approach minimized false positives, which is evident from the proper calibration of the Q-Q plots [41, 56].

3.5. From Discovery to Application: A Molecular Toolkit for Sesame Breeding

The integration of meta-QTL, trait-associated SNPs, and functionally annotated candidate genes is useful for sesame breeding. Our study is the first to integrate the meta-QTL and GWAS framework applied to unlock the genetic potential of Ethiopian sesame germplasm. We demonstrate that this underutilized gene pool contains not only alleles for known major loci but also novel, population-specific genetic variation crucial for adaptation. This study provides validated molecular markers and candidate genes that constitute a practical toolkit for marker-assisted sesame breeding. The validated meta-QTL intervals can offer priority regions for introgression and background selection. Trait-associated SNPs with moderate-to-high PVE, such as Chr11_1877114 for PH, Chr06_27694080 for color, can be converted into robust KASP markers for high-throughput screening. The candidate genes, *CYP90B1* and *AP2/ERF*, and the transcription factors *WRKY23* and *DOF3.1*, provide functional targets for gene-editing or allele-specific marker development. The Ethiopian diversity panel itself serves as a valuable source of novel alleles for pre-breeding.

Given the high heritability and significant effects of SNPs, genomic selection models incorporating these markers (3,633 SNPs) could achieve genome-wide prediction accuracy of > 0.7 for both plant height and seed coat color [31, 57, 58]. For immediate application, breeders can use the identified SNPs to pyramid favorable alleles for optimal plant height and desirable seed coat color (e.g., high L for white-seeded types) in elite backgrounds. Functional validation of the identified candidate genes and favorable alleles is necessary to facilitate sesame breeding.

3.6. Limitations and Future Directions

It is known that landrace accessions generally exhibit a high degree of genetic diversity within each accession, and this diversity can pose a challenge when characterizing phenotypes and genotypes. Here, we addressed the challenge by the single-seed descent (SSD) method for two generations in each landrace accession, thereby obtaining homogeneous lines. We are therefore measuring the phenotypes and genotyping of the SNPs of individuals that have largely homozygous genetic backgrounds, which leads to higher mapping precision and less noise from intra-accession heterogeneity. However, a certain amount of heterogeneity may still be present, and subsequent investigations can take advantage of deep sequencing or haplotype-based methods to reveal landrace diversity. Our meta-QTL analysis has combined data from studies that used different types of populations for genetic mapping, different marker systems, and different genetic maps. We aligned all the positions to physical reference to have a common ground, but differences in population size, marker density, and QTL detection power remain in different studies and may affect the stability of consensus intervals. In addition, differences in the resolution of mapping and thresholds for detection that arise from the use of both biparental QTL and GWAS data without limitations cannot be eliminated, even if the data has been handled carefully. By setting stringent hotspot criteria (3 independent QTL within 5 Mb) and performing functional validation through independent GWAS in Ethiopian germplasm, these limitations have been partially counterbalanced.

4. Materials and Methods

4.1. Global Meta-QTL Analysis

A systematic meta-analysis was conducted to identify consensus genomic regions plant height (PH) and seed coat color (SCC) in sesame. The following protocol was implemented to ensure transparency, reproducibility, and comparability across studies. A comprehensive literature search was done for all published QTL mapping and GWAS on PH and SCC in sesame until January 2025.

 doi:10.20944/preprints202601.0381.v1

Search keywords included: “sesame QTL”, “Sesamum indicum plant height”, “seed coat color QTL”, and “sesame genome-wide association”. From an initial pool of over 85 publications, 28 studies met the initial screening criteria of reporting primary QTL or marker-trait association data. After rigorous evaluation for completeness and comparability, eight studies were selected for the final meta-analysis. The inclusion criteria were peer-reviewed publication with primary QTL or GWAS data; clearly defined trait measurements for PH or SCC; reported chromosomal positions, genetic/physical map intervals, logarithm of odds (LOD) scores, and phenotypic variance explained (PVE); and availability of marker sequences or alignment information to allow mapping to a common reference genome. The eight studies included in the meta-analysis were: [11, 21, 23, 26, 29, 32-34]. Data extraction and synthesis followed standard meta-analytic principles to mitigate bias. A summary of these studies, including mapping method, population type, size, genetic map used, and marker system, is provided in Supplementary Table S1. Supplementary Table S1 provides a comprehensive summary of each study, including mapping method, population type and size, genetic map used, marker system, reported QTL intervals, logarithm of odds (LOD) scores, and phenotypic variance explained (PVE).

Data on QTL included trait name, QTL linkage group [31]. markers, genetic position (cM), LOD score, and PVE. All the genetic positions were converted to the physical coordinates of the reference sesame genome version 3.0 [35] based on the sequence information of the markers. The meta-analysis was done using BioMercator v3.0 [36]. For each trait, QTL were gathered based on physical positions. Meta-QTL were found through a two-step process: (1) choosing the number of meta-QTL on each chromosome using model choice criteria (AIC, AICc, BIC), and (2) finding the consensus position and confidence interval for each meta-QTL. A genomic area was called a "meta-QTL hotspot" if it had three or more independent QTL from different investigations in a 5 Mb area. Candidate genes within these hotspot intervals were retrieved from the S. indicum v3.0 genome annotation [35] and functionally annotated.

4.2. Plant Materials and Field Experimental Design

A total of 200 sesame samples were obtained from the Ethiopian Biodiversity Institute gene bank in Addis Ababa. The samples consisted mainly of landraces from five regional states in Ethiopia, which are major sesame production regions: Tigray (n = 56), Amhara (n = 50), Oromia (n = 44), Benishangul Gumuz (n = 32), and Gambella (n = 18). Three released cultivars, 'Adi', 'Humera-1', and 'Kelafo-74', were included as checks to evaluate performance and environmental effects. Kelafo-74 is a semi-dwarf, late-maturing, medium-yielding sesame with black seeds. 'Adi' is a tall, early-maturing, high-yielding sesame with white seeds, and 'Humera-1' is a medium-height, early-maturing, high-yielding sesame with white seeds and high oil content. To address the genetic heterogeneity typical of landraces, each accession was purified through two generations of single-seed descent (SSD) prior to field trials. This process ensured that each accession was represented by a genetically uniform line, minimizing within-accession variance and enhancing the accuracy of both phenotyping and genotyping. Bulk seed from the SSD-derived lines was used for field experiments and DNA extraction.

Field experiments were carried out for two growing seasons, i.e., 2024 and 2025, at Werer Agricultural Research Center (WARC), Afar Region, Ethiopia (9°36′N, 40°05′E, 570 m above sea level). The location is characterized by semi-arid conditions, with an annual rainfall of 650 mm, silt loam soil containing 1.2% organic carbon, and a pH of 7.8. An augmented block design with eight blocks was used. All 200 test samples and three check cultivars were allocated to every block. The plot contained four 4 m rows with 30 cm spacing between rows and 10 cm between plants, with a total plot size of 3.6 m². Standard practices were followed, including irrigation, weeding, fertilizer, and pest management.

4.3. Phenotyping

 doi:10.20944/preprints202601.0381.v1

Phenotyping was done at maturity. Plant height was included as one of the target traits because it is a key determinant of plant architecture and lodging resistance and is related to agronomic performance and yield potential. Plant height (PH) was measured in centimeters from the soil to the top of the main stem. Ten plants per plot were measured in centimeters, and the sample's mean PH was recorded. Seed coat color (SCC) was evaluated because it is an important quality and market trait with clear phenotypic contrast among sesame cultivars, making it highly informative for genetic analysis. Seed coat color was measured using a Konica Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan). Color measurement had three samples of 50 grams of seeds. Before each session, the chroma meter was calibrated using a standard white calibration tile (L* = 93.7, a* = 0.3160, b* = 0.3323). Color was recorded in the CIELAB color space, defined by three parameters: lightness (L*, 0=black to 100=white), green-red axis (a*; negative values are green, positive values are red), and blue-yellow axis (b*; negative values are blue, positive values are yellow. Three technical replicates per accession and parameter (L*, a*, and b*) were averaged and used in subsequent analysis. The coefficient of variation between values was < 1%, meaning the measurement was precise.

4.4. SNP Data Processing

Whole-genome resequencing data for the 200 Ethiopian accessions were obtained from publicly available whole-genome resequencing data from BioProject PRJNA626474, which includes 705 global sesame accessions [35]. Our panel represents a subset of these accessions, specifically those of Ethiopian origin. Raw sequencing reads were aligned to the S. indicum v3.0 reference genome using BWA-MEM v0.7.17. Variant calling was performed using GATK v4.2 following best practices for germline short variant discovery. Given the SSD-derived nature of the lines, within-accession heterogeneity was minimal; however, to ensure accuracy, genotype calling was performed using a pooled allele frequency threshold of ≥0.8 for homozygous calls. Genotype data in VCF format were filtered using PLINK v1.9 and VCFtools with the following criteria: minor allele frequency (MAF) ≥ 0.03; individual genotype missing rate ≤ 20%; SNP call rate ≥ 80%; Hardy–Weinberg equilibrium p-value > 1×10^{-6}; and removal of indels and multi-allelic sites. SNPs with a minor allele frequency (MAF) < 0.03 were excluded to remove rare variants that could produce spurious associations. After filtering, 3,633 high-confidence biallelic SNPs were retained for downstream population genomic and GWAS analyses.

4.5. Comparative Genomic Analysis

Given the limited availability of publicly deposited raw variant data specifically for African sesame germplasm, we performed comparative analysis by referencing published findings and summary statistics from major sesame genomics studies. We focused on data from [37], who re-sequenced 705 global sesame accessions, including 62 from Ethiopia, data available under BioProject PRJNA626474. A summary of key public genomic resources used and referenced in this study is provided in Supplementary Table S1. From their published supplementary materials and results, we extracted published summary statistics including allele frequencies, population differentiation (FST), and nucleotide diversity (π) for genomic regions corresponding to our GWAS hits. This approach allowed us to contextualize our Ethiopian-specific accessions within global sesame diversity without requiring reprocessing of raw sequencing data.

4.6. Population Structure, Kinship and Linkage Disequilibrium Analysis

Population structure analysis was carried out using the algorithm in ADMIXTURE v1.3.0 [38]. Runs were carried out for values of K ranging between 1 and 10, using cross-validation with 10 folds for each K [39]. K with the lowest cross-validation error was selected. Ancestry proportions as estimated by the Q-matrix output from K=2 were incorporated as covariates in the GWAS model to account for population stratification. The K-matrix was calculated to model genetic relatedness

 doi:10.20944/preprints202601.0381.v1

among individuals. The K-matrix was calculated to model genetic relatedness among individuals. The K-matrix was generated using the identity-by-state (IBS) algorithm in TASSEL v5.2 [40]. Genome-wide linkage disequilibrium (LD) was found using PLINK to measure the correlation (r^2) between all pairs of SNPs in a 1 Mb window. r^2 values were plotted against the distance in kilobases between SNP pairs. The distance at which the smoothed curve, fitted with a LOESS regression, dropped to half its maximum value was taken as the LD decay distance and used to define the candidate gene search window around significant SNPs.

4.7. Genome-Wide Association Analysis

GWAS analysis between the 3,633 SNPs and the traits (PH, L*, a*, and b*) was done using the Fixed and Random Model Circulating Probability Unification (FarmCPU) method [41], in the GAPIT3 R package v3.1.0 (Wang and Zhang, 2021). FarmCPU uses a Fixed-Effect Model (FEM) to test SNPs for association and a Random-Effect Model [6, 10] to control the background, reducing false positives. Population structure and kinship matrix were used. Marker-trait associations were significant at a level decided by Bonferroni correction at $\alpha = 0.05$, or $-\log_{10} \geq 4.86$ [2]. Manhattan plots and quantile-quantile (Q-Q) plots were drawn to show GWAS results and measure model fit.

4.8. Candidate Gene Identification and In Silico Functional Annotation

For each SNP, a candidate genomic area was defined as the region ± the LD decay distance (~204 kb). All annotated genes in these areas were taken from the S. indicum v3.0 GFF3 file. Protein sequences were taken and studied using BLASTP searches against the NCBI non-redundant (nr) protein database (E-value cutoff $< 1 \times 10^{-5}$). Protein domain structure was studied using InterProScan v5.52-86.0 [42]. Candidate genes were chosen based on known functions, mostly genes in plant hormone production/signaling (e.g., PH) and phenylpropanoid/flavonoid production (e.g., SCC).

4.9. Phenotypic Data Analysis

For both traits, the mean, range, standard deviation, and coefficient of variation were calculated. Pearson's correlation coefficients between traits were also estimated. Principal component analysis (PCA) was done in R on the trait matrix (PH, L*, a*, and b*) using the prcomp function. The FactoMineR and factoextra packages were used for PCA visualization. Broad-sense heritability (H^2) for each trait across the seasons was measured using variance components from a linear mixed model:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/(er)}$$

where $\sigma^2 g$ is the genotypic variance, $\sigma^2 ge$ is the genotype-by-environment interaction variance, $\sigma^2 \varepsilon$ is the residual error variance, e is the number of environments (seasons), and r is the number of replicates per environment. For test entries, replication was derived from the research design, and variance components were measured using the lme4 package in R.

5. Conclusions

This study demonstrates the power of combining global meta-analysis with population-specific GWAS to dissect the genetic architecture of complex traits within underutilized germplasm. We identified and validated six conserved meta-QTL hotspots for plant height and seed coat color, pinning the stability of those genomic regions across diverse sesame populations. More importantly, our GWAS on Ethiopian landraces has unraveled novel trait-associated loci on chromosomes 12 and 13, thus pointing out some unique allelic variation from the African gene pool, which was missed in previous Asian-centric studies. The high-priority candidate genes identified include CYP90B1 and AP2/ERF for plant architecture and WRKY23, DOF3.1, and SBP-like genes related to pigmentation, that may provide a functional target for further validation. The rapid LD decay (~204 kb) and clear population structure (K=2) of the Ethiopian panel facilitate fine-mapping and allele mining. Collectively, this work provides a validated molecular toolkit comprising meta-QTL intervals, trait-

 doi:10.20944/preprints202601.0381.v1

associated SNPs, and candidate genes that can be immediately deployed in marker-assisted selection programs to accelerate the improvement of sesame, particularly by introgressing favorable alleles from Ethiopian germplasm into elite breeding lines.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org, Table S1: Summary of studies included in the meta-QTL analysis.

Author Contributions: Conceptualization: A.G., R.O. and R.V.; Methodology: A.G. and R.O.; Software: A.G.; Validation: A.G., R.O. and R.V.; Formal Analysis: A.G.; Investigation: A.G. R.O. and R.V.; Resources: R.O. and R.V.; Data Curation: A.G. and R.O.; Writing -- Original Draft Preparation: A.G.; Writing -- Review and Editing: A.G., R.V. and R.O.; Visualization: A.G.; Supervision: R.O. and R.V.; Project Administration: R.O. and R.V.; Funding Acquisition: R.O. and R.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Carl Tryggers Stiftelse för Vetenskaplig Forskning, grant number CTS 23:2654. The APC was funded by the Swedish University of Agricultural Sciences.

Data Availability Statement: The curated phenotypic dataset and the filtered SNP dataset (VCF format) for the Ethiopian panel are available from the corresponding author upon reasonable request. The plant materials are maintained by the Ethiopian Biodiversity Institute (EBI), Addis Ababa, and may be requested according to EBI's material transfer agreements. Public resequencing data used for comparative analysis is available under BioProject PRJNA626474.

Acknowledgments: We thank the Carl Tryggers Stiftelse for funding this research and covering the APC. We thank the Ethiopian Biodiversity Institute (EBI), Addis Ababa, for the resources. We thank the staff at the Werer Agricultural Research Center (WARC) for their help. The authors thank SLU Bioinformatics Infrastructure (SLUBI) for technical help during the bioinformatic data analysis. We acknowledge the researchers who generated the global sesame resequencing dataset (PRJNA626474), which enabled valuable contextualization of our Ethiopian-specific findings.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

AIC	Akaike Information Criterion
AICc	Corrected Akaike Information Criterion
AP2/ERF	APETALA2/ETHYLENE RESPONSE FACTOR
BIC	Bayesian Information Criterion
BSA	Bulked Segregant Analysis
BWA-MEM	Burrows-Wheeler Aligner-Maximal Exact Matches (bioinformatics tool)
Chr	Chromosome
cM	Centimorgan
CR-400	Model of the Konica Minolta Chroma Meter
CV	Coefficient of Variation
CYP90B1	Cytochrome P450 90B1 (DWF4 gene)
DIR	*DIR*igent (gene family)
DOF	DNA-binding One Zinc Finger
EBI	Ethiopian Biodiversity Institute
F_2	Second Filial Generation
F_3	Third Filial Generation
F_7	Seventh Filial Generation
F_8	Eighth Filial Generation
FAO	Food and Agriculture Organization
FarmCPU	Fixed and Random Model Circulating Probability Unification
FEM	Fixed-Effect Model
FST	Fixation Index (population genetic statistic)
GAPIT	Genome Association and Prediction Integrated Tool
GATK	Genome Analysis Toolkit

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GBS	Genotyping-by-Sequencing
GFF3	General Feature Format version 3
GWAS	Genome-Wide Association Study
H^2	Broad-sense Heritability
HTRX	Haplotype Trend Regression with eXclusion
IBS	Identity-by-State
K	Number of genetic clusters (in population structure)
KASP	Kompetitive Allele-Specific PCR
kb	Kilobase
LD	Linkage Disequilibrium
LOD	Logarithm of Odds
LOESS	Locally Estimated Scatterplot Smoothing
L*	Lightness (CIELAB color space parameter)
a*	Green-Red component (CIELAB color space parameter)
b*	Blue-Yellow component (CIELAB color space parameter)
MAF	Minor Allele Frequency
MAS	Marker-Assisted Selection
Mb	Megabase
MYB	v-*MYB* avian myeloblastosis viral oncogene homolog (transcription factor family)
bHLH	Basic Helix-Loop-Helix (transcription factor family)
NCBI nr	National Center for Biotechnology Information non-redundant (database)
PCA	Principal Component Analysis
PEG	Polyethylene Glycol
PH	Plant Height
PLINK	Whole genome association analysis toolset
PPO	Polyphenol Oxidase
PVE	Phenotypic Variance Explained
Q-matrix	Ancestry proportion matrix (from population structure)
Q-Q plot	Quantile-Quantile plot
QTL	Quantitative Trait Locus/Loci
r	Number of replicates
r^2	Squared correlation coefficient (measure of LD)
RAD-seq	Restriction-site Associated DNA Sequencing
REM	Random-Effect Model (mentioned as background model in FarmCPU)
RIL	Recombinant Inbred Line
SBP	SQUAMOSA Promoter-Binding Protein
SCC	Seed Coat Color
SIACS9	Sesamum indicum 1-aminocyclopropane-1-carboxylic acid synthase 9
SICEN2	Sesamum indicum Centroradialis 2
Sindi	Sesamum indicum (gene prefix in genome annotation)
SLAF	Specific-Length Amplified Fragment
SLUBI	Swedish University of Agricultural Sciences Bioinformatics Infrastructure
SLU	Swedish University of Agricultural Sciences
SNP	Single Nucleotide Polymorphism
SSD	Single-Seed Descent
SSR	Simple Sequence Repeat
STY8	Serine/Threonine-protein kinase STY8
TASSEL	Trait Analysis by aSSociation, Evolution and Linkage
TF	Transcription Factor
VCF	Variant Call Format
VCFtools	Variant Call Format tools
WARC	Werer Agricultural Research Center
WRKY	Transcription factor family named after conserved *WRKY* domain

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