



Article

Glycogen Synthase Kinase 3 (GSK3) Gene Family in *Glycine max* Under the Effect of Manganese Stress

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Abstract

Glycogen synthase kinase 3 (GSK3/SHAGGY-like kinase) plays a pivotal role in regulating plant growth, development, and stress responses. To elucidate the characteristics of the GSK family in *Glycine max*, this study employed whole-genome data combined with bioinformatic and gene expression analyses to investigate the gene structure, chromosomal localization, collinearity, phylogenetic evolution, promoter cis-elements and differential gene expression analysis. Additionally, the expression patterns of *GmGSK* genes under manganese (Mn) stress and their associated phenotypic alterations were analyzed. A total of 22 *GmGSK* family members were identified, all harboring the characteristic GSK kinase domain. These members are distributed across 16 chromosomes, encoding proteins ranging from 380 to 802 amino acids (aa) in length. Phylogenetic analysis classified the *GmGSK* family into four evolutionary clades, consistent with patterns observed in *Arabidopsis* and *Oryza sativa*. Members within the same clade share identical exon-intron structures and conserved motifs. Collinearity analysis revealed that segmental duplication events have been crucial in the functional expansion of the *GmGSK* family through intraspecific collinearity. In recent years, alongside industrial development and fertilizer imbalance, the effective manganese concentration in agricultural soils has risen abnormally in some regions of China, leading to toxic effects on crops. Soybean, an oilseed crop relatively sensitive to manganese, has been adversely impacted. Clarifying the response mechanisms of soybean seedlings to manganese stress is therefore of significant importance for improving both yield and quality. Manganese stress treatment induced significant up-/down-regulation of specific *GmGSK* members in soybean, concomitant with pronounced inhibition of root elongation and leaf growth. This study provides a theoretical framework for deciphering the molecular regulatory mechanisms by which the *GmGSK* gene family mediates plant responses to Mn stress, offers insights into soybean Mn tolerance mechanisms, and establishes a foundation for genetic improvement of Mn-tolerant traits in crops.



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1. Introduction

Glycogen synthase kinase-3 (GSK-3) proteins constitute a group of highly conserved multifunctional kinases, defined by a conserved serine/threonine protein kinase catalytic domain (PF0069) [1]. GSK-3 regulates fundamental biological pathways through inhibitory

mechanisms and acts downstream of diverse cellular signaling factors in eukaryotes [2,3]. Glycogen synthase kinase-3 (GSK-3) was initially characterized as a kinase in mammals [4]. Two isoforms exist in mammals: GSK-3 α and GSK-3 β , both of which are involved in diverse physiological processes, including cell cycle regulation, induction of cell differentiation, and glycogen metabolism [5]. In contrast, GSK-3 kinase genes have been demonstrated to exhibit broader functional diversity in plants. GSK kinases participate in regulating diverse biological processes, including plant growth and development, physiological metabolism, and stress resistance responses [6]. Glycogen synthase kinase 3 (GSK3) is frequently involved in signal transduction networks and may indirectly influence responses to heavy metal stress. For example, GSK3 can regulate the activity of transcription factors, such as those in the MYB family, which play critical roles in heavy metal tolerance. However, direct links between GSK3 and heavy metal stress-specific target genes remain unclear. However, functional characterization of other members within the GSK gene family remains primarily limited to *Arabidopsis* and *Oryza sativa*. Systematic investigations into the stress-responsive properties, phylogenetic relationships, and expression patterns of GSK genes in diploid soybean have not yet been conducted.

Since the Industrial Revolution, heavy metal pollution has become increasingly severe, often being linked to potential health risks. Consumption of Mn-contaminated food and drinking water can lead to neurological damage, osteoporosis, and liver or kidney failure [7]. Manganese (Mn) is an essential micronutrient for plant mineral nutrition, required for normal growth, development, and metabolic functions [8]. *Glycine max* primarily acquires micronutrients through root uptake from the soil solution. These nutrients are subsequently translocated to the shoot via the xylem and allocated to various tissues to support physiological processes and growth requirements. The bioavailability of manganese (Mn), an essential micronutrient, is governed by a complex interplay of edaphic factors [9]. Crucially, Mn solubility and availability exhibit a strong inverse relationship with soil pH, decreasing significantly as pH rises. Furthermore, soils with low moisture content or high porosity often present conditions of low Mn bioavailability due to reduced mobility and diffusion rates [10]. This geochemical principle underpins a clear regional dichotomy in Mn-related nutritional disorders affecting soybean cultivation in China. In the northern regions, the predominance of calcareous soils, characterized by alkaline pH and frequently low soil moisture, leads to prevalent conditions of Mn deficiency. This deficiency can limit crop growth and yield [11]. Conversely, in the acidic soils of southern China, low pH coupled with high soil humidity promotes the excessive solubilization and availability of Mn. Under these conditions, Mn accumulation can reach toxic levels, inducing Mn toxicity stress that impairs plant metabolism, growth, and ultimately, productivity [12]. However, when soil Mn concentration exceeds a critical threshold, it becomes toxic, impairing plant growth, development, and physiological–biochemical processes [13,14]. Manganese toxicity damages plant physiological processes, mainly including inhibiting photosynthesis, chlorophyll biosynthesis, destroying key enzyme activities, interfering with the absorption and transport of other essential mineral elements, and, through unrestricted production of reactive oxygen species, inducing oxidative stress [15,16]. Under Mn stress, plant responses vary across growth stages [17]. Excess Mn²⁺ suppresses leaf photosynthesis, manifesting visible toxicity symptoms including chlorosis, brown spots, and leaf crinkling [18]. Beyond leaves, roots are also affected—lateral root number and dry weight decrease [19,20], and root architecture disruption further compromises nutrient uptake and distribution. Critically, Mn toxicity impacts shoot tissues more severely than roots, with leaves being the primary target; nearly 90% of absorbed Mn translocates to aboveground tissue. Therefore, manganese toxicity constitutes a major constraint limiting plant growth and productivity. It is imperative to investigate the mechanisms by which manganese stress affects plant growth

and strategies for mitigating such stress. Research on the effects of excessive manganese on soybean growth and transcriptomics will facilitate the breeding of manganese-tolerant crop varieties through genetic improvement, thereby enhancing crop yield and quality.

Soybean (*Glycine max* L.) is a globally significant economic crop. Compared to other legume crops and animal proteins, soybean serves as a cost-effective and superior source of high-quality edible oil and protein for humans [21], provides supplementary feed for livestock [22], and has industrial applications in biodiesel production and renewable energy development [23]. With rapid population growth, the demand for soybean production continues to increase [24]. However, its yield and quality are hindered by various environmental stressors, such as drought, high salinity, heavy metals, and other biotic stresses. Mn stress is a critical constraint on soybean yield and quality. Therefore, investigating the molecular mechanisms of soybean responses to diverse environmental stresses is essential for breeding stress-resistant cultivars. In recent years, omics approaches have been employed to investigate the response mechanisms of soybean to manganese toxicity. Research findings demonstrate that root growth is significantly inhibited under Mn stress conditions. Proteomic analyses further reveal substantial alterations in protein synthesis/metabolism, cell wall composition, and signal transduction pathways [23]. Nevertheless, metabolomic studies on soybean Mn toxicity responses remain scarce, and no reports exist on metabolomic profiling of soybean leaves and roots exposed to Mn toxicity. This experiment analyzed phenotypic changes and corresponding physiological indicators in soybean leaves and roots under high-manganese treatment. To further investigate regulatory patterns under manganese stress, transcriptomic approaches were employed to compare gene expression profiles in soybeans under normal and high-manganese conditions. This work provides foundational data for understanding manganese tolerance in soybeans and establishes a basis for genetic improvement of manganese-tolerance traits in crops.

2. Results

2.1. Identification and Physicochemical Characterization of the GmGSK Gene Family in Soybean

Through integrated BLAST and HMM analyses in TBtools-II (v2.390), followed by the removal of sequences lacking the characteristic GSK kinase domain, we identified 22 high-confidence *GmGSK* family members in the soybean genome. Chromosomal localization analysis revealed that the 22 *GmGSK* genes are distributed across all 16 soybean chromosomes (Figure 1). Notably, chromosomes 4, 10, and 12 each harbor two or more *GmGSK* members, while the remaining 13 chromosomes contain single-copy genes. Physicochemical characterization of the soybean *GmGSK* family members revealed substantial variation among the 22 proteins (Additional S1 and S7). Key findings include polypeptide length (380–802 amino acids), gene size (43,089.95–87,841.92 bp), molecular weight (42.88–87.84 kDa), and pI (6.47–9.03 with 4 members exhibiting acidic pI < 7). These findings demonstrate that the majority (81.8%) of *GmGSK* proteins are basic, while a minor fraction (18.2%) exhibits acidic properties.

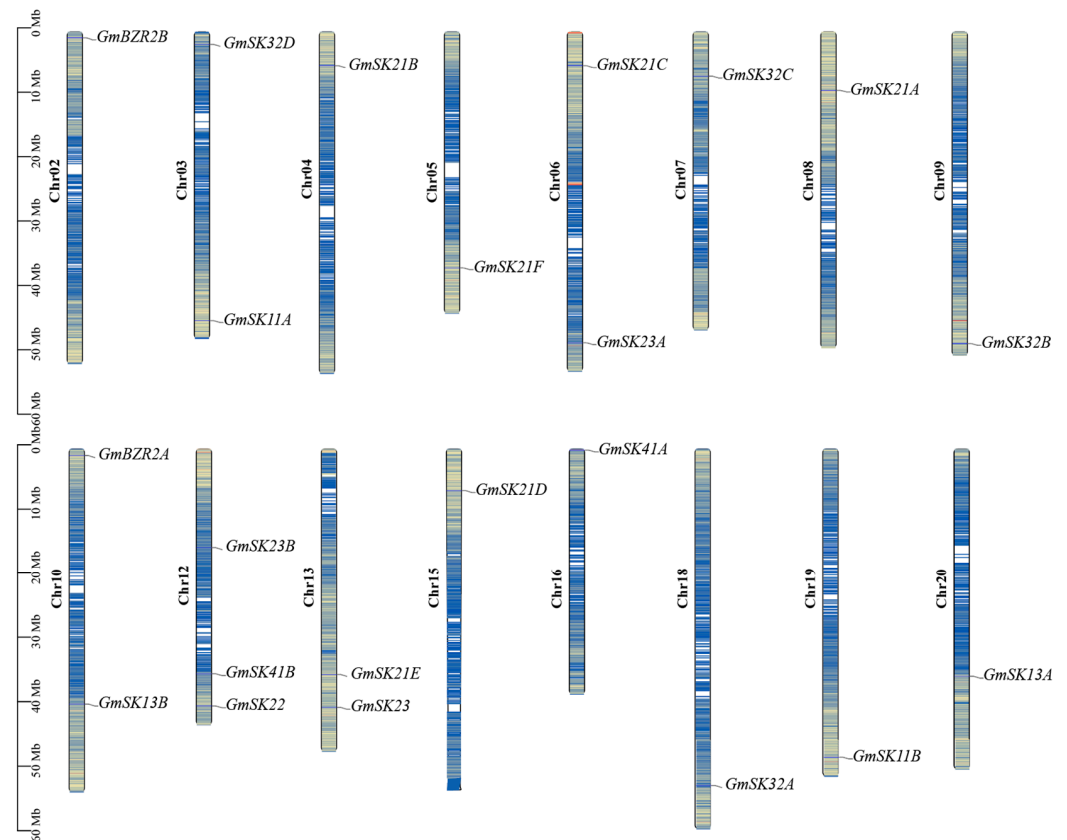


Figure 1. Localization analysis on chromosomes of *Glycine max* GmGSK gene family members.

2.2. Phylogenetic Analysis of the Soybean GmGSK Gene Family

To elucidate the evolutionary relationships between the soybean GmGSK gene family and GSK orthologs from other species, we performed multiple sequence alignment of GSK proteins from *Arabidopsis thaliana*, *Oryza sativa*, and *Glycine max*. Phylogenetic reconstruction revealed consistent clustering into four distinct subfamilies in all three species, mirroring the subfamily classification observed in soybean-specific analysis (Figure 2). The phylogenetic tree demonstrates significant homology among GSK gene families across these three species.

2.3. Structural Organization, Conserved Motifs, and Functional Domains of the Soybean GmGSK Gene Family

Conserved protein motif analysis of soybean GmGSK family members was performed using the MEME suite, which revealed identical composition and sequential organization across all 22 family members, with each protein containing the same 10 motifs in conserved order (Figure 3A). Protein structural domain analysis of the soybean GmGSK gene family revealed that all 22 members contain a highly conserved C-terminal S_TKc domain (Figure 3B). To characterize the genomic architecture of soybean GmGSK genes, we performed a comparative analysis of exon-intron organizations using TBtools visualization. The analysis revealed conserved exon-intron organization among the 22 GmGSK genes, with exon numbers ranging from 11 to 18 and primary structural variations occurring in the spatial distribution and length polymorphisms of introns/exons (Figure 3C).

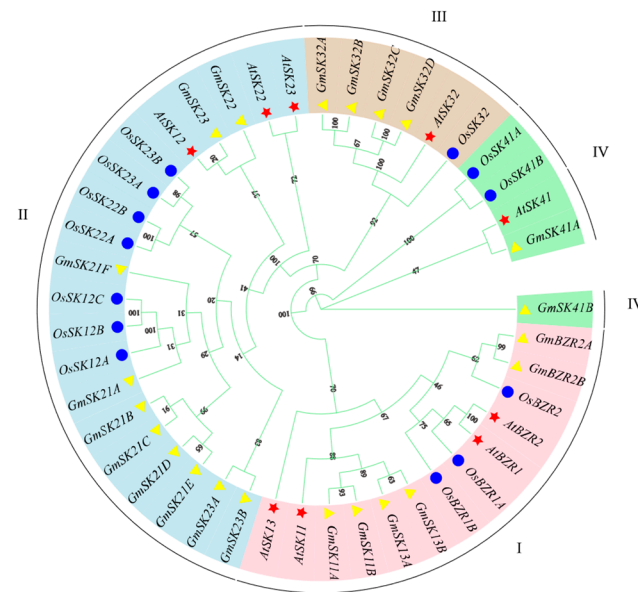


Figure 2. Phylogenetic tree analysis of the *GmGSK* gene family in *Glycine max*. Note: *Gm*, *Glycine max*; *At*, *Arabidopsis thaliana*; *Os*, *Oryza sativa*; The numbers in the circles represent the genetic relationships among members of the gene family.

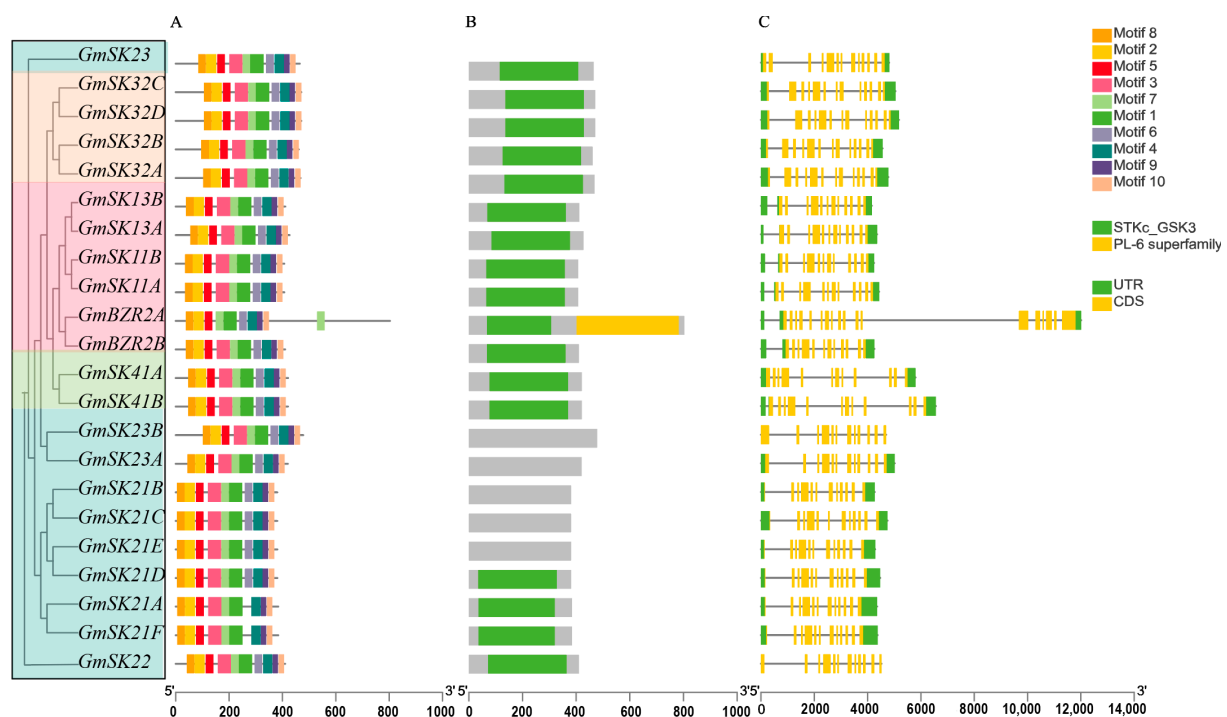


Figure 3. Analysis of conserved motifs, conserved domains and genetic structures of *Glycine max* *GmGSK*. Note: (A) The distribution of all motifs identified by MEME detected the distribution of conserved domains of 10; (B) Distribution of conserved domains of proteins; (C) exon-intron structures, with the significance of each color block marked on the right.

2.4. Analysis of Cis-Regulatory Elements (CREs) in Promoter Regions of Soybean *GmGSK* Gene

Bioinformatic analysis of promoter cis-regulatory elements in the soybean *GmGSK* gene family (Figure 4) classified the functional motifs into four major categories: hormone-responsive elements, light-responsive elements, abiotic stress-responsive elements, and growth and development-related regulatory elements. The presence of light-responsive cis-elements suggests potential photoregulation of the *GmGSK* gene family, possibly

linking these genes to circadian rhythm modulation and photoperiod adaptation in plants. Furthermore, four hormone-associated cis-regulatory elements were identified: auxin-responsive elements (AuxREs), abscisic acid-responsive elements (ABREs), gibberellin-responsive elements (GAREs), and jasmonate-responsive elements (JAREs). Low-temperature-responsive elements (LTRs) were predominantly localized in *GmSK23A*, *GmSK21A*, *GmSK13B*, *GmSK21E*, *GmSK21D*, *GmSK32A*, and *GmSK13A*, suggesting their specialized roles in cold stress adaptation; drought-responsive elements (MBS) were enriched in *GmSK32D*, *GmSK21F*, *GmSK21A*, *GmSK23B*, and *GmSK13A*, implicating their functions in drought response; and anaerobic-responsive elements (AREs) showed broad distribution across multiple promoters, collectively indicating the *GmGSK* family's pivotal involvement in abiotic stress responses.

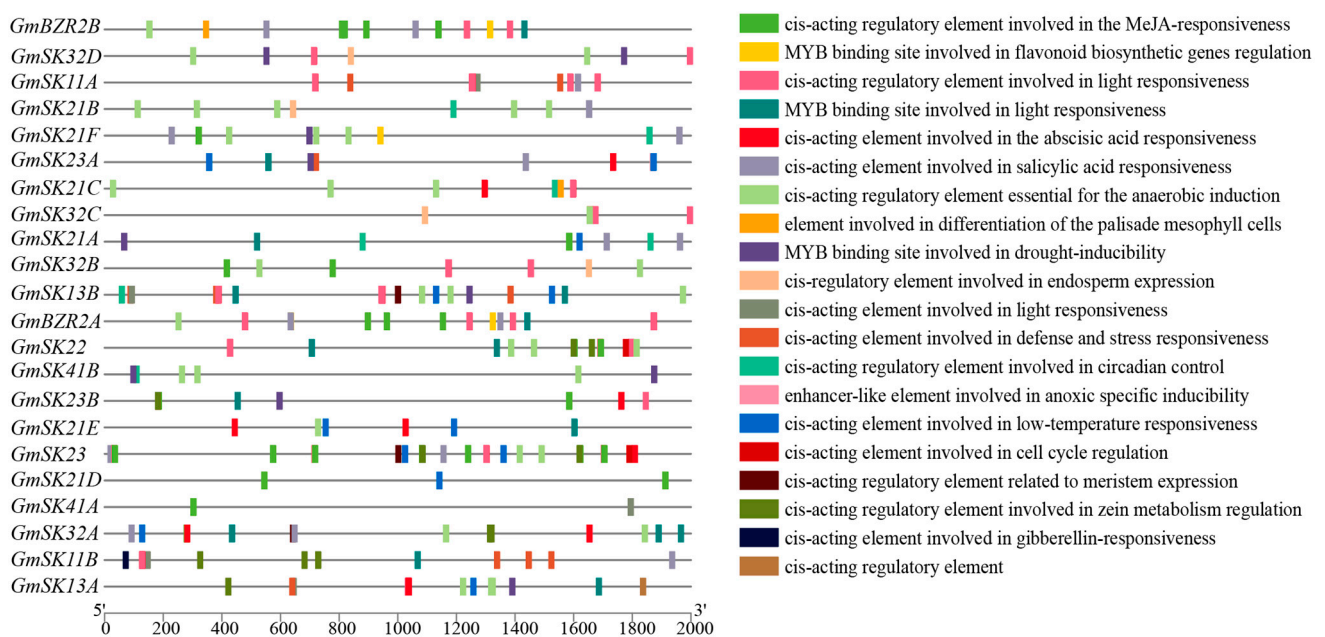


Figure 4. Analysis of promoter cis-acting elements in the *GmGSK* gene family of *Glycine max*.

2.5. Collinearity Analysis of the Soybean *GmGSK* Gene Family

Through collinearity analysis of soybean genomic data, this study revealed tandem duplication events in two pairs of *GmGSK* genes (*GmSK41B*/*GmSK23B* and *GmSK23*/*GmSK21E*), which are located on chromosomes 12 and 13, respectively (Figure 5A). Furthermore, extensive segmental duplication events were identified in the *GmGSK* gene family, with a total of 36 duplicated gene pairs. Notably, chromosomes 10/12 and 13/20 harbored 7 and 8 segmental duplication pairs, respectively, demonstrating that segmental duplication served as both a key evolutionary mechanism and the primary driver for *GmGSK* family expansion. The *GmGSK* genes exhibited collinear homologous relationships with both *Oryza sativa* and *Arabidopsis thaliana* (Figure 5B), sharing 18 homologous gene pairs with *Arabidopsis thaliana* and 21 with *Oryza sativa*. Most GSK genes in soybean exhibit clear one-to-one orthologous relationships with those in *Arabidopsis* and *Oryza sativa*, demonstrating widespread and highly conserved synteny across these species. This indicates that the GSK gene family has remained relatively stable and is highly conserved during plant evolution.

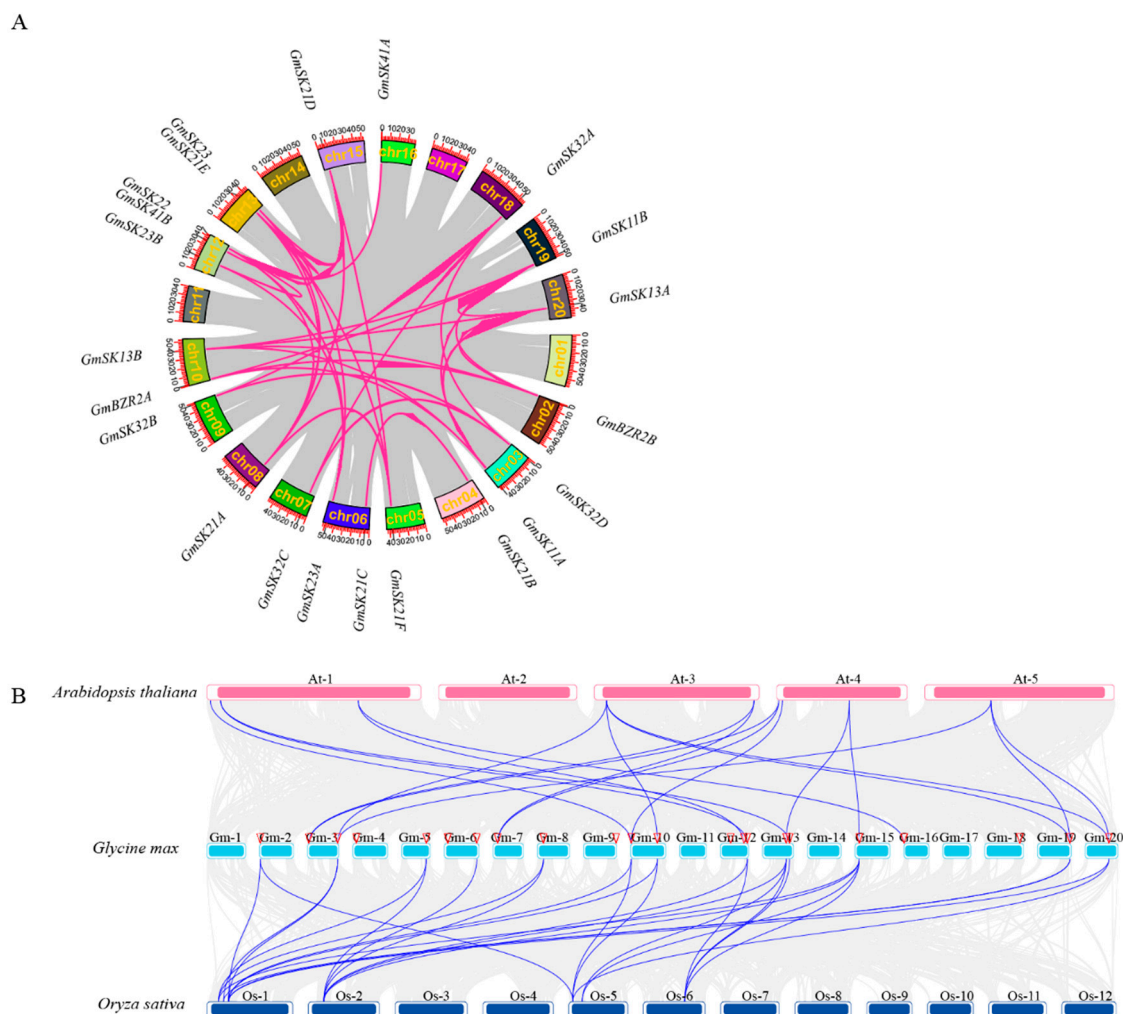


Figure 5. Synteny analysis of the *GmGSK* gene family in *Glycine max* with Arabidopsis and rice. Note: (A) Intra-genomic synteny analysis of the soybean *GmGSK* gene family; (B) Inter-species synteny analysis between soybean and Arabidopsis/rice; The pink and blue lines represent members of the *GmGSK* gene family, while the gray lines represent members of the *GSK* family.

2.6. Phenotypic Analysis of Soybean *GmGSK* Gene Family Under Manganese Stress

Manganese (Mn^{2+}) is an essential micronutrient for plant development and participates in maintaining metabolic activity in various plant cell types. However, under conditions of excess Mn^{2+} exposure, cellular Mn^{2+} homeostasis is disrupted, leading to Mn toxicity. Different crop species exhibit distinct sensitivity and tolerance mechanisms to Mn toxicity. To investigate the response of *GmGSK* genes to Mn stress, soybean plants were subjected to Mn treatments at concentrations of 0, 20, 50, 100, 200, and 300 mg/L. After 12 days of cultivation, when the two primary leaves had fully expanded, the seedlings were carefully removed from the nutrient pots. The roots were thoroughly rinsed with deionized water, and surface moisture was blotted dry before morphometric measurements. Hypocotyl length and primary root length were measured using a calibrated ruler. Control plants (0 mg/L Mn) exhibited maximal root length and leaf area, whereas increasing Mn^{2+} concentrations progressively inhibited soybean growth, resulting in significant reductions in both root elongation and leaf expansion ($p < 0.05$). The results demonstrated that elevated soil Mn^{2+} concentrations significantly reduced soybean leaf biomass (Figure 6). When the manganese concentration exceeded 50 mg/L, both root and shoot length were significantly reduced compared to those in the control group (Figure 6B,C). A statistically significant difference in manganese accumulation was observed between the 50 mg/L treatment group

and the controls, demonstrating a clear concentration-dependent trend (Figure 6D). Mn toxicity impaired root and leaf development in a dose-dependent manner, while exhibiting minimal effects on chlorophyll content. These findings indicate that excessive Mn stress inhibits soybean growth and development, suggesting that this crop species is highly sensitive to Mn toxicity.

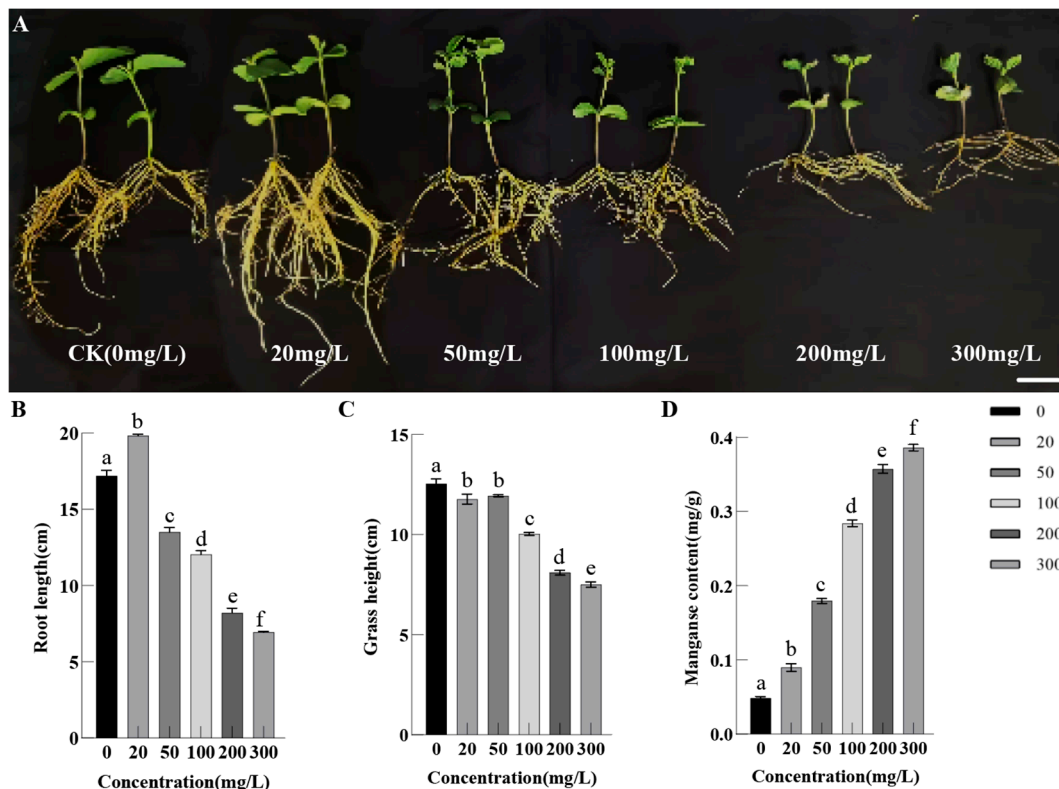


Figure 6. (A). Phenotypic effects of manganese stress on *Glycine max* growth; (B). The root length of Zhonghuang 13 soybean under different manganese concentrations; (C). The stem length of Zhonghuang 13 under different manganese concentrations; (D). Measurement results of manganese content in soybean roots under manganese stress at different concentrations. Note: Manganese concentrations were adjusted using manganese (II) chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$); the bar is 5 cm; within the same group, identical letters indicate no significant difference between groups ($p > 0.05$), while different letters indicate significant differences between groups ($p \leq 0.05$).

2.7. GO and KEGG Enrichment Analysis of Differentially Expressed Genes

In this study, root tips were collected for transcriptome analysis to investigate transcriptional changes under manganese stress at the concentration (50 mg/L) that exhibited incipient inhibitory effects. Statistical analysis of differentially expressed genes (DEGs) identified 541 significant DEGs between treated and control groups, comprising 390 up-regulated and 151 down-regulated genes (Figure 7a). The predominance of up-regulated genes suggests that Mn stress triggers complex molecular response mechanisms in plants. Also, in the case of black, all selected genes are displayed at once and overlap, so it appears black like this. These results demonstrate substantial differential gene expression between the two sample groups. GO term enrichment analysis was performed using a threshold of $|\log_2(\text{fold change})| \geq 1$ and $\text{FDR} < 0.05$. The differentially expressed genes were analyzed for enrichment across three major GO categories: Molecular Function, Cellular Component, and Biological Process. GO enrichment analysis of the control and treatment groups revealed that the most significantly enriched terms were “response to external stimulus,” followed by “defense response” and “biological process involved in interspecies interaction between organisms.” (Figure 7b). KEGG enrichment analysis of

the control and treatment groups revealed that the most significantly enriched pathways were “phenylpropanoid biosynthesis” and “plant hormone signal transduction” (Figure 7c). Among plant hormones, the primary defense hormones were jasmonic acid and salicylic acid, while abscisic acid, gibberellins, auxins, brassinosteroids, ethylene, and jasmonic acid may function as immunomodulators in plant manganese stress responses (Figure S1) [23]. A total of 679 differentially expressed genes (DEGs) were annotated in plant hormone signal transduction pathways, including genes involved in auxin, brassinosteroid, abscisic acid, ethylene, salicylic acid, jasmonic acid, and gibberellin signaling. Among the soybean homologous genes enriched in the plant hormone signal transduction pathway, 10 *GmGSK* genes were identified, with 3 showing upregulation and 7 exhibiting downregulation. Furthermore, heatmap analysis of differentially expressed genes (DEGs) revealed both up-regulated and down-regulated genes in manganese-treated groups compared to the control (Figure 6D).

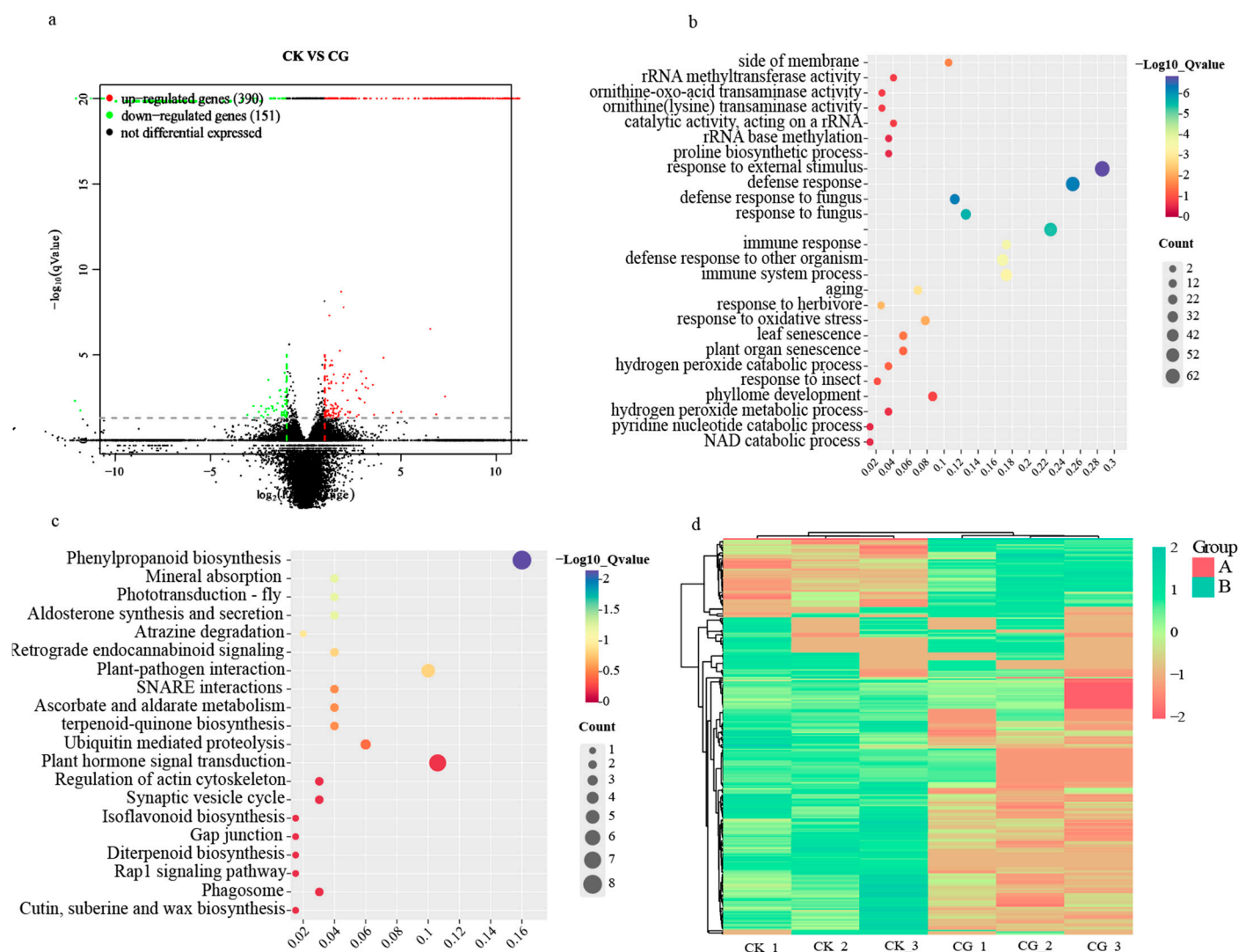


Figure 7. Differential gene expression analysis. Note: (a) Volcano plot of differentially expressed genes (DEGs) between CK and CG groups; (b) GO enrichment scatter plot of DEGs (CK vs. CG); (c) KEGG enrichment scatter plot of DEGs (CK vs. CG) (The horizontal coordinate indicates the rich factor of each pathway, the vertical coordinate is the pathway name, and the dot color is the p value; the redder it is, the more significant the enrichment. The size of the dots represents the number of differential metabolites enriched); (d) Differential gene expression analysis in manganese-treated soybean.

2.8. qRT-PCR Validation of Soybean *GmGSK* Family Genes Under Manganese Stress

Transcriptome sequencing analysis of Mn-treated soybean plants revealed distinct expression patterns of *GmGSK* family genes under manganese stress, with qRT-PCR validation confirming significant upregulation of *GmBZR2A*, *GmBZR2B*, and *GmSK32B* alongside downregulation of *GmSK21A*, *GmSK11B*, and *GmSK21F* (Figure 8b; Additional S8). This differential expression likely reflects a coordinated stress adaptation mechanism, where upregulated genes may enhance antioxidant defenses through GSK-mediated signaling pathways, while downregulated members potentially alleviate growth suppression via modulation of brassinosteroid signaling. The observed expression changes suggest that Mn stress triggers a complex regulatory network involving kinase activity modulation, hormone crosstalk, and possible epigenetic modifications, highlighting the functional diversification of *GmGSK* family members in soybean's manganese stress response. These findings not only advance our understanding of plant metal stress adaptation but also identify promising genetic targets for developing Mn-resistant soybean cultivars through precise manipulation of GSK-related pathways. Based on the correlative findings of this study, further investigations employing functional genomics and proteomics approaches are warranted to build upon and clarify the observed molecular associations within this stress response system.

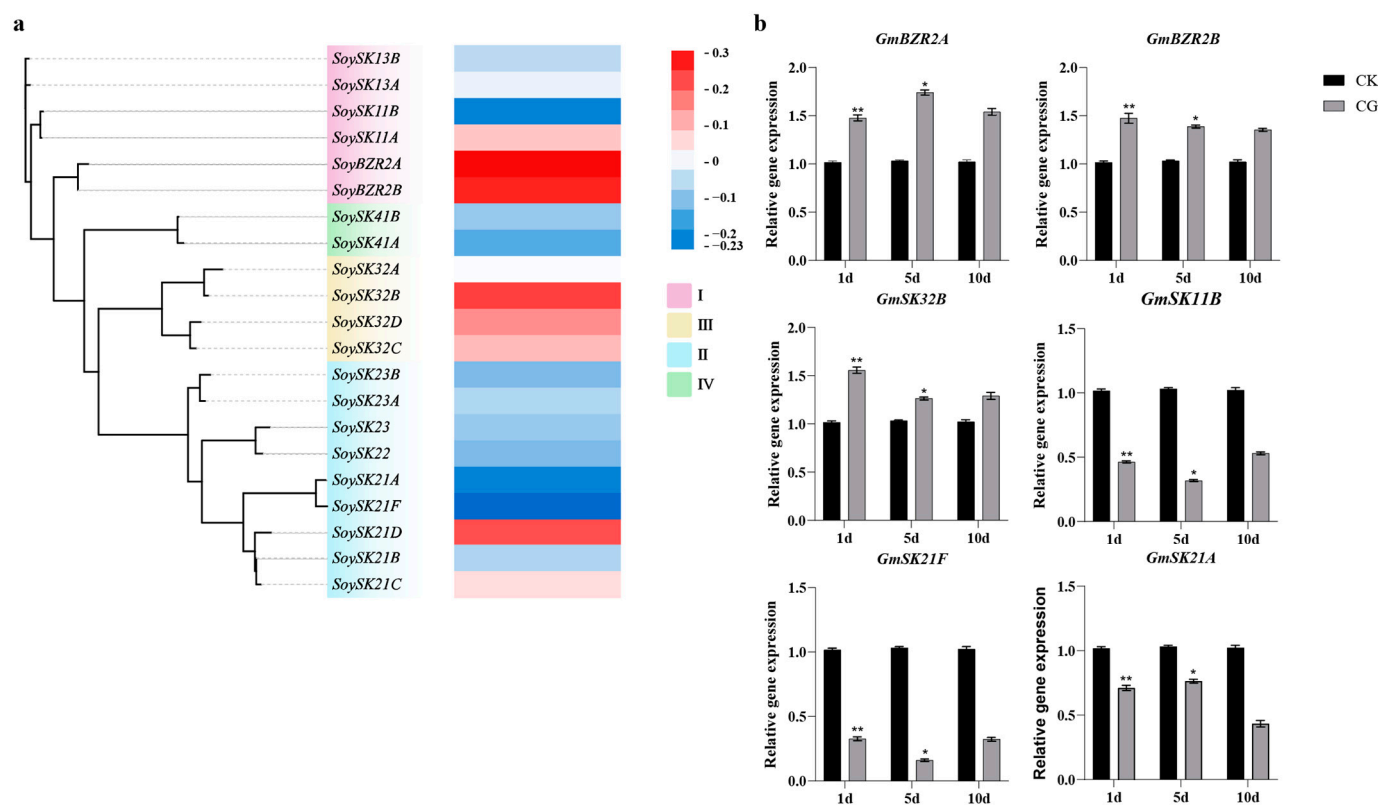


Figure 8. Expression profiles of the *Glycine max* *GmGSK* gene family under manganese stress. Note: (a) The four subfamilies of *GmGSK* are designated as I, II, III, and IV, respectively; (b) The six genes analyzed by qRT-PCR. Analysis of variance (ANOVA) was used to determine the significance of differences in the obtained data. * indicates a statistically significant difference ($p < 0.05$), and ** indicates a highly statistically significant difference ($p < 0.01$).

3. Discussion

GSK protein kinases serve as key regulatory factors extensively involved in various physiological and biochemical processes, including cell growth, tissue development, and stress responses [25]. The GSK gene family has been systematically identified in multiple

plant species, with 10, 9, 7, and 7 members characterized in *Arabidopsis thaliana*, *Oryza sativa*, *Hordeum vulgare* [26], and *Vitis vinifera* [27], respectively. These studies have demonstrated that GSK proteins play crucial roles in regulating plant physiology and developmental processes [5]. Through comprehensive bioinformatic analysis of whole-genome data, we identified 22 *GmGSK* family members in soybean, representing a significant expansion compared to other species. This expansion likely results from two whole-genome duplication (WGD) events during soybean's evolutionary history [28], which provided raw genetic material for gene family diversification. Moreover, the expansion of the soybean *GmGSK* family primarily originated from segmental duplication. As a relatively stable mode of genomic evolution, segmental duplication not only effectively avoids potential deleterious interference from gene clusters associated with tandem duplication, thereby enhancing environmental adaptability, but also generates redundant gene copies that may undergo subsequent functional divergence or subfunctionalization [29]. Phylogenetic analysis revealed that the soybean *GmGSK* family segregates into four distinct clades, maintaining evolutionary patterns consistent with those observed in *Arabidopsis* and rice. This cross-species conservation suggests potential functional universality of GSK genes in core plant biological processes. Genes within the same subfamily exhibited conserved exon/intron architectures and motif arrangements, indicating high functional conservation and possible functional redundancy among these members. Promoter cis-acting element analysis further strengthens the prediction of their involvement in stress responses. The promoter regions of most *GmGSKs* are enriched with elements associated with abiotic stress, such as ABRE (abscisic acid-responsive element), MBS (MYB-binding site involved in drought induction), and a substantial number of hormone-responsive elements.

Manganese ions (Mn^{2+}), serving as essential cofactors for multiple enzymes, play vital roles in maintaining chloroplast ultrastructure, participating in photosynthetic redox reactions, and activating enzymatic activities. Consequently, maintaining appropriate Mn^{2+} concentrations in root systems is critical for sustaining normal physiological processes in plants [30]. The root system not only mediates absorption and transport of water, nutrients, and mineral elements but also serves as a critical site for substance assimilation and conversion, directly influencing overall plant growth and development [31]. The correlation between gene expression changes and physiological responses to manganese stress observed in this study shares methodological similarities with prior research on manganese's impact on soybean or other plant growth. Specifically, these studies commonly employ experimental designs that establish growth environments with graded metal concentrations to analyze associated biological outcomes. However, there are also notable differences. This study was conducted under hydroponic conditions during the seedling stage. Hydroponic experiments not only allow precise control of the plant growth environment but also offer higher efficiency, making them more conducive to investigating the effects of specific elements or compounds on plant growth and development. In terms of exploring the impact of metallic manganese on soybean growth, the study found that appropriate manganese application significantly increases soybean yield and improves various yield-related traits. In contrast, high manganese treatment markedly inhibits both yield and yield-related traits, consistent with the "low-promotion and high-inhibition" phenomenon. This study determined that 50 mg/L Mn^{2+} treatment was the critical concentration inducing initial inhibition of root tips, manifested by typical toxic phenotypes such as slowed root elongation and root tip swelling. Transcriptome sequencing was performed on Zhonghuang 13 soybean seedlings treated with manganese concentrations of 0 mg/L and 50 mg/L. Analysis revealed an upregulation in the expression of stress-related genes associated with functions such as respiration and defense, including amino acid metabolism, terpenoid and polyketide metabolism, while the expression of key genes related to membrane transport,

auxin regulation, and other processes was downregulated, such as those in the membrane transport pathway, cell motility pathway, and cell population pathway. A total of 541 significantly differentially expressed genes were obtained. KEGG pathway enrichment analysis showed that these differentially expressed genes were primarily involved in the “Phenylpropanoid biosynthesis” and “Plant hormone signal transduction” pathways.

The present study demonstrated a significant correlation between observed changes in gene expression and the physiological response of soybeans to manganese stress. Notably, the expression patterns of GSK-related genes were associated with tolerance phenotypes. These findings provide clues for deciphering the potential molecular underpinnings of manganese stress adaptation in soybeans and suggest that the GSK-related pathway may represent a noteworthy aspect for subsequent investigation. Building upon this correlative analysis, future research should focus on characterizing protein–protein interactions, conducting functional validation experiments, and integrating multi-omics data to explore and confirm potential regulatory relationships within this stress response system.

4. Materials and Methods

4.1. Plant Material Cultivation and Manganese Treatment Protocol

The experimental material consisted of the *Glycine max* cultivar ‘Zhonghuang 13’. Seedlings were cultivated hydroponically in Hoagland’s nutrient solution containing all essential macronutrients and micronutrients. Manganese treatments were established by supplementing the nutrient solution with varying concentrations of manganese chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$). The experiment was conducted using specialized heavy-metal plant culture chambers, each accommodating 50 uniformly grown soybean seedlings under controlled environmental conditions ($25 \pm 1^\circ\text{C}$, 16/8 h light/dark photoperiod, $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity). Nutrient solutions were renewed every 3 days to maintain stable manganese concentrations and solution pH was monitored and adjusted to 5.5–6.0 to ensure manganese bioavailability. Physiological parameters, including root elongation, leaf chlorophyll content, and biomass accumulation, were monitored throughout the treatment period.

This study established both control and experimental groups. The control group received 0 mg/L manganese treatment, representing normal growth conditions for soybean seedlings and serving as the reference standard for the treatment groups. The experimental groups were subjected to 50 mg/L manganese treatment for 1 d, 5 d, and 10 d durations, with three biological replicates per treatment group. Each replicate consisted of 50 uniformly grown soybean seedlings to effectively minimize experimental error and enhance the reliability. Throughout the entire hydroponic cultivation process, the nutrient solution was replaced daily at scheduled intervals (hydroponic box volume: 750 cm^3) to ensure sufficient nutrient supply and stable manganese concentration. All cultivation pots were maintained in a growth chamber under controlled environmental conditions: light intensity of 30,000 lux with a 16 h/d photoperiod, constant temperature of 27°C , and relative humidity of 75%.

4.2. Identification and Selection of GmGSK Gene Family

Soybean genome data (ZH13_IGA1005.a1) was downloaded from the Soybase database (<https://legacy.soybase.org/dlpages/>, accessed on 20 December 2024; Additional S4 and S5) and screened via the BlastP tool (Genes-NCBI) for comparative analysis. The hidden Markov model (HMM) profile of GSKs (PF006500) was obtained from the Pfam database (<http://pfam.xfam.org/>, v35.0). Preliminary identification of GmGSK family members was performed using the Simple HMM Search function in TBtools (II-v2.390) software. The identification results were submitted to the NCBI-CDD online

database for verification, and the conserved domain (STKc_GSK) in the sequence was analyzed to obtain all *GmGSK* genes.

4.3. Chromosomal Distribution and Physicochemical Properties Analysis of Soybean *GmGSK* Gene Family

The physicochemical properties of *GmGSK* protein sequences were analyzed using the ProtParam online tool (<https://web.expasy.org/protparam/>, accessed on 20 December 2024); chromosomal localization analysis was performed via TBtools with gene density set to 100,000.

4.4. Gene Structure, Protein Domain, and Conserved Motif Analysis of Soybean *GmGSK* Gene Family

Protein domain prediction was conducted using the NCBI Conserved Domain Database (<https://www.ncbi.nlm.nih.gov/cdd/>); conserved motifs of *GmGSK* proteins were analyzed by MEME 5.5.3 (<https://meme-suite.org/meme/>, accessed on 20 December 2024) with maximum motif number set to 10 (other parameters default), and motif visualization with gene structure was implemented by TBtools; domain prediction was performed via Web CD Search Tool (<https://www.ncbi.nlm.nih.gov/Structure/>, accessed on 20 December 2024), and data visualization was completed using TBtools.

4.5. Cis-Regulatory Element Analysis of Soybean *GmGSK* Gene Family

Sequences 2000 bp upstream of transcription factors were extracted, and cis-acting elements were predicted by PlantCARE; a distribution heatmap of cis-acting element quantities was generated and visualized using TBtools.

4.6. Phylogenetic and Collinearity Analysis of Soybean *GmGSK* Gene Family

A phylogenetic tree was constructed by MEGA 11 software and visualized via Evolview (<https://evolgenius.info/evolview-v2>, accessed on 20 December 2024); chromosomal length, gene density, and collinearity files were obtained by TBtools (collinearity file parameters default), and intra-species collinearity visualization of the *GmGSK* family was achieved using the Advanced Circos function in TBtools; collinearity files between soybean and Arabidopsis/rice were acquired by TBtools to identify collinear gene pairs.

4.7. Phenotypic Analysis and Physiological Parameter Determination in Soybean Under Manganese Stress

To investigate the response of soybean (*Glycine max*) to manganese (Mn) stress, we conducted Mn stress treatments on plants at 12 days after germination and leaf emergence. The control group (CK) was irrigated with normal water (0 mg/L Mn), while the experimental groups (CG) were treated with $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ solutions at five different concentrations (20, 50, 100, 200, and 300 mg/L Mn) for 12 days. All plants were maintained under controlled environmental conditions with a 16 h photoperiod, day/night temperatures of $25 \pm 1^\circ\text{C}/22 \pm 1^\circ\text{C}$, and 65% relative humidity. The nutrient solution was renewed every 48 h to maintain stable Mn bioavailability. Following the treatment period, we systematically evaluated phenotypic responses by measuring leaf area and root morphological parameters (primary root length and lateral root density) while documenting whole-plant growth characteristics and visible stress symptoms through high-resolution photography. This experimental design, which implements a gradient of Mn concentrations for dose-response analysis, adheres to standard heavy metal stress protocols while incorporating soybean-specific growth stage considerations, enabling a comprehensive assessment of Mn tolerance thresholds across multiple physiological parameters.

For sample preparation, three biological replicates of uniformly grown soybean seedlings were selected from each treatment group. Approximately 0.2 g of dried tissue powder from different plant parts was accurately weighed into digestion tubes. After moistening the samples with 500 μ L distilled water, 5 mL concentrated nitric acid was added, and the mixtures were left at room temperature overnight for pre-digestion. The samples were then subjected to a two-stage thermal digestion protocol: first at 180 °C for 0.5 h, followed by 350 °C for 1 h. When the solutions turned homogeneous brown, an appropriate amount of hydrogen peroxide (H_2O_2) was added to complete catalytic oxidation. The digestion was considered complete when the solutions became colorless or transparent. After cooling, the digested samples were diluted to a final volume of 50 mL with deionized water. Using blank-digested solutions as controls, Mn^{2+} concentrations in different plant tissues were quantitatively determined by inductively coupled plasma mass spectrometry (ICP-MS) following standard operating procedures with appropriate quality control measures, including calibration standards and spike recovery tests.

4.8. Screening, Functional Annotation, and Enrichment Analysis of Differentially Expressed Genes

In this study, RNA-seq analysis was conducted with three independent biological replicates. The sequencing was performed by Huazhi Biotechnology Co., Ltd. (Changsha, China) using the Illumina NovaSeq platform, with raw data deposited in the National Genomics Data Center. Clean reads were aligned to the soybean reference genome (<https://legacy.soybase.org/dlpages/>, accessed on 20 December 2024; Additional S4 and S5) using Hisat2,-2.2.1, and gene expression levels were quantified using HTSeq-count-2.0.9. Differential expression analysis was performed using DESeq2 (v1.30.1) with raw counts data normalized by the median-of-ratios method. Differentially expressed genes (DEGs) were identified using thresholds of $|\log_2\text{FoldChange}| \geq 1$ and false discovery rate (FDR) < 0.05 . Gene Ontology (GO) and KEGG enrichment analyses were carried out using clusterProfiler (v4.0), with an FDR < 0.05 considered statistically significant. Based on the analytical results, visualization plots including volcano plots and heatmaps were generated, and candidate genes were subsequently selected for qRT-PCR validation.

4.9. Validation of the GmGSK Gene Family in Soybean by Quantitative Real-Time PCR

To elucidate the expression patterns of the GmGSK gene family, we performed quantitative real-time PCR (qRT-PCR) analysis on soybean seedlings subjected to different manganese (Mn) concentrations. One-month-old uniformly grown soybean seedlings were treated daily with 0 mg/L (CK) or 50 mg/L Mn solutions. Leaf samples (≈ 1 g) were collected at 1, 5, and 10 days post-treatment, flash-frozen in liquid nitrogen, and stored at -80 °C. Three biological replicates were included to ensure experimental reliability. Total RNA was extracted using TRIzol reagent, with quality verified by Clear ribosomal RNA bands on denaturing agarose gels and A260/A280 ratios between 1.8 and 2.1. Following genomic DNA removal, RNA was reverse-transcribed into cDNA using PrimeScript RT reagent Kit and stored at -20 °C for subsequent qPCR. The reagents and reaction conditions of the reverse transcription qRT-PCR can be found in the Supplementary Tables (Additional S3). Gene-specific primers for six target genes were designed via NCBI Primer-BLAST, with soybean EF1- α (elongation factor 1-alpha; XM_002850842) serving as the internal reference (Table 1). qPCR was performed in technical triplicate using SYBR Premix Ex Taq on a QuantStudio 6 Flex system, with reaction conditions as previously described. All experiments were conducted with three biological replicates and three technical replicates. Data visualization was performed using GraphPad Prism 10 to clearly demonstrate expression patterns. For all experimental conditions in this study, a minimum of three parallel replicates were maintained, with results expressed as mean $\pm$ standard

deviation. The relative expression levels of *GmGSK* genes were calculated using the $2^{-\Delta\Delta CT}$ method [32]. Statistical significance of observed differences was determined by analysis of variance (ANOVA), where * indicates significant differences ($p < 0.05$) and ** denotes highly significant differences ($p < 0.01$).

Table 1. Real-time fluorescence quantitative PCR amplification primers.

Gene ID	Gene Name	Forward Primer (5'→3')	Reverse Primer (5'→3')
LOC100817819	<i>GmBZR2A</i>	TGTATATCCCTGCCTCCCCTT	TCCTTTCTCTCACCAGCCCC
LOC100788046	<i>GmBZR2B</i>	TCCACGTCTAGATCTGTTGT	TGTTGCAGCTTCGATTCAACT
LOC100784150	<i>GmSK32B</i>	ACCACACCAGCAATGCTACC	TGGCGTGTGGGTCCTTGATA
LOC100812801	<i>GmSK21A</i>	ATACTTCGTTTCATCACGGCG	AGGACGTTAATGGGATGGGA
LOC100795659	<i>GmSK11B</i>	TTGACAGTCACAAGCTCACC	TGAGTTGTGAGGATGAGGAAGT
LOC100810237	<i>GmSK21F</i>	TTCTCCTCCTCACAACCTCACA	TGAAGCCATTTTGCACCTTCC
-	<i>EF1-α</i>	TGCAAAGGAGGCTGCTAACT	CAGCATCACCGTTCTTCAAA

5. Conclusions

This study presents the first comprehensive genomic characterization of the soybean *GmGSK* gene family, revealing its phylogenetic segregation into four distinct subfamilies with evolutionarily conserved domain architectures. In silico promoter analysis identified an enrichment of cis-acting elements associated with light responsiveness, hormonal signaling, and stress regulation, suggesting functional diversification among subfamily members. In the concentration gradient experiment using Zhonghuang 13 as the model cultivar, six treatment levels were established: 0, 20, 50, 100, 200, and 300 mg/L. Both root length and shoot length of soybean seedlings were promoted at a manganese concentration of 20 mg/L, while inhibition was observed at 50 mg/L and higher concentrations. Combined with overall growth performance, the results consistently demonstrated a pattern of low-concentration promotion and high-concentration inhibition. Under 50 mM Mn treatment, qRT-PCR analysis of six representative *GmGSK* genes demonstrated subfamily-specific expression patterns, with three members showing significant upregulation and three exhibiting downregulation. Although these expression changes are associated with manganese treatment, it must be emphasized that the current findings are based on a single experimental condition and have not yet been functionally validated through kinase activity assays or genetic complementation experiments. Therefore, the results of this study primarily reflect a correlation between manganese exposure and alterations in *GmGSK* gene expression, rather than establishing a definitive causal relationship. These findings provide a foundational framework for further investigation into the potential roles of the *GmGSK* family in metal ion regulation, nutrient homeostasis, and stress signaling networks in soybean.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27052118/s1>.

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Abbreviations

Mn	Manganese
GSK3	Glycogen synthase kinase 3
qRT-PCR	Quantitative real-time PCR
pI	Isoelectric point

References

1. Finn, R.D.; Coghill, P.; Eberhardt, R.Y.; Eddy, S.R.; Mistry, J.; Mitchell, A.L.; Potter, S.C.; Punta, M.; Qureshi, M.; Sangrador-Vegas, A.; et al. The Pfam protein families database: Towards a more sustainable future. *Nucleic Acids Res.* **2016**, *44*, D279–D285. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Patel, P.; Woodgett, J.R. Glycogen Synthase Kinase 3: A Kinase for All Pathways? In *Current Topics in Developmental Biology*; Academic Press: Cambridge, MA, USA, 2017; Volume 123, pp. 277–302.
3. Qiu, A.; Wu, J.; Lei, Y.; Cai, Y.; Wang, S.; Liu, Z.; Guan, D.; He, S. CaSK23, a Putative GSK3/SHAGGY-Like Kinase of *Capsicum annuum*, Acts as a Negative Regulator of Pepper's Response to *Ralstonia solanacearum* Attack. *Int. J. Mol. Sci.* **2018**, *19*, 2698. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Ali, A.; Hoefflich, K.P.; Woodgett, J.R. Glycogen synthase kinase-3: Properties, functions, and regulation. *Chem. Rev.* **2001**, *101*, 2527–2540. [\[CrossRef\]](#)
5. Li, J.; Zhang, W.; Jiao, R.; Yang, Z.; Yuan, Y.; Wu, Q.; Hu, Z.; Xiang, S.; Tang, Q. DIM attenuates TGF- β 1-induced myofibroblast differentiation in neonatal rat cardiac fibroblasts. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 5121–5128. [\[PubMed\]](#)
6. Youn, J.-H.; Kim, T.-W. Functional insights of plant GSK3-like kinases: Multi-taskers in diverse cellular signal transduction pathways. *Mol. Plant* **2015**, *8*, 552–565. [\[CrossRef\]](#)
7. Liu, R.; Wang, M.; Chen, W.; Peng, C. Spatial pattern of heavy metals accumulation risk in urban soils of Beijing and its influencing factors. *Environ. Pollut.* **2016**, *210*, 174–181. [\[CrossRef\]](#)
8. Hou, L.; Wang, Z.; Gong, G.; Zhu, Y.; Ye, Q.; Lu, S.; Liu, X. Hydrogen Sulfide Alleviates Manganese Stress in Arabidopsis. *Int. J. Mol. Sci.* **2022**, *23*, 5046. [\[CrossRef\]](#)
9. Jiang, S.G.; Zhan, H.M.; Liu, G.M.; Zheng, A.J.; Lu, D.Q.; Wang, Z.G.; Gong, S.J. Available Concentrations of Major Micronutrients and Their Influencing Factors in Soils of the Tianjin Xiaozhan Rice Cultivation Area. *Soil* **2021**, *53*, 1215–1220.
10. Hu, H.J.; Wan, S.H.; He, X.Y. A Brief Analysis of Factors Influencing the Availability of Copper, Iron, Manganese, Zinc, and Molybdenum in Soil. *Agric. Technol.* **2014**, *34*, 23–24.
11. Ding, Y.F.; Zhao, F.X.; Mi, L.; Wang, H.T.; Song, X.L.; Zhu, J.W.; Zhang, M.; He, J. Relationships Between Available Trace Elements and pH/Organic Matter in Tobacco-Growing Soils of Central Henan. *Soil* **2022**, *54*, 88–94.
12. Yu, H.M.; Yi, L.S.; Zhu, Z.Y. Advances and Prospects in Soil Manganese Research. *Crop Res.* **2005**, *19*, 137–142.
13. Wang, Y.; Li, J.; Pan, Y.; Chen, J.; Liu, Y. Metabolic Responses to Manganese Toxicity in Soybean Roots and Leaves. *Plants* **2023**, *12*, 3615. [\[CrossRef\]](#)
14. Wege, S. Manganese management in plants: Golgi transporter determines manganese allocation and cell wall composition. *Plant Physiol.* **2022**, *190*, 2077–2079. [\[CrossRef\]](#)
15. Santos, E.F.; Kondo Santini, J.M.; Paixão, A.P.; Júnior, E.F.; Lavres, J.; Campos, M.; Reis, A.R.D. Physiological highlights of manganese toxicity symptoms in soybean plants: Mn toxicity responses. *Plant Physiol. Biochem.* **2017**, *113*, 6–19. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Alejandro, S.; Höller, S.; Meier, B.; Peiter, E. Manganese in Plants: From Acquisition to Subcellular Allocation. *Front. Plant Sci.* **2020**, *11*, 300. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Xue, S.; Zhu, F.; Kong, X.; Wu, C.; Huang, L.; Huang, N.; Hartley, W. A review of the characterization and revegetation of bauxite residues (Red mud). *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 1120–1132. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Xu, Y.; Liu, J.; Qin, H.; Liu, S.; Wang, R.; Cao, X. Genome-wide identification and expression analysis of the MBD gene family in Broomcorn millet (*Panicum miliaceum*) and its response to abiotic stress. *BMC Genom.* **2025**, *26*, 991. [\[CrossRef\]](#)
19. Vaculíková, M.; Vaculík, M.; Šimková, L.; Fialová, I.; Kochanová, Z.; Sedláková, B.; Luxová, M. Influence of silicon on maize roots exposed to antimony—Growth and antioxidative response. *Plant Physiol. Biochem.* **2014**, *83*, 279–284. [\[CrossRef\]](#)

20. Feng, R.; Lei, L.; Su, J.; Zhang, R.; Zhu, Y.; Chen, W.; Wang, L.; Wang, R.; Dai, J.; Lin, Z.; et al. Toxicity of different forms of antimony to rice plant: Effects on root exudates, cell wall components, endogenous hormones and antioxidant system. *Sci. Total Environ.* **2020**, *711*, 134589. [[CrossRef](#)]
21. Vianna, G.R.; Cunha, N.B.; Rech, E.L. Soybean seed protein storage vacuoles for expression of recombinant molecules. *Curr. Opin. Plant Biol.* **2023**, *71*, 102331. [[CrossRef](#)]
22. Chen, Z.; Yan, W.; Sun, L.; Tian, J.; Liao, H. Proteomic analysis reveals growth inhibition of soybean roots by manganese toxicity is associated with alteration of cell wall structure and lignification. *J. Proteom.* **2016**, *143*, 151–160. [[CrossRef](#)]
23. Foley, J.A.; Ramankutty, N.; Brauman, K.A.; Cassidy, E.S.; Gerber, J.S.; Johnston, M.; Mueller, N.D.; O'Connell, C.; Ray, D.K.; West, P.C.; et al. Solutions for a cultivated planet. *Nature* **2011**, *478*, 337–342. [[CrossRef](#)]
24. Rajpoot, R.; Srivastava, R.K.; Rani, A.; Pandey, P.; Dubey, R.S. Manganese-induced oxidative stress, ultrastructural changes, and proteomics studies in rice plants. *Protoplasma* **2021**, *258*, 319–335. [[CrossRef](#)]
25. Youn, J.-H.; Kim, T.-W.; Kim, E.-J.; Bu, S.; Kim, S.-K.; Wang, Z.-Y.; Kim, T.-W. Structural and functional characterization of Arabidopsis GSK3-like kinase AtSK12. *Mol. Cells* **2013**, *36*, 564–570. [[CrossRef](#)] [[PubMed](#)]
26. Kloc, Y.; Dmochowska-Boguta, M.; Zielezinski, A.; Nadolska-Orczyk, A.; Karlowski, W.M.; Orczyk, W. Silencing of HvGSK1.1-A GSK3/SHAGGY-Like Kinase-Enhances Barley (*Hordeum vulgare* L.) Growth in Normal and in Salt Stress Conditions. *Int. J. Mol. Sci.* **2020**, *21*, 6616. [[CrossRef](#)]
27. Zeng, J.; Haider, M.S.; Huang, J.; Xu, Y.; Pervaiz, T.; Feng, J.; Zheng, H.; Tao, J. Functional Characterization of VvSK Gene Family in Grapevine Revealing their Role in Berry Ripening. *Int. J. Mol. Sci.* **2020**, *21*, 4336. [[CrossRef](#)] [[PubMed](#)]
28. Schmutz, J.; Cannon, S.B.; Schlueter, J.; Ma, J.; Mitros, T.; Nelson, W.; Hyten, D.L.; Song, Q.; Thelen, J.J.; Cheng, J.; et al. Genome sequence of the palaeopolyploid soybean. *Nature* **2010**, *463*, 178–183. Erratum in *Nature* **2010**, *465*, 7294. <https://doi.org/10.1038/nature08670>. [[CrossRef](#)]
29. Roulin, A.; Auer, P.L.; Libault, M.; Schlueter, J.; Farmer, A.; May, G.; Stacey, G.; Doerge, R.W.; Jackson, S.A. The fate of duplicated genes in a polyploid plant genome. *Plant J.* **2012**, *73*, 143–153. [[CrossRef](#)]
30. Faria, J.M.; Teixeira, D.M.; Pinto, A.P.; Brito, I.; Barrulas, P.; Alho, L.; Carvalho, M. Toxic levels of manganese in an acidic Cambisol alters antioxidant enzymes activity, element uptake and subcellular distribution in *Triticum aestivum*. *Ecotoxicol. Environ. Saf.* **2020**, *193*, 110355. [[CrossRef](#)]
31. Jumrani, K.; Bhatia, V.S.; Kataria, S.; Alamri, S.A.; Siddiqui, M.H.; Rastogi, A. Inoculation with Arbuscular Mycorrhizal Fungi Alleviates the Adverse Effects of High Temperature in Soybean. *Plants* **2022**, *11*, 2210. [[CrossRef](#)] [[PubMed](#)]
32. Chen, N.; Zhan, W.; Shao, Q.; Liu, L.; Lu, Q.; Yang, W.; Que, Z. Cloning, Expression, and Functional Analysis of the MYB Transcription Factor SIMYB86-like in Tomato. *Plants* **2024**, *13*, 488. [[CrossRef](#)] [[PubMed](#)]

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