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Genome-Wide Association Study Uncovers Loci and Candidate Genes Underlying Phytosterol Variation in Sesame (*Sesamum indicum* L.)

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Abstract: Sesame is one of the most important oilseed crops grown worldwide. It provides diverse nutraceuticals—including lignans, unsaturated fatty acids (UFA), phytosterols, etc.—to humans. Among sesame's nutraceuticals, phytosterols have received less attention from sesame breeders, although their biological and pharmacological functions have been recorded. Therefore, in the present study, we evaluated the variation of phytosterol contents in 402 sesame accessions grown in two environments and revealed their associated loci and candidate genes. Gas chromatography (GC) analysis unveiled that sesame mainly contains four phytosterols: campesterol, stigmasterol, β-sitosterol, and Δ5-avenasterol. β-sitosterol (1.6–4.656 mg/g) was the major phytosterol, followed by campesterol (0–2.847 mg/g), stigmasterol (0.356–1.826 mg/g), and Δ5-avenasterol (0–1.307 mg/g). The total phytosterol content varied from 2.694 to 8.388 mg/g. Genome-wide association study identified 33 significant associated single nucleotide polymorphism (SNP) loci for the four traits, of which Ch6-39270 and Ch11-142842 were environmentally stable and simultaneously linked with campesterol and stigmasterol content variation. Candidate genes screening indicated that *SINPZ1100015* encoding a NAC domain-containing protein 43 is likely the major candidate effect gene of phytosterol variation in sesame. The results of this study extend knowledge of phytosterol variation in sesame and provide important resources for markers-assisted breeding of high-phytosterol content varieties.

Keywords: sesame; plant sterols; GWAS; SNP loci; NAC protein; SiNST1

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1. Introduction

Phytosterols, or plant sterols, are a group of natural products that exist in various organs at all stages of plants' development [1]. Phytosterols have received broad concern as a critical diet component and possess huge applications in foods, cosmetics, medicines, etc. [2]. They have shown a wide range of biological and pharmacological abilities, including promotion of lipids metabolism [3]; lowering cholesterol level [4,5]; and anticancer [6,7], anti-neurodegenerative [2], antioxidant and anti-microbial [8], and anti-cardiovascular disease activity [9]. Consequently, they have become compounds of countless research interests aiming to understand their physiological functions and improve their natural levels in seed oils through genetic engineering.

Structurally, sterols are based on 1,2-cyclopentane perhydrophenantrene and are distinguished by a hydroxyl moiety at the C-3 position and a side chain at the C-17 position [10]. Compared to animals, which contain only one main sterol type (cholesterol), plants possess diversified and complex sterol compositions [11]. Hitherto, over 250 types of phytosterols

have been identified, among which β-sitosterol, stigmasterol, and campesterol are the most abundant in diverse plant species [11,12]. β-sitosterol and stigmasterol differ from campesterol in terms of the alkyl group in the side chain at the C-24 position. Campesterol has a methyl at C-24 (24-methylsterol), while the others have an ethyl group (24-ethylsterols) [12]. The diversity of phytosterols is associated with various *in planta* functions. They are precursors of a class of plant hormones, namely brassinosteroids (BRs), which regulate plant growth and development [12]. Besides their conversion into BRs, phytosterols are basically integral components of biological membranes. They regulate membrane fluidity and permeability to influence its functions, properties, and structure [13,14]. In addition, they are involved in transmembrane signal transduction via lipid microdomain formation and by modulating the activity of membrane-bound enzymes [12,14]. Accordingly, they play important roles during embryogenesis, cell proliferation and elongation, hormone signaling, vascular differentiation, and plant tolerance to environmental stresses [13,15–17]. Furthermore, they are required for the coordinated assembly of lipid droplets, which are intracellular organelles essential for energy storage and lipid metabolism in developing seeds [18].

The biosynthesis of phytosterols occurs in the endoplasmic reticulum mainly via the cycloartenol pathway and consists of three steps (Figure 1) [11,12]. The first step is the mevalonate pathway consisting of the conversion of acetyl-CoA into squalene via the formation of mevalonate and many intermediate reactions [11,12]. The second step consists of squalene cyclization up to the formation of cycloartenol [11,12]. The final step is the conversion of cycloartenol into phytosterols via a series of oxidative demethylation reactions [11,12]. The plant sterols biosynthetic pathway involves many enzymes, among which 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), C24-sterol methyltransferase (SMT), and C22-sterol desaturase (CYP710A) are the key ones [1,12]. The three major phytosterols are biosynthesized principally via SMT, sterol-Δ^{24}-isomerase/reductase, or Dimunito/Dwarf1 (DWF1), and CYP710A [11].

Sesame (*Sesamum indicum* L., 2n = 26) is one of the most ancient oil crops widely cultivated in Asia, Africa, and South America [19]. Its oil contains important nutraceuticals such as UFA, lignans, vitamin E, and phytosterols. Therefore, with regard to nutraceutical functions, efforts are being made for the crop's quality improvement. Accordingly, genetic markers and candidate genes associated with fatty acids (FAs), lignans, vitamin E have been detected [20–23]. However, despite their huge pharmacological capacities, no study has yet focused on phytosterol content variation in large sesame germplasms, and the genetic basis of its natural variation in sesame is undetermined. Therefore, it is necessary to reveal the variation and dissect the genetic basis of phytosterol content in sesame for quality-breeding purposes. This study investigated the composition and natural variation in phytosterol contents of 402 sesame accessions grown in two environments. Moreover, we performed a genome-wide association study (GWAS) for phytosterol traits in sesame for the first time and identified several significant SNP loci and candidate genes. These findings represent valuable resources for functional studies towards the genetic improvement of phytosterol contents in sesame.

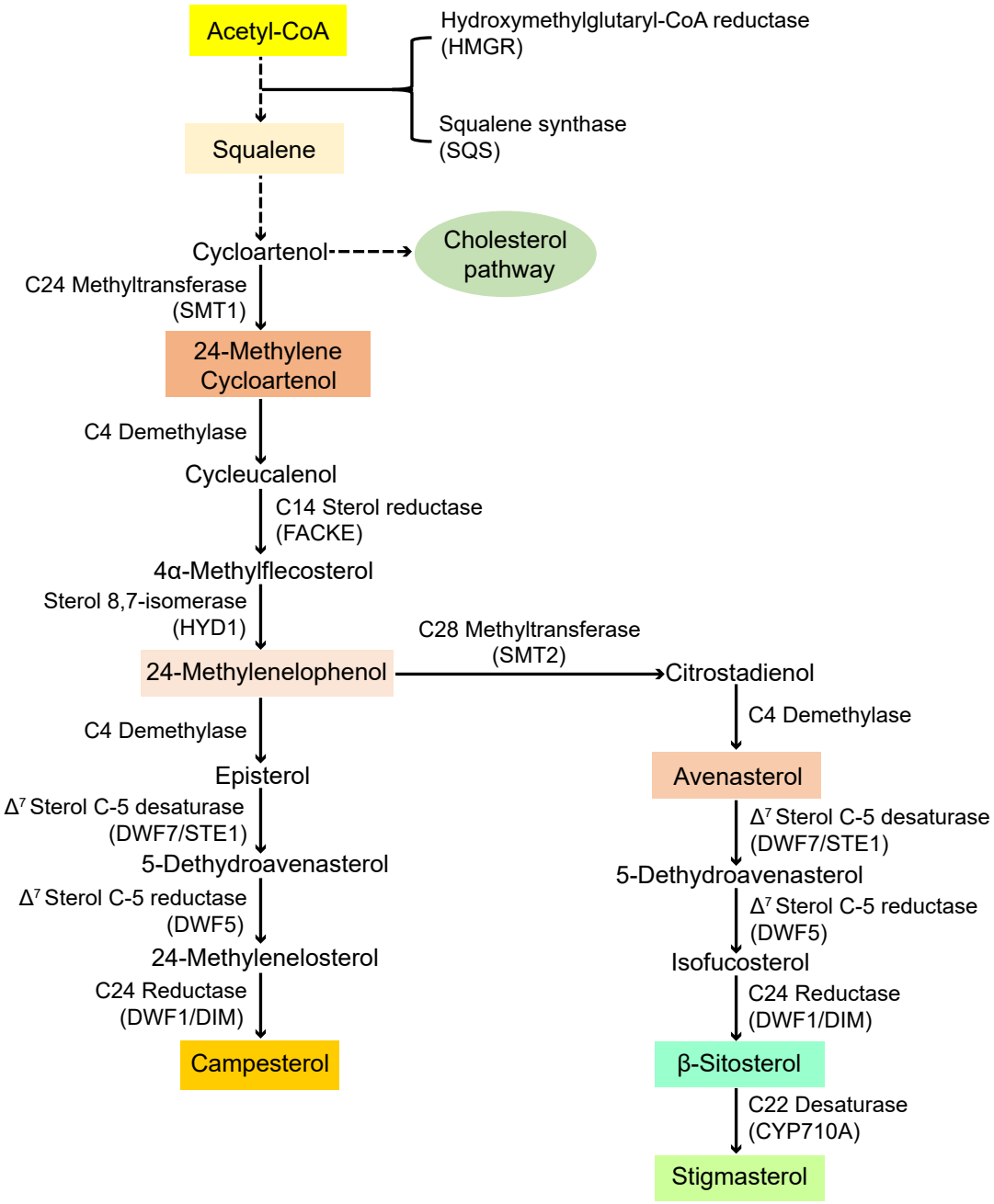

Figure 1. A diagram of the phytosterol biosynthesis pathway in plants. Adapted from [11,12,24].

2. Materials and Methods

2.1. Plant Materials

The 402 sesame accessions were given by the National Medium-Term Sesame Gene Bank of China (Wuhan, China). The population was cultivated at two experimental field stations of the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences (OCRI-CAAS) located in Wuhan (WH) and Zhumadian (ZMD). Wuhan belongs to the Yangtze River valley with a subtropical humid monsoon climate, while Zhumadian belongs to the Huang-Huai River valley with a warm temperate climate. Standard agronomic practices were applied in field management, and the experiments were conducted in a completely randomized block design, with three replications at each location. When the seeds reached maturity, they were harvested and dried. Seeds from the three replications were mixed and stored at the seed room for phytosterol content analysis.

2.2. Phytosterol Content Analysis

The sesame oil was extracted from 5.5 g of each seed sample in triplicate via a manual press. First, the primary extracts were centrifuged at 13,000 rpm for 10 min, and the clear oil supernatants were collected. Next, to 0.2 g of each seed pure oil extract, 0.5 mL of alpha-cholestanol was added as internal standard. Then, the samples were saponified by adding 10 mL of 2 M KOH–ethanol solution, followed by shaking at 60 °C for 1 h. After cooling at room temperature, 10 mL of n-hexane and 4 mL of distilled water were added to the saponification solution, vortex mixed, and the reaction was allowed to stand for 15 min. Next, the upper n-hexane extract was collected. This step was repeated twice, and the collected n-hexanes were pooled, following dehydration by anhydrous Na_2SO_4 and then evaporation to dryness. Finally, 100 µL of trimethylsilylation reagent (N, O-Bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane) was added to the dried sample followed by derivatization at 105 °C for 15 min, and dissolution in 1 mL n-hexane.

The dissolved solution was analyzed using an Agilent 6890N gas chromatography (GC, Wilmington, NC, USA) equipped with a flame ionization detector (FID) and a DB-5HT (30 m × 0.22 mm × 0.1 µm) capillary column. The column temperature was initially set at 60 °C and held for 1 min and then increased to 310 °C at the rate of 40 °C/min and maintained at this temperature for 10 min. The FID temperature was set at 320 °C. The injection volume was 1 µL with a split ratio of 1:25. Helium served as the carrier gas at a 2 mL/min flow rate. The composition and concentration of the sterols were analyzed by the relative retention time and standard internal methods [25].

2.3. Statistical Analysis

The descriptive statistical analysis was performed by Microsoft Excel 2010. The frequency distributions were explored by SPSS (version 25.0, SPSS Inc, Chicago, IL, USA). The Pearson correlation coefficients for different phytosterols were calculated using the R package 'ggplot', and the statistical differences were determined by Student's *t*-test.

2.4. Genome-Wide Association Analysis

5,385,583 single-nucleotide polymorphisms (SNPs) generated from the whole-genome resequencing of 402 worldwide sesame accessions data in our group (unpublished data) were used for the genome-wide association study (GWAS). The population structure (Q) analysis was carried out using the Admixture software, and the kinship (K) matrix was implemented using the Tassel 5.0 software [26]. It was reported that the linkage disequilibrium (LD) windows in the sesame genome were 88 kb [20,27]. The significance threshold was set as $-\log_{10}(P) \geq 5$. The GWAS for phytosterol content was conducted using the EMMAX software with the MLM (mixed linear model). The R package 'qq-man' was used to visualize GWAS results as Manhattan and QQ plots [28]. The PVE (R^2) of each significant associated peak SNP was calculated by the regression model in Microsoft Excel 2010.

2.5. Identification of Candidate Gene and Haplotype Analysis

The candidate genes were extracted from the LD widow (±88 kb) of the significant associated SNP loci detected in the two environments [20,27]. Gene Ontology (GO) enrichment analysis was performed using an online tool at https://www.omicshare.com/tools/ (accessed on 1 December 2021). The LD (linkage disequilibrium) analysis and drawing were carried out with LDBlockShow software [29]. The haplotype analysis was performed using CandiHap software [30]. Haplotypes were classified according to all SNPs with MAF > 0.05 in the candidate gene. Haplotypes containing at least 10 accessions were used to perform comparative analysis, and the difference in the corresponding phytosterol content among the haplotypes was analyzed using Student's *t*-test.

2.6. Gene Expression Analysis of Candidate Genes

Two contrasting sesame accessions in campesterol and stigmasterol contents, G268 (campesterol, 1.547 mg/g; stigmasterol, 0.498 mg/g) and G580 (campesterol, 0.758 mg/g; stigmasterol 1.419 mg/g) were selected for gene expression analysis. They were grown at the Wuhan experimental station from May to September 2021. Seed samples were collected at 20 days post-anthesis (DPA) on ice, frozen in liquid nitrogen, and then stored at −80 °C for RNA extraction.

The total RNA of the seed samples was extracted using EASYspin Plus Plant RNA Kit (Vazyme Biotech, Nanjing, China). The quantity and quality of RNA were evaluated by the ND-1000 NanoDrop spectrometer (NanoDrop Technologies, Wilmington, DE USA). The RNA was reverse-transcribed into cDNA with HiScriptIIQRT SuperMix (Vazyme Biotech, Nanjing, China). The qRT-PCR was conducted in triplicated with ChamQ™ SYBR1 qPCR Master Mix (Vazyme Biotech, Nanjing, China) on LightCycler480 real-time (RT) PCR system (Roche, Basel, Switzerland) following the manufacturer's instructions. The specific primers for the selected genes were designed using Primer Premier 5.0 (PREMIER Biosoft, San Francisco, CA, USA), and the sesame H3.3 gene was used as an internal control (Table S1). The relative expression levels of genes were calculated by the comparative $2^{-\Delta\Delta Ct}$ method [31].

3. Results

3.1. Phenotypic Variation and Correlation Analysis of Primary Phytosterols in Sesame

To reveal the natural variation in phytosterol content in sesame seeds, 402 sesame accessions were cultivated in two different environments located in Wuhan (WH) and Zhumadian (ZMD), and mature seed samples were collected and analyzed by GC. The results indicated that sesame contains four phytosterols: campesterol, stigmasterol, β-sitosterol, and Δ5-avenasterol. The summary of the descriptive statistics of the four phytosterol variation in sesame is presented in Table 1. The total phytosterol content varied from 2.694 to 8.206 mg/g and 2.986 to 8.388 mg/g in WH and ZMD, respectively. β-sitosterol and campesterol exhibited the highest contents with an average range of 2.662–3.003 mg/g and 1.129–1.298 mg/g, respectively. The average content of Δ5-avenasterol was the lowest in the two environments, with a range of 0.467–0.544 mg/g. Among them, campesterol and Δ5-avenasterol exhibited higher variations, and the coefficient of variation (CV) at the two locations was 28.85 and 31.14 for campesterol and 28.04 and 38.72 for Δ5-avenasterol. The lowest CV at the two locations of 15.87 and 17.58 was observed for β-sitosterol, indicating it is less affected by environmental conditions. The stigmasterol content varied from 0.356 to 1.825 mg/g, with CV ranging from 22.24 to 24.38. The four phytosterol contents in the two environments were almost normally distributed (Figure S1), indicating that they might be under the control of several loci. To facilitate the overview of differences in the variation of the four phytosterol contents in the two environments, we graphed the data and performed a one-way analysis of variance (ANOVA (Figure 2a)). The results confirmed that environmental conditions significantly influence phytosterol content in sesame.

Table 1. Variation of primary phytosterol contents in sesame

Trait	Environment	Min (mg/g)	Max (mg/g)	Mean (mg/g)	SD	CV (%)
Campesterol	2018_WH	0	2.847	1.129	0.352	31.14
	2019_ZMD	0	2.751	1.298	0.375	28.85
Stigmasterol	2018_WH	0.415	1.826	0.867	0.193	22.24
	2019_ZMD	0.356	1.682	0.793	0.193	24.38
β-Sitosterol	2018_WH	1.794	4.656	3.003	0.477	15.87
	2019_ZMD	1.6	4.114	2.662	0.468	17.58
Δ5-Avenasterol	2018_WH	0	1.307	0.467	0.181	38.72
	2019_ZMD	0.155	1.012	0.544	0.153	28.04
Total phytosterol	2018_WH	2.694	8.206	5.496	0.798	14.6
	2019_ZMD	2.986	8.388	5.297	0.853	16.11

Min, minimum; Max, maximum; SD, standard deviation; CV, coefficient of variation.

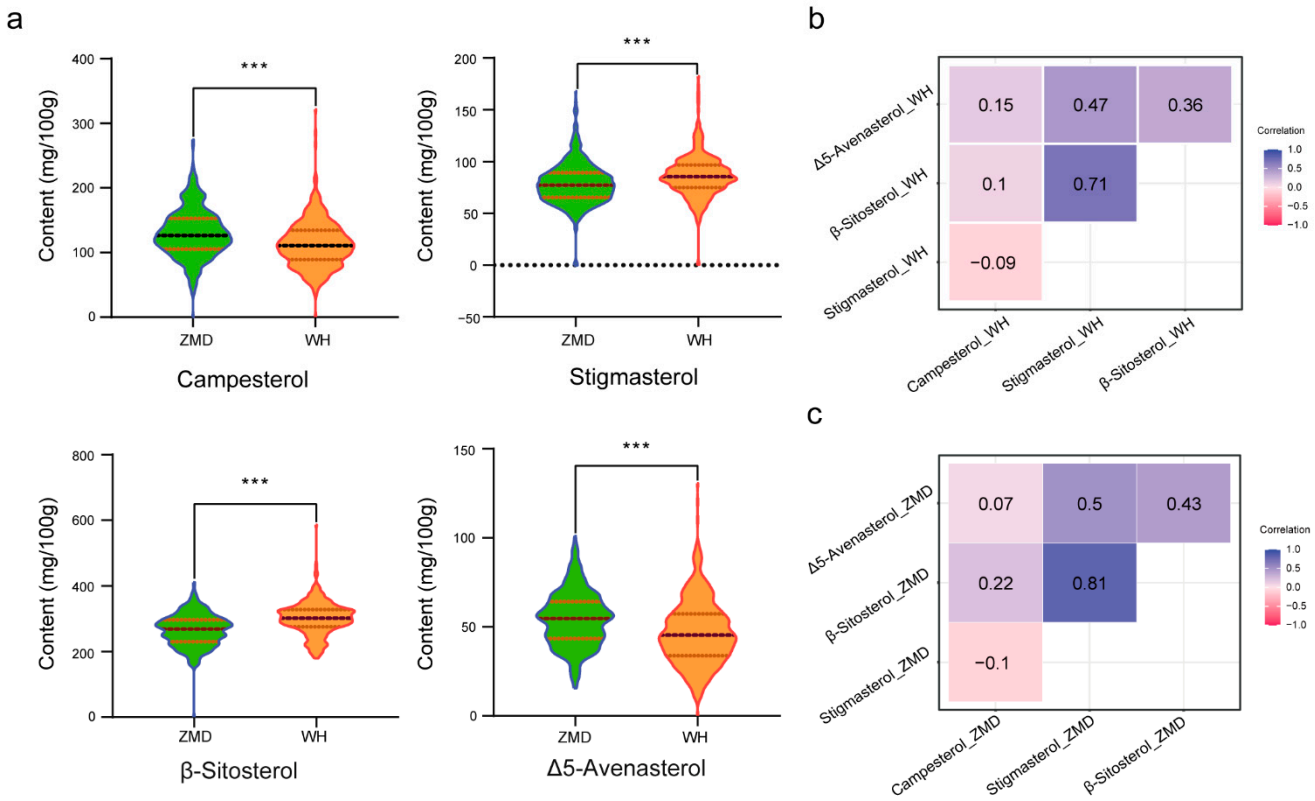

Figure 2. Variation and correlation of four phytosterol contents in sesame. Variation of four phytosterol contents in two different environments (**a**). *** indicates significant difference at $p < 0.001$. The correlation between the four phytosterols in Wuhan, WH (**b**) and Zhumadian, ZMD (**c**).

We performed correlation analysis to investigate whether it would be possible to improve the four phytosterol contents simultaneously in sesame. The correlation trends among the four phytosterols in the two environments were consistent (Figure 2b,c). β-sitosterol, Δ5-avenasterol, and stigmasterol exhibited positive correlations with the most significant positive correlation observed between β-sitosterol and stigmasterol (r = 0.71 and 0.84 in WH and ZMD, respectively). The campesterol and stigmasterol contents were negatively correlated in the two environments.

3.2. GWAS for Four Phytosterol Contents in Sesame

The GWAS revealed 33 significant SNP loci explaining a PVE ranging from 1.37 to 31.02% associated with the four phytosterols (Table 2). The Manhattan and QQ plots for all traits at the two locations are shown in Figure S2. These loci are distributed on all the 13

chromosomes of the sesame genome. Notably, we detected 9, 12, 5, and 4 significant SNP loci for campesterol, stigmasterol, β-sitosterol, and Δ5-avenasterol, respectively. It is worth noting that two loci, Ch6-39270 and Ch11-142842, were associated with campesterol and stigmasterol in the two environments.

Table 2. List of the significant SNP loci associated with four phytosterol contents variation in two environments in sesame

Environment	Trait	Locus	Chr	Position	Ref	Alt	*p*-Value	PVE (%)
WH	Campesterol	Chr2-370158	2	370158	C	T	5.603	8.94
		Chr3-18989758	3	18989758	C	G	5.744	6.47
		Chr4-10138424	4	10138424	C	T	6.095	9.60
		Chr5-9706516	5	9706516	C	T	5.312	7.76
		Chr6-39270	6	39270	A	C	5.424	1.63
		Chr8-5486783	8	5486783	C	A	6.005	2.86
		Chr11-142842	11	142842	C	A	9.079	7.95
		Chr13-12554381	13	12554381	G	A	6.257	9.03
	Stigmasterol	Chr-1-13179445	1	13179445	G	A	5.207	4.53
		Chr3-15930743	3	15930743	G	A	6.098	5.22
		Chr4-18569755	4	18569755	G	A	6.668	11.40
		Chr8-25004528	8	25004528	C	A	5.954	3.03
		Chr10-15465843	10	15465843	T	C	5.282	6.67
		Chr11-142842	11	142842	C	A	19.675	28.24
		Chr12-10292195	12	10292195	C	T	7.182	9.44
	β-Sitosterol	Chr4-4016241	4	4016241	T	C	5.033	5.98
		Chr8-3623152	8	3623152	G	A	5.076	3.33
		Chr11-1227222	11	1227222	T	C	5.321	8.35
		Chr13-14801278	13	14801278	A	G	5.124	4.80
	Δ5-Avenasterol	Chr2-7188241	2	7188241	C	A	5.607	2.61
		Chr11-5738482	11	5738482	G	A	10.211	6.42
		Chr12-10290279	12	10290279	G	A	5.115	7.19
ZMD	Campesterol	Chr4-13279017	4	13279017	C	T	5.182	1.37
		Chr6-39270	6	39270	A	C	7.070	8.20
		Chr11-142842	11	142842	C	A	14.887	17.87
	Stigmasterol	Chr3-23741	3	23741	G	T	12.830	22.01
		Chr6-39270	6	39270	A	C	12.154	19.11
		Chr11-142842	11	142842	C	A	18.351	22.11
		Chr7-17668358	7	17668358	G	A	10.523	19.41
		Chr11-111895	11	111895	G	A	21.507	31.02
		Chr13-19899439	13	19899439	A	G	10.635	19.54
	β-Sitosterol	Chr3-14800933	3	14800933	G	A	5.301	4.41
	Δ5-Avenasterol	Chr9-6530662	9	6530662	C	T	5.238	6.75

PVE, phenotypic variance explained.

3.3. Candidate Genes Associated with Phytosterol Contents

The common loci (Chr11:142842 and Chr06:39270) of campesterol and stigmasterol detected in the two environments were selected for candidate genes identification. We screened the ±88 kb window of each locus and disclosed a total of 37 genes (Table S2). Of the 37 candidate genes, only 14 were annotated. Gene ontology (GO) enrichment analysis revealed that these genes are mainly involved in the metabolic process (eight genes), cellular process (seven genes), binding (eight genes), and catalytic activity (five genes) (Figure 3). We selected the eight genes assigned into the metabolic process and examined their expression levels at a critical sesame seed development stage of 20 DPA [32,33], in G268 (high campesterol and low stigmasterol contents sesame accession) and G580 (low campesterol and high stigmasterol contents sesame accession) via qRT-PCR. The results indicated that six of these genes are differentially expressed during seed development in G580 and G268 (Figure S3). Among them, SINPZ1100015 exhibited the most differential expression in the two sesame accessions.

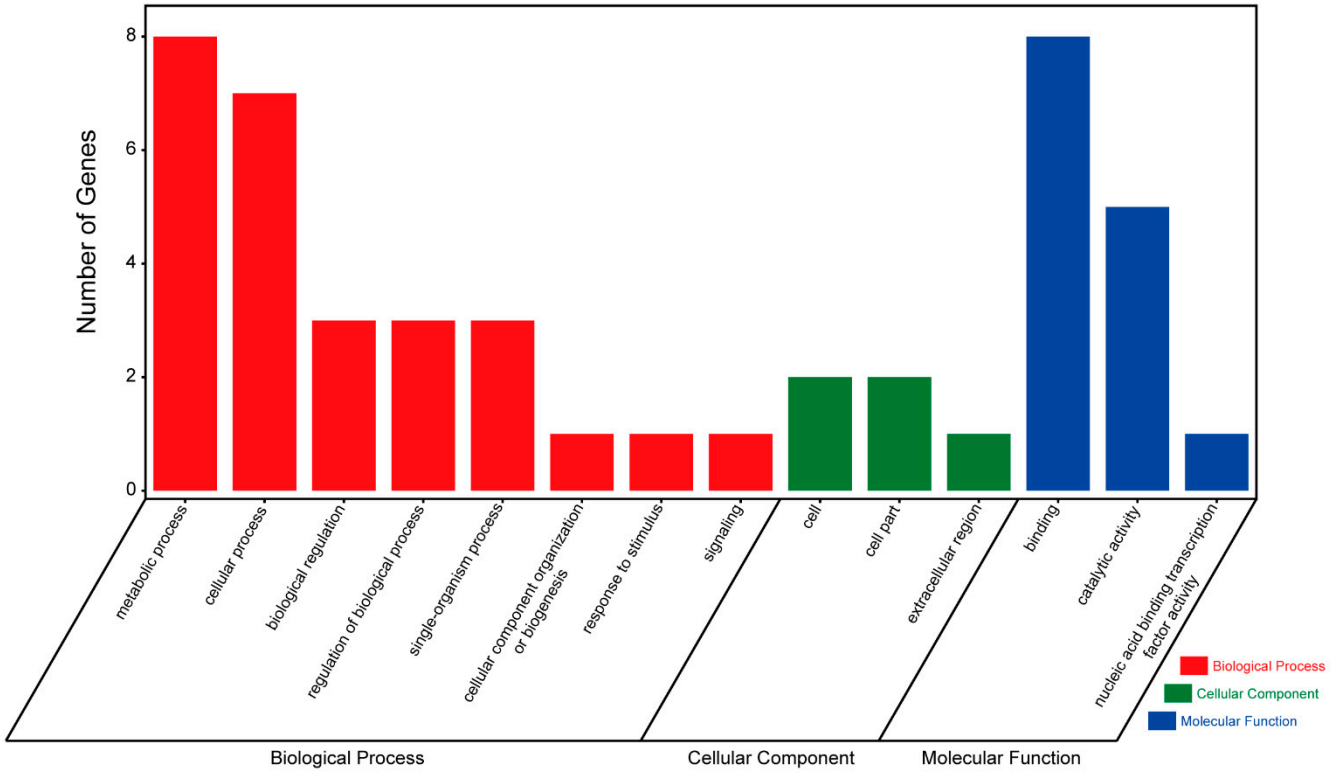

Figure 3. Gene ontology (GO) enrichment analysis of the 37 candidate genes. The red, green, and blue bars represent the biological process, cellular component, and molecular function, respectively.

SINPZ1100015 locate at the pleiotropic locus Chr11:142842 of campesterol and stigmasterol (Figure 4a,b). Interestingly, it may also contribute significantly to oil and lignans contents variation in sesame [20,23]. SINPZ1100015 encodes a NAC domain-containing protein 43 and is known to substantially influence the elongation of cells and deposition of secondary cell walls [34–36]. The LD analysis results corroborated the association analysis (Figure 4c,d). SINPZ1100015 contains three exons and two introns and harbored one type of SNP mutation (Figure 4e). The SNP mutation is a nonsynonymous mutation in the exon of the gene. It consists of allele change from C to A, leading to amino acid change from threonine (Thr) to lysine (Lys). The expression level of SINPZ1100015 in the seed of G268 at 20 DPA was significantly higher than that of G580 (Figure 4f). We identified two haplotypes of this gene, Hap1 (C/C) and Hap2 (A/A). Hap1 is associated with high campesterol content and low stigmasterol content, while Hap2 is associated with low campesterol content and high stigmasterol content (Figure 4g,h).

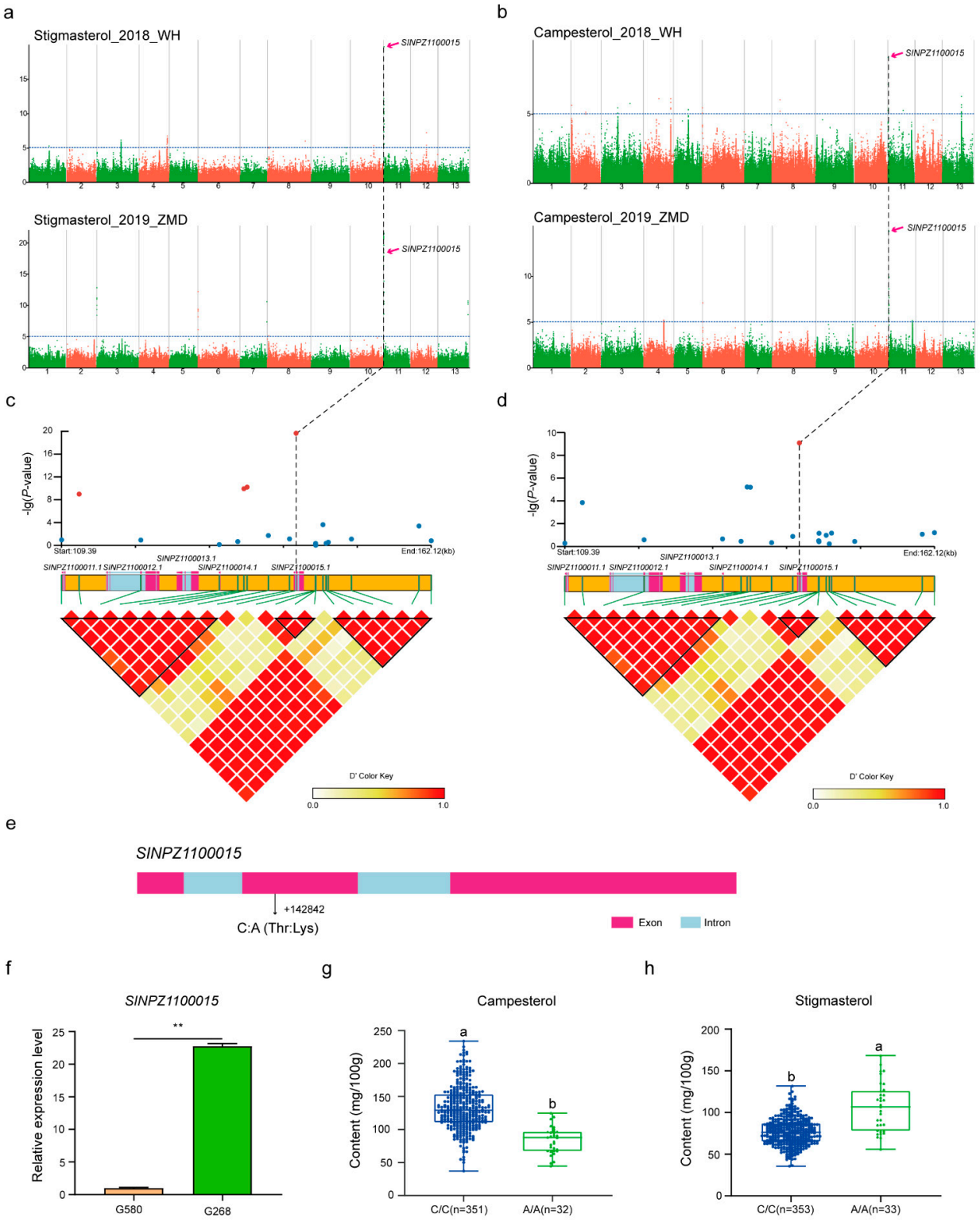

Figure 4. Colocalization, expression, and haplotype analysis of the candidate gene *SINPZ1100015* associated with campesterol and stigmasterol content variation in sesame. Manhattan plots of stigmasterol (**a**) and campesterol (**b**) in WH and ZMD. Locus Chr11:142842 LD heatmap for stigmasterol (**c**) and campesterol (**d**). The gene's structure (**e**). The red and grey rectangles line indicate upstream, exons, and introns, respectively. The bottom left is the synonymous SNP, and the right is the non-synonymous SNP. Expression levels of *SINPZ1100015* in G268 and G580 (two contrasting campesterol and stigmasterol content sesame accessions) seed at 20 DPA (**f**). ** indicates significant difference at $p < 0.01$. Campesterol (**g**) and stigmasterol (**h**) contents in the two haplotypes of *SINPZ1100015*. The different letter indicates significant differences at $p < 0.05$.

4. Discussion

It is essential for breeders to understand quantitative agronomic trait variation in crop germplasms under various environments as it helps select genotypes. In sesame, the variability of phytosterol contents was still not well understood. Ryan et al. [37] have tested one sesame variety and found that the content of β-sitosterol, campesterol, and stigmasterol was 1.39, 0.223, and 0.415 mg/g, respectively. Liu et al. [38] have evaluated five sesame oil samples and found that they contained brassicasterol, β-sitosterol, campesterol, and stigmasterol in the range of 0.0031–0.0044, 0.746–1.131, 0.112–0.182, and 0.113–0.129 mg/g, respectively. In this study, we analyzed 402 sesame accessions grown in two environments and found that sesame mainly contains four phytosterols, including β-sitosterol, stigmasterol, campesterol, and Δ5-avenasterol. This results suggest that the brassicasterol detected by Liu et al. might be due to contamination during experimental processes [38]. In agreement with previous studies, we found that β-sitosterol (1.909–4.287 mg/g) is the most predominant phytosterol in sesame. Campesterol, stigmasterol, and Δ5-avenasterol content varied from 0 to 2.847, 0.356 to 1.826, and 0 to 1.307 mg/g, respectively. Compared with other crops, the composition and content of phytosterols in sesame are different. In rice, only three phytosterols, namely campesterol, stigmasterol, and β-sitosterol, have been detected [13]. In pecan nut, Δ5-avenasterol (1.139–2.144 mg/g) has been reported as the second most abundant sterol [39], while in sesame, we found that Δ5-avenasterol is the least abundant sterol. In rapeseed oil, it is reported that the main phytosterols include β-sitosterol (0.976–2.148 mg/g), followed by campesterol (0.636–1.364 mg/g), and brassicasterol (0.375–0.678 mg/g) [40]. Consistent with studies in wheat and sunflower, we found that the variability of phytosterol content in sesame is attributable to genotypes and environmental conditions [41,42]. The content of phytosterols gradually increased in response to changes in the external environment [43]. β-sitosterol content exhibited the lowest CV of 15.87 and 17.58 at the two locations, indicating it might be easier to improve its content through genetic engineering. Taken together, these findings confirm that β-sitosterol is the most prevalent and abundant phytosterol in plants and imply that it might play critical roles in the growth and development of plants. It is reported that the ratio of campesterol to sitosterol influences *Arabidopsis* plant growth [44]. β-sitosterol may also affect the physical and chemical properties of ordered microdomains to modulate signal pathways of biological membranes [45].

Phytosterols are naturally occurring compounds essential for plant species survival and human health [2,3,8,9,11,12]. In sesame, GWAS has become the method of choice for identifying genetic variants associated with complex agronomic traits [46,47]. Herein, we performed GWAS for the four phytosterols in sesame and detected 33 significant SNP loci, among which Ch6-39270 and Ch11-142842 were associated with campesterol and stigmasterol in the two environments. We searched for candidate genes for phytosterol variation at these two loci and selected 37 genes for future functional studies. Campesterol and stigmasterol contents were negatively correlated at the two locations, indicating that it might not be possible to breed for high content of campesterol and stigmasterol in sesame. The biosynthetic pathways of the two phytosterols represent support of this finding. In plants, the ratio between 24-methylcholesterol (campesterol) and 24-ethylcholesterol (β-sitosterol) is of great importance for maintaining membranes' properties and cell survival, which in turn influence normal growth and development of the whole plant [12]. A decrease in the content of campesterol may negatively affect BRs biosynthesis leading to a dwarfism phenotype [44]. Meanwhile, the stigmasterol/β-sitosterol ratio in membranes may influence plants' response to various abiotic and biotic stresses [12]. β-sitosterol is the precursor of stigmasterol. At the locus Ch11-142842, associated with campesterol and stigmasterol, locate the gene *SINPZ1100015* (SIN_1005755/ SiNST1). It encoded a NAC domain-containing protein 43 and was previously associated with oil and lignans contents variation in sesame [20,23]. Its homologous AT2G46770 in *Arabidopsis* is a master regulator of secondary wall synthesis [34–36,48]. As integral components of biological membranes, variation in phytosterol contents may significantly influence secondary cell walls formation.

In cotton, it was shown that changes in the composition and content of phytosterols affect fiber cell elongation and secondary cell wall deposition [49]. The qRT-PCR analysis showed that *SINPZ1100015* is significantly expressed in high campesterol content sesame varieties compared to high stigmasterol varieties. Haplotype analysis showed that the Hap1 (C/C) of *SINPZ1100015* are favorable for higher campesterol accumulation in seeds. In contrast, the Hap2 (A/A) is favorable for high stigmasterol accumulation in seeds. These results indicate that *SINPZ1100015* is the major candidate effect gene of phytosterol contents variation in sesame. Targeting this gene may help improve sesame seed quality. Therefore, functional studies are required to decipher the molecular mechanisms underlined by this gene for exploitation in sesame breeding.

5. Conclusions

In summary, this study characterized the composition and variation of phytosterols in sesame. Sesame seeds contain four phytosterols—β-sitosterol, campesterol, stigmasterol, and Δ5-avenasterol according to their order of abundance. Although these traits are significantly influenced by environmental conditions, they could be improved through genetic engineering. Thirty-three (33) significant SNP loci associated with the four phytosterols were detected, and 37 candidate genes were selected for future studies. Among them, *SINPZ1100015* was associated with stigmasterol and campesterol contents variation and may represent the major candidate effect gene for phytosterol variation in sesame. Our findings represent key resources for future studies regarding the dissection of molecular mechanisms involved in phytosterol biosynthesis and regulation in sesame and for crop improvement.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12030392/s1, Figure S1: Histograms for the frequency distribution of phytosterols in WH and ZMD; Figure S2: Manhattan plots and QQ plots of the four phytosterol traits content in WH and ZMD; Figure S3: Expression levels of eight selected candidate genes in two contrasting campesterol and stigmasterol contents sesame accessions; Table S1: List of the primers used for the qRT-PCR analysis; Table S2: List of the 37 candidate genes detected at the environmentally stable loci associated with campesterol and stigmasterol variation.

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