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Soybean steroids improve crop abiotic stress tolerance and increase yield

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Summary

Sterols have long been associated with diverse fields, such as cancer treatment, drug development, and plant growth; however, their underlying mechanisms and functions remain enigmatic. Here, we unveil a critical role played by a GmNF-YC9-mediated CCAAT-box transcription complex in modulating the steroid metabolism pathway within soybeans. Specifically, this complex directly activates squalene monooxygenase (GmSQE1), which is a rate-limiting enzyme in steroid synthesis. Our findings demonstrate that overexpression of either *GmNF-YC9* or *GmSQE1* significantly enhances soybean stress tolerance, while the inhibition of SQE weakens this tolerance. Field experiments conducted over two seasons further reveal increased yields per plant in both *GmNF-YC9* and *GmSQE1* overexpressing plants under drought stress conditions. This enhanced stress tolerance is attributed to the reduction of abiotic stress-induced cell oxidative damage. Transcriptome and metabolome analyses shed light on the upregulation of multiple sterol compounds, including fucosterol and soyasaponin II, in *GmNF-YC9* and *GmSQE1* overexpressing soybean plants under stress conditions. Intriguingly, the application of soybean steroids, including fucosterol and soyasaponin II, significantly improves drought tolerance in soybean, wheat, foxtail millet, and maize. These findings underscore the pivotal role of soybean steroids in countering oxidative stress in plants and offer a new research strategy for enhancing crop stress tolerance and quality from gene regulation to chemical intervention.

Introduction

With the advent of climate change, abiotic stresses such as drought, salt, heat and cold are exerting increasingly severe influences on plant growth, leading to a reduction in grain production. However, being sessile organisms, plants have evolved a series of defence mechanisms to adapt to environmental stressors. For instance, extensive research has been conducted on the role of abscisic acid (ABA) in enhancing tolerance to abiotic stress conditions, such as drought (Zhang *et al.*, 2022; Zhu, 2016). Cold stress triggers a Ca^{2+}-signal-activated MAP-kinase cascade that activates the expression of cold-responsive genes in plants and can also modulate *HSP* gene expressions to enhance plant tolerance towards heat stress (Sangwan *et al.*, 2002; Zhang *et al.*, 2022; Zhu, 2016). Nevertheless, with advancements in metabolomics technology, several small molecular compounds have been discovered to play crucial roles in promoting plant stress tolerance. For example, recent studies have revealed that high levels of putrescine are induced during cold stress, and its exogenous application improves cold-acclimated freezing tolerance in potato after acclimation to low temperatures (Kou *et al.*, 2018). Additionally, flavonoid accumulation has been found beneficial for enhancing UV tolerance in rice (Liu *et al.*, 2020). However, due to the inherent challenges associated with small metabolite techniques used for their characterisation and quantification purposes, our understanding of their precise roles under abiotic stress conditions remains limited.

One notable family of small metabolites is sterols. While extensive research has been conducted on these compounds in animal systems, progress regarding plant sterols has remained limited. Nonetheless, sterols have been implicated in numerous vital plant processes, encompassing cell division, elongation and polarity (He *et al.*, 2018; Men *et al.*, 2008; Schrick *et al.*, 2000; Willemsen *et al.*, 2003). They also play a crucial role in regulating

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auxin and ethylene signalling (Souter *et al*., 2002), modulating the activity of microRNAs (Brodersen *et al*., 2012), and enhancing tolerance to low-temperature stress (Senthil-Kumar *et al*., 2013). The biosynthesis of sterol requires the initial synthesis of squalene (SQ), a 30-carbon intermediate, through the isopentenyl diphosphate (IPP) pathway (Phillips *et al*., 2006; Rasbery *et al*., 2007). Squalene is subsequently oxidised by squalene epoxidase (SQE, squalene monooxygenase), leading to the formation of 2,3-oxidosqualene (2,3-OSQ) (Phillips *et al*., 2006; Rasbery *et al*., 2007), which then undergoes cyclisation to generate sterols (Benveniste, 2004; Schaller, 2003, 2004; Spanova and Daum, 2011). SQE serves as a crucial rate-limiting enzyme in the sterol biosynthesis pathway. In the model plant *Arabidopsis thaliana*, seven SQE proteins are identified; however, only SQE1, SQE2, and SQE3 exhibit catalytic activity in converting squalene to squalene-2, 3-epoxide (2,3-OSQ) (Rasbery *et al*., 2007). The mutation of *SQE1* results in plants exhibiting impaired root development, hypocotyl and stem elongation defects, as well as inviable seeds (Posé *et al*., 2009; Rasbery *et al*., 2007). The *sqe1-5* mutant exhibits altered sterol composition in roots and impaired stomatal responses (Posé *et al*., 2009). Loss of *SQE2*, which exhibits tissue-specific tissue expression patterns, does not exert any discernible influence on the sterol composition at the whole plant level. However, depletion of *SQE3* results in the squalene accumulation and consequential phenotypic defects (Laranjeira *et al*., 2015). These studies unveil the significance of phytosterols in plant growth and development, however, there remains a dearth of research on the molecular mechanisms governing plant sterol synthesis and their roles in abiotic stress.

In human, SP1 and SREBF2 have been identified as two crucial transcription factors that regulate sterol synthesis by controlling the expression level of SQE (Zhang *et al*., 2019). In addition, research has demonstrated that WsWRKY1, a WRKY transcription factor in *Withania somnifera*, modulates biotic stress tolerance through the regulation of the SQE-mediated triterpenoid system pathway (Singh *et al*., 2017). In *Panax notoginseng*, PnMYB4 exerts a repressive effect on saponin biosynthesis by modulating the transcript level of SQE (Man *et al*., 2023). These findings sequentially unveil the regulatory role of a transcription factor in sterol synthesis. However, among these transcription factors, the nuclear factor Y (NF-Y) garners significant attention due to its distinctive structure and functionality. The NF-Y transcription factor, composed of NF-YA, NF-YB, and NF-YC subunits, exerts crucial regulatory functions in diverse plant developmental processes by binding to the CCAAT cis-element in target gene promoters (Mantovani, 1999; Shen *et al*., 2020; Zanetti *et al*., 2010). Moreover, NF-Y transcription factors also actively participate in enhancing plant stress tolerance. For instance, a recent study demonstrates that *NF-YB21* plays a crucial role in enhancing drought tolerance and modulating ABA-mediated IAA transport to positively regulate root growth in *Populus trichocarpa* (Zhou *et al*., 2020). In our previous research, we discovered that *GmNF-YC14* conferred enhanced tolerance to drought and salt stresses by regulating the PYL-mediated ABA signalling pathway in soybean (Yu *et al*., 2021). Recent research has unveiled the interaction between NF-YCs and BIN2, which indirectly regulates the biosynthesis of brassinosteroids, crucial components of phytosterols. This interaction consequently influences light-regulated hypocotyl elongation in *Arabidopsis* (Zhang *et al*., 2021). This highlights the significance of NF-Y transcription factors in finely modulating phytosterol composition and plant development. However, the intricate interplay between the NF-Y transcription factor, phytosterol, and abiotic stress remains shrouded in ambiguity.

In our previous study, we identified two NF-YC family genes, *GmNF-YC9* and *GmNF-YC14*, through a comprehensive analysis of the molecular characteristics of this gene family in soybean (Yu *et al*., 2021). These two genes exhibit significant induction under drought and salt stresses in soybean. Our current findings reveal that, unlike *GmNF-YC14*, the GmNF-YC9-mediated NF-Y transcription complex directly regulates the expression of *GmSQE1* under stress conditions. This regulation leads to an enhancement in the squalene metabolic pathway and ultimately enhances plant abiotic stress responses. Our findings have revealed a previously unidentified regulatory module (NF-Y-SQE) that governs the biosynthesis pathway of plant sterols and confers stress tolerance, thereby establishing a theoretical basis for enhancing soybean variety improvement. Furthermore, our comprehensive analysis of transcriptome and metabolome association has demonstrated a significant upregulation of multiple soybean sterols under stressful conditions. Notably, exogenous application of soybean sterols has been shown to significantly enhance crop stress tolerance, highlighting the potential utility of plant steroids in improving crop resilience to environmental challenges. These results provide insights into a non-GMO strategy for modulating crop stress tolerance through chemical intervention.

Results

GmNF-YC9-mediated CCAAT-box transcriptional complex can improve stress tolerance in soybean

In our previous investigation, we observed a significant induction of *GmNF-YC9* expression in response to drought and salt treatments (Yu *et al*., 2021). To further elucidate the potential role of *GmNF-YC9* in drought tolerance, we generated transgenic soybean plants overexpressing *GmNF-YC9* (*GmNF-YC9*-OE1, *GmNF-YC9*-OE5, and *GmNF-YC9*-OE8) (Figure S1). Our findings revealed that the overexpression of *GmNF-YC9* in soybean resulted in increased biomass, longer root and hypocotyl lengths compared to wild-type (WT) plants under stress conditions (Figure 1a–d). Microscopic analysis of root tip tissue sections further demonstrated an enhanced root tip differentiation rate in *GmNF-YC9*-OE plants subjected to 200 mM mannitol treatment (Figure 1e,f). These results highlighted the superior growth performance of *GmNF-YC9*-OE plants under stress conditions, which had been consistently observed in pot experiments (Figure 1g). Furthermore, *GmNF-YC9*-OE plants exhibited elevated proline levels, reduced malondialdehyde (MDA) content, and higher biomass compared to WT plants under drought and salt stresses (Figure 1h–j). Notably, these effects were more pronounced under stressful conditions, firmly establishing that the overexpression of *GmNF-YC9* enhances soybean's tolerance to osmotic and salt stresses.

To further elucidate the function of GmNF-YC9, we conducted a screening of a soybean cDNA library to identify candidate proteins. This screening led to the identification of several positive clones, including two additional subunits of the CCAAT-box complex, GmNF-YA2 and GmNF-YB24. Subsequent yeast two-hybrid (Y2H) assays confirmed interactions among protein pairs GmNF-YC9/GmNF-YA2, GmNF-YC9/GmNF-YB24, and GmNF-YA2/GmNF-YB24 (Figure 2a). These interactions were further validated through bimolecular fluorescence complementation (BiFC) assays in *Arabidopsis* protoplast cells (Figure 2b–d). Additionally, luciferase complementary imaging (LCI) assays

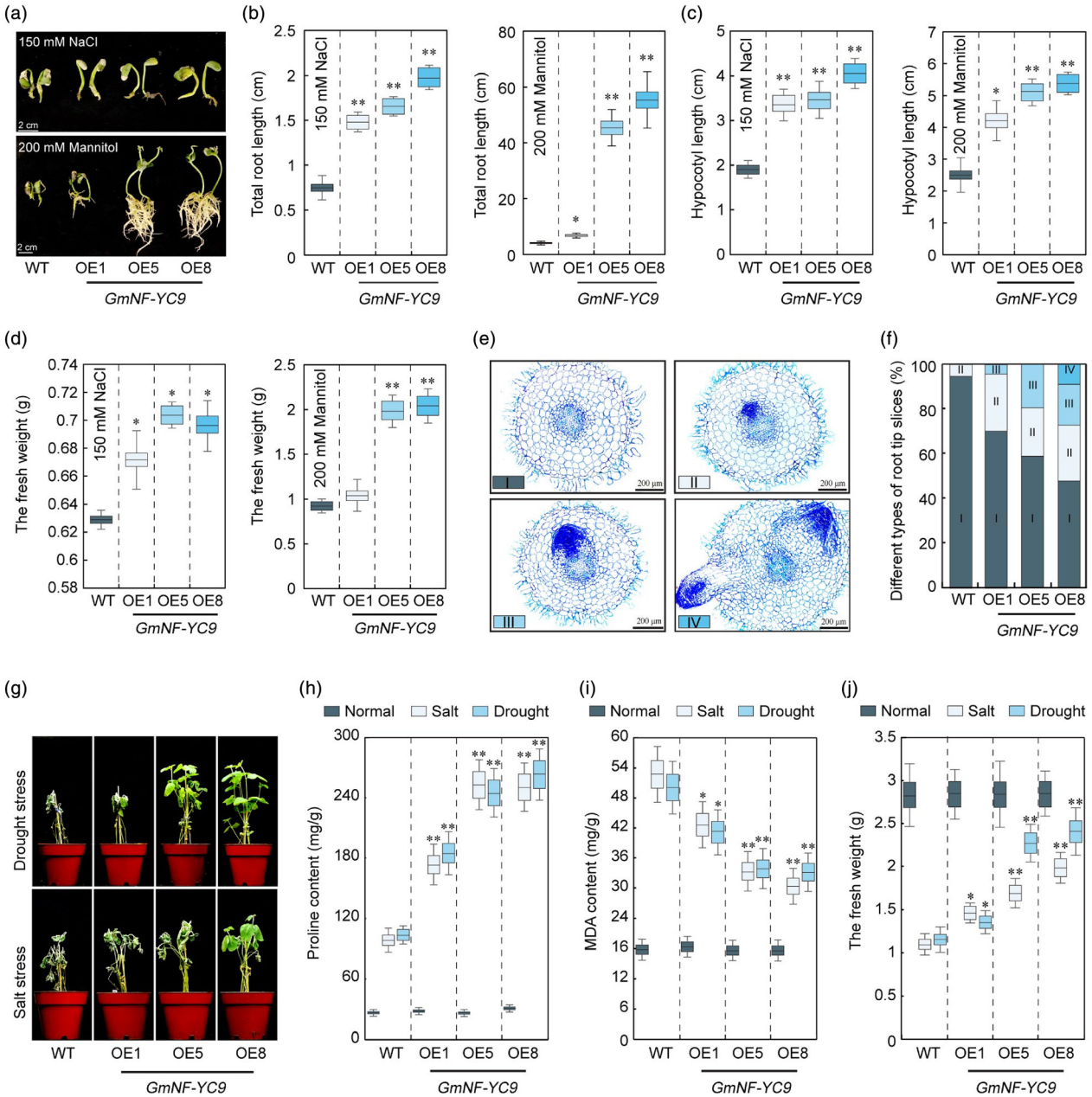


Figure 1 Stress tolerance analysis of *GmNF-YC9* overexpressing soybean plants. (a) Phenotype analysis of 7-day-old WT and *GmNF-YC9*-OE soybean seedling plants after 7 days of different stress treatments. (b–d) Total root length (b), hypocotyl (c), and fresh weight (d) analysis of 7-day-old WT and *GmNF-YC9*-OE soybean plants after 7 days of different stress treatments. (e) The different slice samples of the root tip elongation zone after 7 days of 200 mM mannitol treatment. (f) The percentage of different types of root tip slice samples. (g) Phenotypic analysis of *GmNF-YC9*-OE and WT plants at seedling stage under drought and salt stress conditions. (h, i) Proline (h) and MDA (i) content analysis of *GmNF-YC9*-OE and WT plants at seedling stage under drought and salt stress conditions. (j) The fresh weight of *GmNF-YC9*-OE and WT plants at seedling stage under drought and salt stress conditions.

detected LUC signal in tobacco leaf areas co-expressing GmNF-YA2-cLUC/GmNF-YC9-nLUC, GmNF-YB24-cLUC/GmNF-YC9-nLUC, or GmNF-YB24-cLUC/GmNF-YA2-nLUC (Figure 2e–g). GST-pulldown assays demonstrated physical interactions among GmNF-YC9, GmNF-YB24, and GmNF-YA2 proteins (Figure 2h–j). These results unequivocally indicated that GmNF-YC9, GmNF-YB24, and GmNF-YA2 proteins interacted with each other both *in vivo* and *in vitro*. Subsequent analysis of *GmNF-YA2* and *GmNF-YB24*, through the generation of transgenic hairy-root composite plants, confirmed their involvement in abiotic stress tolerance (Figure S2a–e). Under stress conditions, the transgenic hairy roots expressing either *GmNF-YA2* or *GmNF-YB24* exhibited improved growth characteristics, elevated proline content, reduced MDA content, and decreased relative electrical conductivity compared to those transformed with an empty vector control (Figure S2f–m). These findings collectively suggested that, similar to GmNF-YA9, GmNF-YA2 and GmNF-YB24 actively participate in abiotic stress responses.

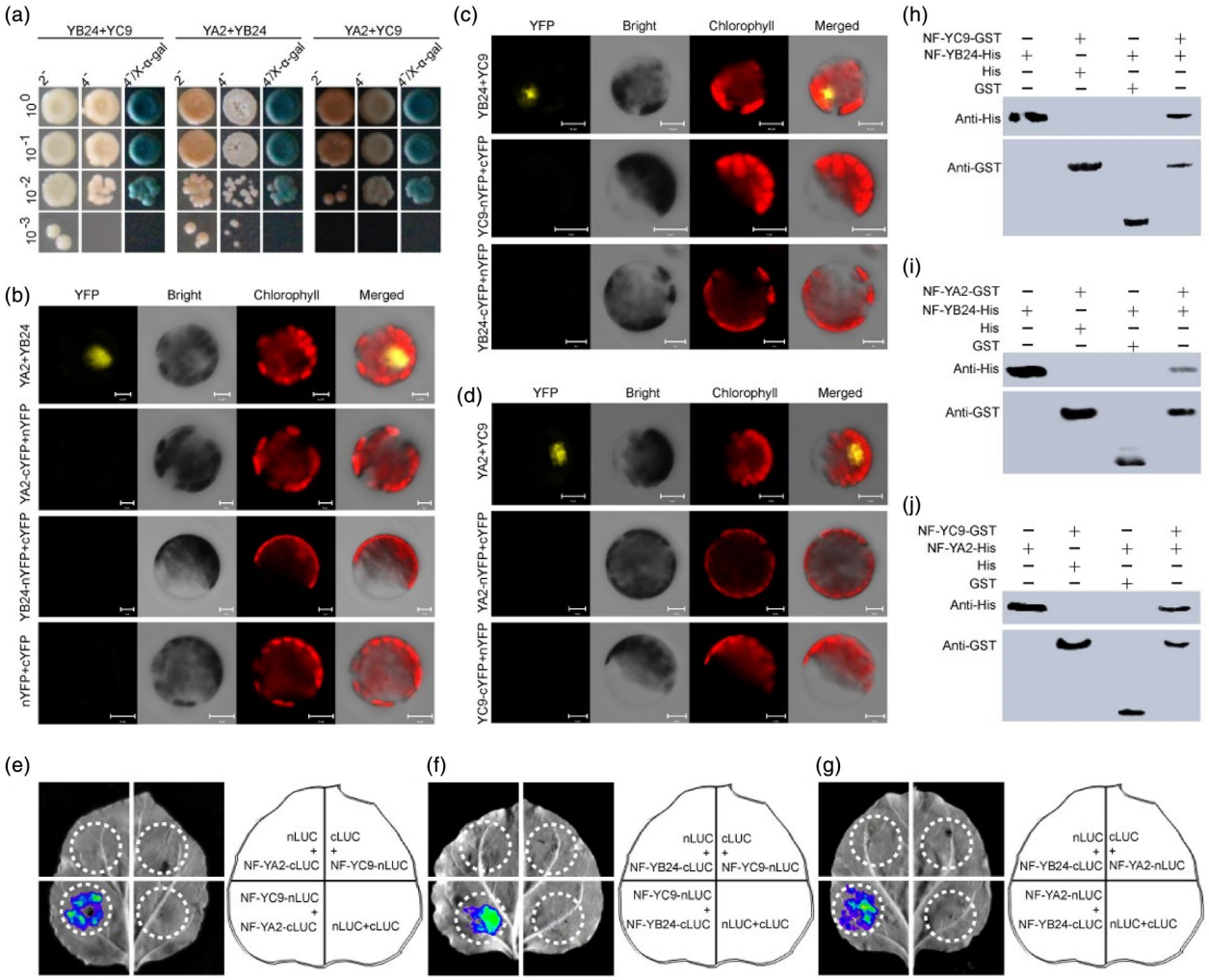


Figure 2 Interaction verification of GmNF-YC9 and its candidate proteins. (a–j) Interaction verification between GmNF-YC9 and its candidate proteins (GmNF-YB24 and GmNF-YA2) by yeast two-hybrid (a), BiFC (b–d), LCI (e–g), and pull-down (h–j) assays. YB24 and NF-YB24, GmNF-YB24; YA2 and NF-YA2, GmNF-YA2; YC9 and NF-YC9, GmNF-YC9.

GmNF-YC9-mediated CCAAT-box transcriptional complex can activate the expression of *GmSQE1*

To elucidate the molecular mechanism underlying GmNF-YC9--mediated stress tolerance in soybean, we conducted RNA-seq assays on *GmNF-YC9*-OE and WT plants. The analysis revealed upregulation of numerous stress-related genes in *GmNF-YC9*-OE plants. Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis identified significant enrichment in genes associated with sterol metabolism, including biosynthetic processes, transporters, and responses to steroid hormones (Figure 3a,b, Figure S3a,b). Among these genes, squalene monooxygenase (GmSQE1), which encodes the enzyme responsible for the rate-limiting step in sterol synthesis, displayed significant induction in *GmNF-YC9*-OE plants under stress conditions (Figure 3b). This suggested that the GmNF-YC9 transcription factor regulated the sterol synthesis pathway by modulating GmSQE1 activity. Our qRT-PCR results further confirmed elevated transcript levels of GmSQE1 in *GmNF-YC9*-OE plants (Figure S3c), which were significantly induced by drought and salt stress treatments (Figure S3d). Notably, *GmSQE1* exhibited higher expression in *GmNF-YA2* and *GmNF-YB24* transgenic hairy roots (Figure S4a,b), suggesting that it could be a potential target gene of GmNF-YC9-mediated CCAAT-box transcriptional complex.

To validate the regulatory role of GmNF-YC9 on GmSQE1 expression, luciferase (LUC) activity analysis assays were conducted. These assays revealed a substantial increase in *GmSQE1* promoter-induced LUC activity in the presence of GmNF-YA2, GmNF-YB24, or GmNF-YC9 proteins (Figure 3c,d). Electrophoretic mobility shift assays (EMSA) further demonstrated the binding of the GmNF-YA2/GmNF-YB24/GmNF-YC9 transcription complex to the CCAAT-box of the *GmSQE1* gene promoter (Figure S4c). These findings conclusively established that *GmSQE1* was a downstream target gene of a trimeric nuclear transcriptional activation complex. Moreover, enzyme-linked immunosorbent assay (ELISA) was employed to analyse SQE enzyme activity and its catalytic product, 2,3-oxidosqualene (2,3-OSQ), in both *GmNF-YC9*-OE and WT plants. The results demonstrated a significant increase in SQE enzyme and 2,3-OSQ content in salt and drought-treated *GmNF-YC9*-OE plants compared to WT plants (Figure S4d–g), indicating that the regulation of GmSQE1 activity by GmNF-YC9 can affect the sterol synthesis pathway.

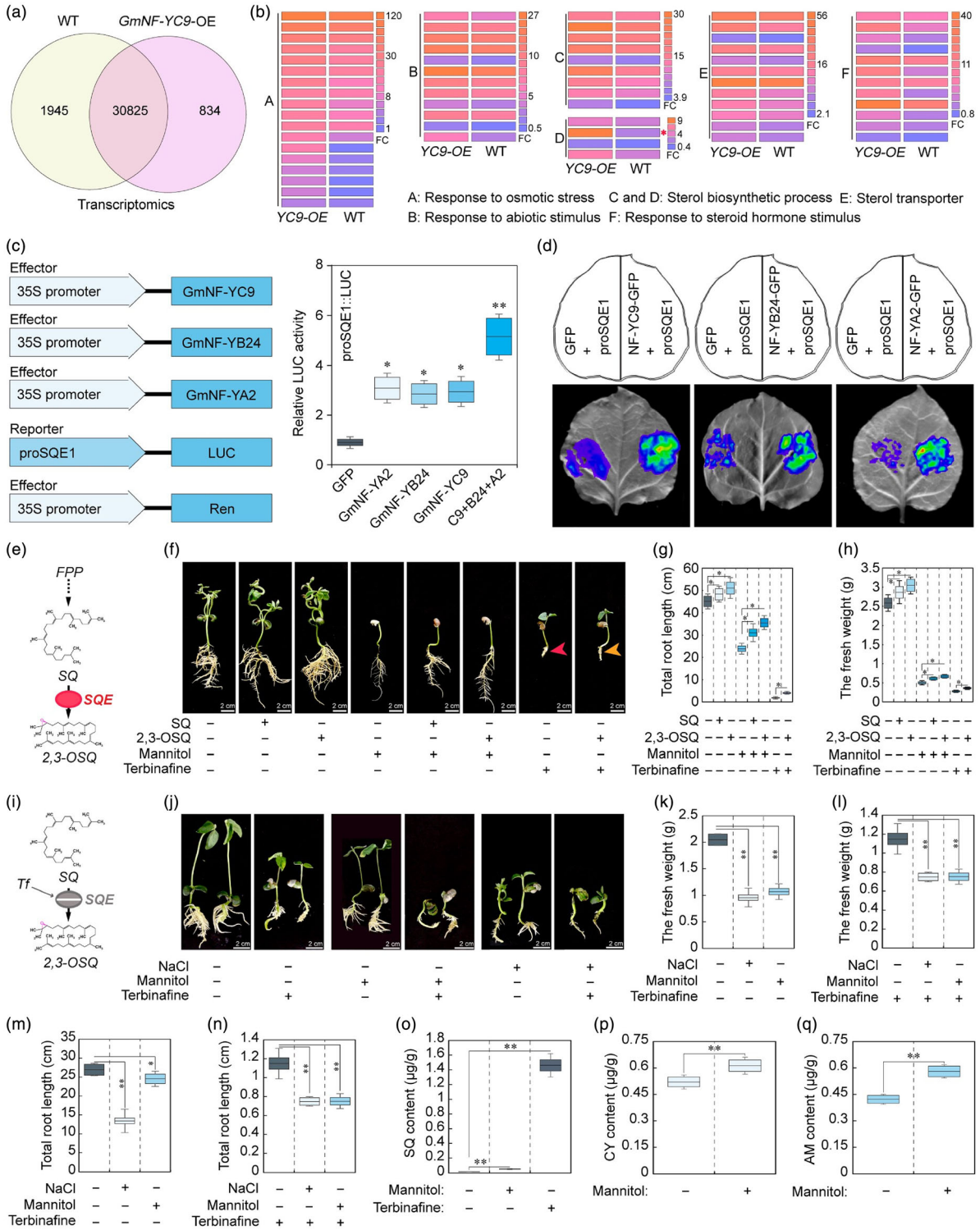


Figure 3 Downstream target gene and its regulation pathway analysis of GmNF-YC9 transcription factor. (a) The Venn diagram shows gene co-expression analysis of WT and *GmNF-YC9*-OE soybean plants under drought conditions. (b) GO enrichment analysis of differentially expressed genes (DEGs) in *GmNF-YC9*-OE soybean plants. Red asterisk indicates squalene monooxygenase (GmSQE1). (c, d) Interaction analysis between the NF-Y transcription factor and *GmSQE1* promoter by LUC activity assay. (e) The module of SQE-mediated catalysation to squalene. (f) Phenotype analysis of 7-day-old soybean seedling plants after 10 days of SQ (600 μg/L), 2,3-OSQ (300 μg/L), mannitol (200 mM), and terbinafine (100 μM) treatments, respectively. (g, h) Total root length (g) and fresh weight (h) of soybean seedlings under different treatments. (i) The inhibition of terbinafine on SQE-mediated catalysation to SQ. (j) Phenotype of 7-day-old soybean seedlings after 10 days of 100 mM NaCl, 100 mM mannitol, and 25 μM terbinafine treatments. (k–n) Fresh weight (k, l) and total root length (m, n) of 7-day-old soybean seedlings after 10 days of 100 mM NaCl, 100 mM mannitol, and 25 μM terbinafine treatment conditions. (o) Squalene content analysis of soybean seedling roots under different treatment conditions (25 μM terbinafine and 100 mM mannitol). (p, q) Cycloartenol (p) and beta-amyrin (q) content analysis of soybean seedling roots under 100 mM mannitol treatment conditions. AM, beta-amyrin; CY, cycloartenol.

Squalene and squalene-2, 3-epoxide, two key components in SQE-mediated catalytic pathway, can affect plant stress tolerance

To elucidate the contribution of enhanced GmSQE1 expression to plant stress tolerance, we investigated the correlation between stress tolerance and two key components of the SQE-mediated catalytic pathway: squalene (SQ) and 2,3-oxidosqualene (2,3-OSQ) (Figure 3e). 7-day-old soybean seedlings were subjected to different treatments. After a treatment period of 10 days, we observed the phenotypes of the soybean seedlings under different treatment conditions. The results revealed that exogenous application of SQ (600 μg/L) and 2,3-OSQ (300 μg/L) promoted soybean growth under normal conditions (Figure 3f–h). Moreover, these compounds significantly enhanced soybean stress tolerance under 200 mM mannitol conditions (Figure 3f–h). In contrast, terbinafine, a specific inhibitor of SQE widely used to inhibit sterol biosynthesis pathways (Figure 3i), conditionally impeded the development of normal root structures in soybean seedlings exposed to mannitol-induced stress (Figure 3f). Notably, this inhibitory effect was alleviated by providing exogenous concentrations of 2,3-OSQ at various levels, which partially prevented the occurrence of short-thick root phenotype (Figure 3f–h, Figure S5a–d). These results underscored the crucial role played by SQE-mediated plant sterol synthesis pathways in both growth and stress tolerance.

Furthermore, we employed the SQE inhibitor terbinafine to investigate the significance of squalene production in plant stress tolerance. Similarly, 7-day-old soybean seedlings were exposed to various stress treatments. After a duration of 10 days, the phenotypes of soybean seedlings were observed under different treatments. Our findings clearly demonstrated that the application of terbinafine compromised soybean's tolerance to stress (Figure 3j–n). Additionally, we performed liquid chromatography-mass spectrometry (LC–MS) analysis to examine changes in key metabolites from the SQE-mediated plant sterol synthesis pathway in soybean roots under 100 mM mannitol-induced stress. These analyses revealed a significant increase in squalene content in soybean roots upon exposure to 100 mM mannitol (Figure 3o, Figure S5e). Interestingly, treatment with terbinafine led to a substantial accumulation of squalene in soybean roots (Figure 3o, Figure S5e). Moreover, there was a notable elevation in the levels of cycloartenol and β-amyrin in soybean roots-two crucial products derived from 2,3-OSQ – after treatment with 100 mM mannitol (Figure 1p,q, Figure S5f), further highlighting their role in plant stress tolerance. Collectively, these results underscored the indispensable function of SQE in both plant growth and stress tolerance.

Overexpression of the NF-Y target gene *GmSQE1* in soybean improves drought and salt stress tolerance

To assess the potential role of squalene-derived compounds in enhancing plant stress tolerance, we generated three distinct *GmSQE1* overexpression lines (*GmSQE1*-OE1, *GmSQE1*-OE3, and *GmSQE1*-OE6) (Figure S6a–d). The impact of *GmSQE1* overexpression on drought and salt stress tolerance paralleled the observations made for *GmNF-YC9*-OE plants during the seedling stage (Figure S6e–j). Under stress conditions, *GmSQE1*-OE plants exhibited enhanced characteristics such as increased biomass and longer hypocotyl lengths compared to their WT counterparts (Figure S6e–j). These findings were further supported by pot experiments, highlighting the robust adaptation of *GmSQE1*-OE plants to drought and salt stresses (Figure 4a). Moreover, elevated proline and chlorophyll contents along with reduced malondialdehyde (MDA) levels were observed in *GmSQE1*-OE lines compared to WT plants (Figure 4b), providing evidence for the enhanced stress resilience conferred by overexpressing *GmSQE1*.

Furthermore, the stress tolerance effects of *GmSQE1* overexpression were also observed during the flowering stage, as evidenced by increased biomass in *GmSQE1*-OE and *GmNF-YC9*-OE plants compared to WT plants under drought and salt stress conditions (Figure S6k–p). These results confirmed that the high expression of *GmSQE1* and *GmNF-YC9* in soybean provided benefits for enhancing plant stress tolerance. To validate the durability of these effects, we conducted drought tolerance tests in field conditions over two seasons. Under normal irrigation conditions, no discernible differences in agronomic performance were observed between *GmSQE1*-OE, *GmNF-YC9*-OE, and WT plants (Figure S7). However, under drought stress, both *GmSQE1*-OE and *GmNF-YC9*-OE plants exhibited significantly improved agronomic performance compared to WT plants. This was evident through the increases in stem base circumference, grain count per plant, and grain weight per plant (Figure 4c–l, Figure S8a–t). Furthermore, an analysis of seed traits indicated that under drought conditions, *GmSQE1*-OE and *GmNF-YC9*-OE plants produced more high-quality seeds and fewer low-quality seeds than WT plants (Figure 4d–g, Figure S8l–t, Table S1). Importantly, *GmSQE1*-OE and *GmNF-YC9*-OE plants maintained these drought tolerance characteristics and improved agronomic traits at the adult stage under drought stress conditions (Figure 4c–l, Figure S8).

GmSQE1 and *GmNF-YC9* can enhance tolerance to abiotic stress-induced oxidation

A recent study has demonstrated that the depletion of *SQE* in cholesterol auxotrophic lymphomas leads to an accumulation of squalene, thereby preventing oxidative cell death (Garcia-Bermudez *et al.*, 2019). This finding suggests that the involvement of SQE in mediating cellular antioxidant process. However, our results and previous studies indicate that abiotic stress induces increased levels of reactive oxygen species (ROS) in plants (Figure S9a,b). Consequently, we hypothesised that GmSQE1 may enhance plant stress tolerance by improving soybean's resilience to oxidative stress. To test this hypothesis, *GmSQE1*-OE, *GmNF-YC9*-OE, and WT plants were subjected to methyl viologen (MV) and hydrogen peroxide (H_2O_2). After 7 days of treatment, MV induced wilting in WT plants, whereas *GmSQE1*-OE and *GmNF-YC9*-OE plants displayed varying degrees of resistance to this effect (Figure 4m–p). These transgenic plants exhibited a higher biomass compared to WT plants under MV treatment conditions (Figure 4n,p). Additionally, after a 1.5-h exposure to 200 mM H_2O_2, cell damage was evaluated using propidium iodide (PI) staining. While WT plant root cells exhibited PI red fluorescence in their cytoplasm, only a few *GmSQE1*-OE and *GmNF-YC9*-OE plant root cells displayed such fluorescence (Figure 4q,r). This result indicated that *GmSQE1*-OE and *GmNF-YC9*-OE plants experienced less cell damage compared to WT plants under 200 mM H_2O_2 treatment conditions (Figure 4q,r). Moreover, high-temperature (43 °C) stress, known to induce ROS production (Pospíšil, 2016), was applied to four-week-old seedlings. Following a recovery period after 12 h of exposure, both *GmSQE1*-OE and *GmNF-YC9*-OE plants survived at higher rates and accumulated more shoot biomass than the control group, which further highlighted their enhanced tolerance towards oxidative stress (Figure S9c–h). Interestingly, the application of terbinafine

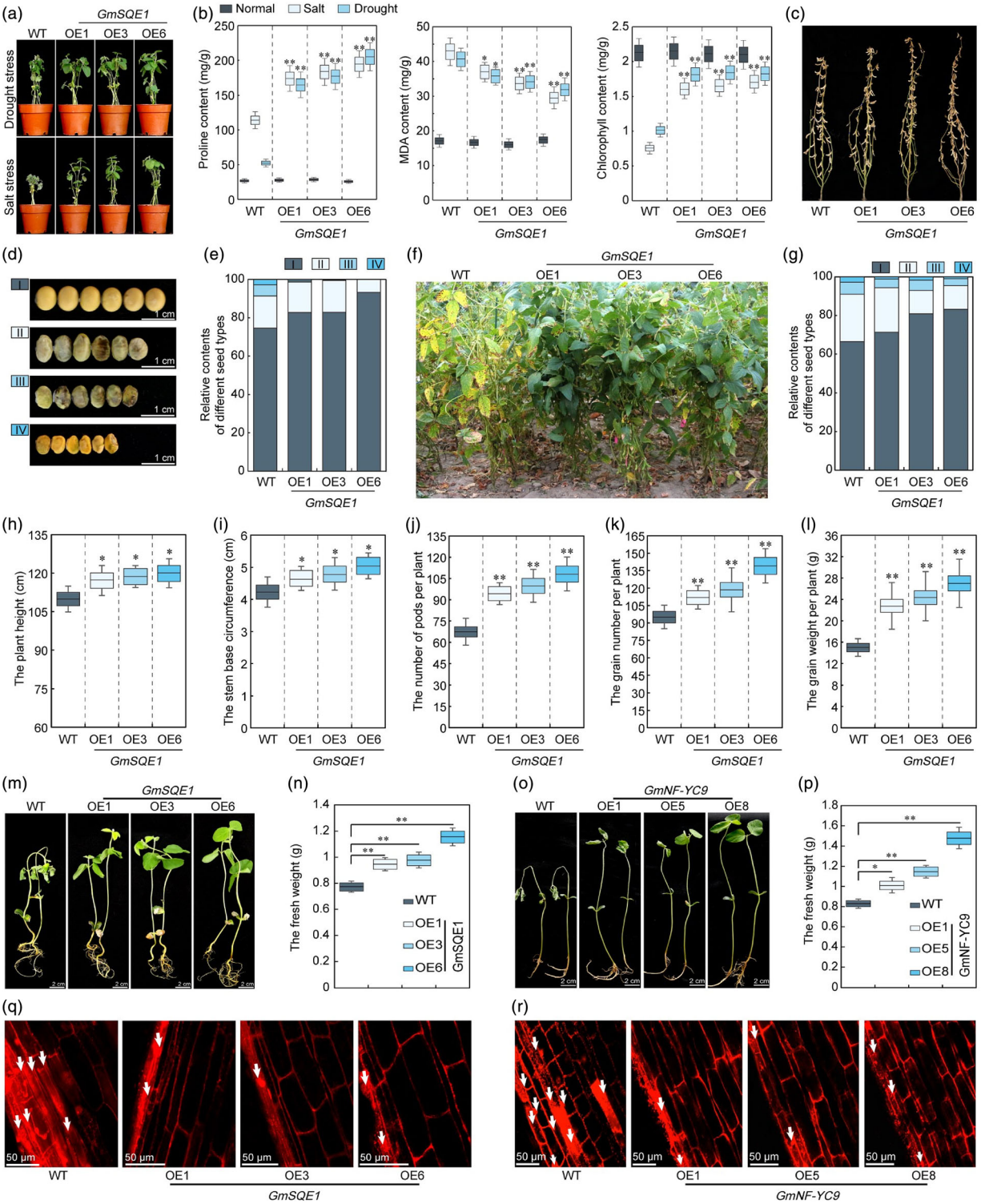


Figure 4 Stress tolerance identification analysis of *GmSQE1* overexpressing soybean plants. (a) Phenotype of WT and *GmSQE1*-OE seedling soybean plants under drought and 200 mM NaCl treatment conditions. (b) Proline, MDA, and chlorophyll content analysis of WT and *GmSQE1*-OE soybean plants under different stress conditions. (c) Phenotype analysis of *GmSQE1*-OE soybean plants at adult stage under drought conditions in the field in the year 2020. (d, e) Phenotype (d) and relative content (e) of different seed types in *GmSQE1*-OE soybean plants under drought conditions in the field in the year 2020. (f) Phenotype analysis of *GmSQE1*-OE soybean plants at adult stage under drought conditions in the field in the year 2021. (g) Relative contents of different seed types in *GmSQE1*-OE soybean plants under drought conditions in the field in the year 2021. (h–l) Agronomic trait analysis of WT and *GmSQE1*-OE soybean plants under drought conditions in the field in the year 2021. (m) Oxidative stress tolerance analysis of *GmSQE1* transgenic soybean plants under 50 μM MV treatment. (n) Fresh weight analysis of *GmSQE1* transgenic soybean plants under 50 μM MV treatment. (o) Oxidative stress tolerance analysis of *GmNF-YC9* transgenic soybean plants under 50 μM MV treatment. (p) Fresh weight analysis of *GmNF-YC9* transgenic soybean plants under 50 μM MV treatment. (q, r) 200 mM H_2O_2-induced cell death analysis of *GmSQE1* (q) and *GmNF-YC9* (r) transgenic soybean plant roots by Pi staining.

compromised soybean's oxidative stress tolerance (Figure S10a–c). Collectively, these results suggested that overexpressing both *GmSQE1* and *GmNF-YC9* significantly improved plant responses to oxidative stress across various contexts.

Exogenous application of soybean steroids can enhance crop stress tolerance

To further investigate the underlying mechanisms of *GmSQE1* and *GmNF-YC9*-mediated plant stress tolerance, we performed metabolomic analyses on *GmSQE1*-OE, *GmNF-YC9*-OE, and WT plants subjected to 100 mM mannitol treatment. The correlation analysis between transcriptome and metabolome data unveiled 134 KEGG pathways enriched in *GmNF-YC9*-OE compared to WT plants, with 132 pathways enriched in the transcriptomics data and 47 pathways in the metabolomics data (Figure 5a–c). Among these, 45 KEGG pathways overlapped (Figure 5c), primarily focus on metabolism and secondary metabolite biosynthesis (Figure 5d). Notably, genes and metabolites related to steroid biosynthesis, including brassinosteroid, were identified. In total, there was a total of 493 upregulated metabolites in *GmNF-YC9*-OE plants and 435 in *GmSQE1*-OE plants, with an overlap of 267 metabolites (Figure 5e). Since SQE catalysis is a rate-limiting step in the synthesis of steroids, their derivatives, and triterpenoids (Figure 5f), we specifically examined these compound classes. Our analysis revealed that a total of 20 upregulated steroids and steroid derivatives along with 22 triterpenoids under stress conditions in either *GmNF-YC9*-OE or *GmSQE1*-OE plants (Figure 5g,h). Notably, fucosterol and soyasaponin II displayed significant upregulation under stress conditions in both *GmNF-YC9*-OE and *GmSQE1*-OE plants (Figure 5g,h), highlighting their significance for plant stress tolerance.

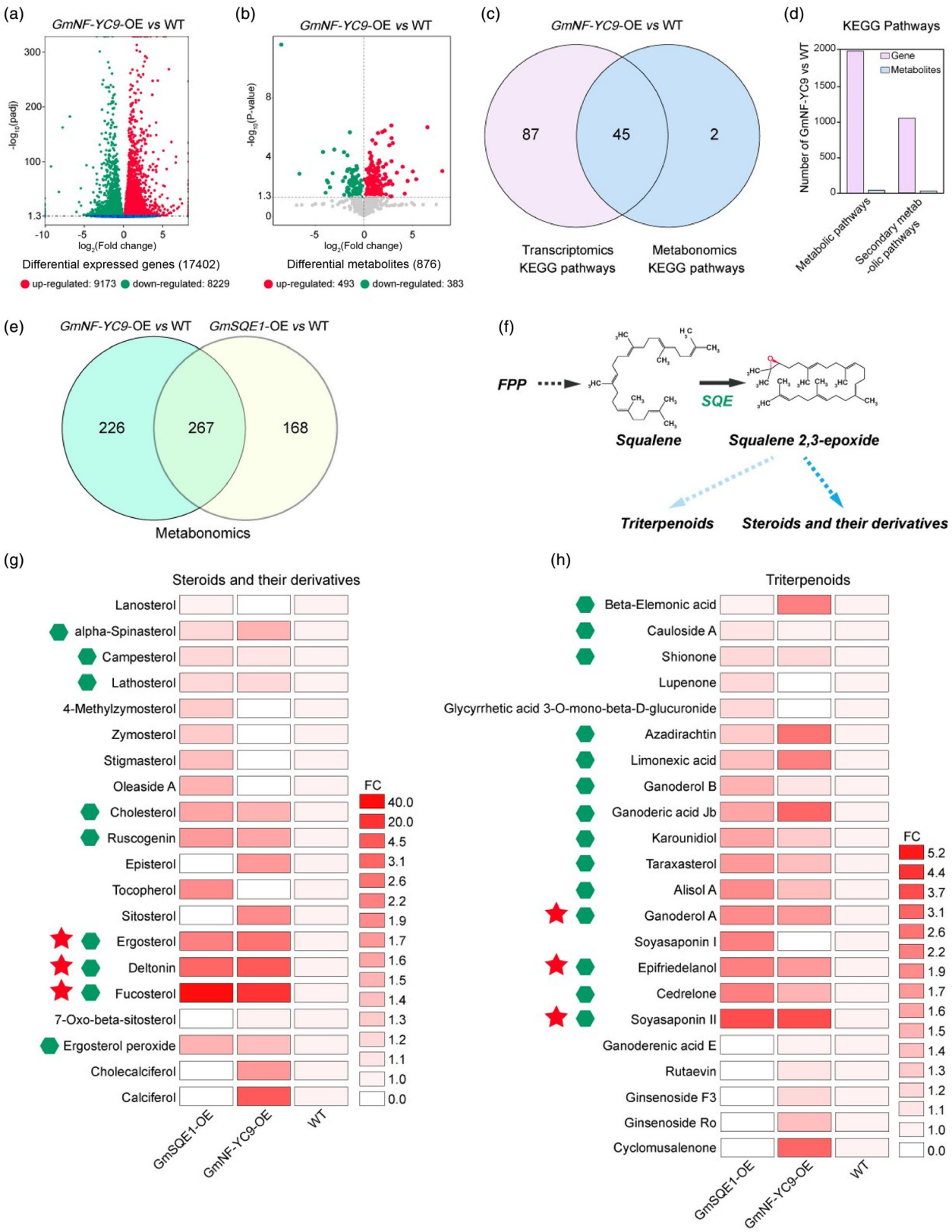
Subsequently, we investigated the effects of exogenously applied fucosterol and soyasaponin II on plant stress tolerance. Interestingly, while higher concentrations of these compounds inhibited soybean growth under normal conditions, and lower concentrations promoted the growth (Figure 6a–d). Furthermore, the application of moderate concentrations of fucosterol and soyasaponin II alleviated stress-induced growth inhibition in soybeans (Figure 6e–j). *In vitro* experiments conducted various crops including soybean, wheat, foxtail millet, and maize demonstrated that the spray application of moderate concentrations of these compounds enhanced drought and osmotic stress tolerance (Figure 6k–m, Figure S11). Additionally, we investigated the impact of beta-amyrin and cycloartenol-key products of the plant sterol synthesis pathway-on crop stress tolerance. Consistent with previous findings, the exogenous application of beta-amyrin and cycloartenol yielded similar results, higher concentrations inhibited soybean growth while lower concentrations not only promoted growth but also enhanced crop stress tolerance (Figure S12). Collectively, these results underscored the potential of these metabolites in enhancing stress tolerance across a variety of crucial crops.

Discussion

Several studies have established a correlation between CCAAT-box transcription factors and drought as well as heat stress (Nelson *et al*., 2007; Sato *et al*., 2019). However, the underlying mechanism remains elusive, and no direct gene targets associated with abiotic stress have been identified yet. In this study, we demonstrated that GmNF-YC9, a member of the NF-Y transcription factor C subunit family protein in soybean, exhibited responsiveness to both drought and salt stresses (Figure 1). Furthermore, our findings revealed that GmNF-YC9 could form a NF-Y transcriptional complex with GmNF-YA2 and GmNF-YB24 through protein–protein interactions to directly activate expression of the key enzyme involved in phytosterol synthesis pathway (*GmSQE1*), thereby promoting sterol synthesis to improve soybean stress tolerance (Figures 2, 3, Figure S4). This discovery revealed an unrecognised role of the NF-Y transcription factors in plants. Additionally, several subunits of NF-Y transcription factors have been implicated in flowering regulation. For instance, the trimeric complex comprising NF-YC and NF-YB subunits can recruit CONSTANS-Like transcription factors to regulate their DNA targets, thus influencing flowering time in *Arabidopsis* (Ben-Naim *et al*., 2006; Wenkel *et al*., 2006). Moreover, NF-Y has been demonstrated to modulate flowering time by mediating the control of H3K27me3 demethylation at the *SOC1* locus (Hou *et al*., 2014). Our previous study revealed that GmNF-YC9 showed homology with OsHAP5H, OsHAP5O, and AtNF-YC11 (Yu *et al*., 2021). Interestingly, research conducted on rice indicates that OsHAP5H (OsNF-YC9) has an association with the heading period, and overexpression of OsHAP5H delays heading under long day conditions (Li *et al*., 2016). However, in our experimental setup, no differences related to flowering time were observed between the *GmNF-YC9* overexpressing lines and WT plants in the growing area. Nevertheless, the role of GmNF-YC9 in regulating flowering time required further validation.

Adaptation to oxidative stress is a fundamental aspect of plant and animal cell physiology, with implications for both crop production and human disease in the face of environmental stress (Devireddy *et al*., 2021; Dixon *et al*., 2012; Forman and Zhang, 2021; Kerchev and Van Breusegem, 2022; Zandalinas *et al*., 2021). Reactive oxygen species (ROS) are the primary

Figure 5 Differentially accumulated sterol metabolite analysis in WT, *GmNF-YC9*-OE, and *GmSQE1*-OE soybean plants under drought stress conditions using transcriptome and metabolome. (a) Analysis of differentially expressed genes (DEGs) between *GmNF-YC9*-OE and WT plants under drought stress conditions using transcriptome technology. (b) Analysis of differentially accumulated metabolites (DAMs) between *GmNF-YC9*-OE and WT plants under drought stress conditions using metabolome technology. (c) Correlation analysis of drought stress-induced transcriptome and metabolome data of *GmNF-YC9*-OE *vs* WT. (d) KEGG pathway correlation analysis of transcriptome and metabolome in *GmNF-YC9*-OE *vs* WT plants under drought stress conditions. (e) Correlation analysis of metabolome data among *GmNF-YC9*-OE, *GmSQE1*-OE, and WT plants under drought stress conditions. (f) Analysis of plant SQE-mediated catalysed pathways. As SQE catalysis is the rate-limiting step in triterpenoid, steroid and steroid derivative biosynthesis, we focused on these compound classes. (g) Differentially expressed steroid and their derivative analysis among *GmNF-YC9*-OE, *GmSQE1*-OE, and WT plants under drought stress conditions. (h) Differentially expressed triterpenoid analysis among *GmNF-YC9*-OE, *GmSQE1*-OE, and WT plants under drought stress conditions. The green hexagon in figure g and h indicates the accumulations of 9 steroid-related compounds and 14 triterpenoids in both *GmNF-YC9*-OE and *GmSQE1*-OE plants under drought stress conditions. The red pentacle in figure g and h indicates the significant accumulations of steroid-related compounds and triterpenoids in both *GmNF-YC9*-OE and *GmSQE1*-OE plants under drought stress conditions.



source of cell oxidisation, leading to the oxidation of proteins, nucleic acids, and lipids (Mittler *et al*., 2022; Oikawa *et al*., 2022). Abiotic stress can cause excessive ROS accumulation in plant cells, compromising their stress tolerance (Castro *et al*., 2021; Kerchev and Van Breusegem, 2022). NF-Y transcription factors have a crucial function in inhibiting the excessive ROS accumulation and enhancing the antioxidant capacity of plant cells. Studies have shown that TaNF-YB1 regulates early wheat grain development by scavenging the excessive ROS to promote grain filling (Liu *et al*., 2023). In rice, OsNF-YC5 alleviates the inhibitory effect of

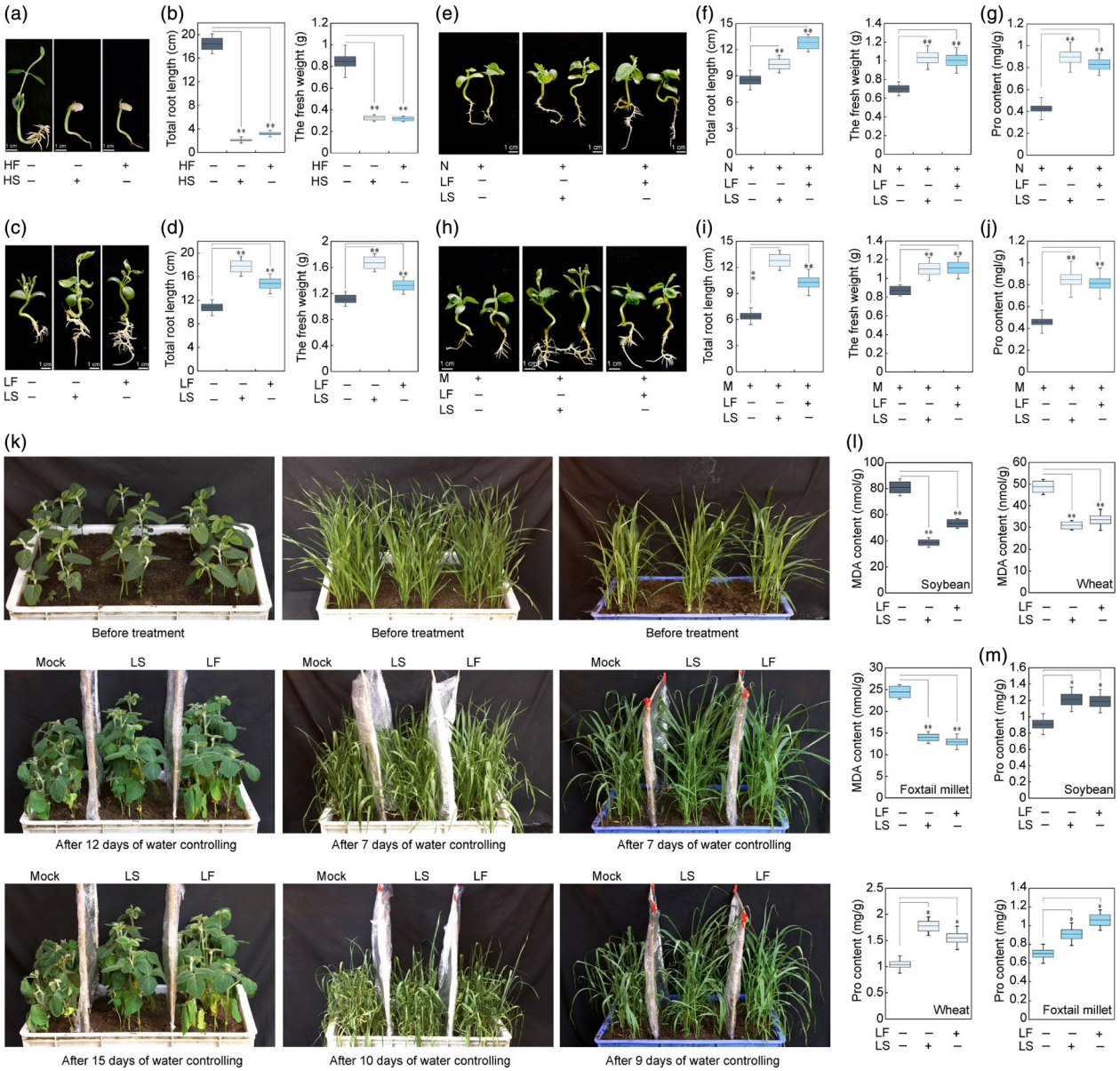


Figure 6 Function analysis of fucosterol and soyasaponin II in plant. (a, b) Phenotype (a) and biomass (b) analysis of soybean seedling plants under high concentrations of fucosterol and soyasaponin II treatment conditions. In figure a and b, HF indicates a high concentration of fucosterol (5 mg/L), while HS indicates a high concentration of soyasaponin II (5 mg/L). (c, d) Phenotype (c) and biomass (d) analysis of soybean seedling plants under moderate concentrations of fucosterol and soyasaponin II treatment conditions. (e–g) Phenotype (e), biomass (f), and proline (Pro) content (g) analysis of soybean seedling plants under fucosterol, soyasaponin II, and 100 mM NaCl treatment conditions. In figure e–g, N indicates 100 mM NaCl. (h–j) Phenotype (h), biomass (i), and proline (Pro) content (j) analysis of soybean seedling plants under fucosterol, soyasaponin II, and mannitol treatment conditions. In figure h–j, M indicates 100 mM mannitol. (k) Phenotype analysis of soybean, wheat, and foxtail millet plants by spraying moderate concentration of fucosterol and soyasaponin II under drought conditions. (l, m) MDA (l) and proline (Pro) content (m) analysis of soybean, wheat, and foxtail millet plants by spraying moderate concentration of fucosterol and soyasaponin II under drought conditions. In figure c–k, LF indicates a moderate concentration of fucosterol (300 μg/L), while LS indicates a moderate concentration of soyasaponin II (300 μg/L).

salt stress on seed germination by reducing ROS levels (Jin *et al*., 2023). Our findings demonstrated that NF-Y transcription factors improved plant oxidation stress tolerance through the regulation of SQE activity (Figure S4, Figure 4m–r). SQE is a rate-limiting enzyme of the IPP pathway and oxidises squalene to produce 2,3-OSQ, which is an important precursor for steroid and triterpenoid synthesis both in plants (Prashant *et al*., 2016; Thimmappa *et al*., 2014). Research has indicated that SQE mutation alters sterol composition in *Arabidopsis* roots, which affects the localisation of NADPH oxidases and subsequent regulation of ROS production (Posé *et al*., 2009). Additionally, in gastric cancer cells, SQE mutation results in an increased squalene level, thereby conferring high antioxidant ability upon cancer cells (Garcia-Bermudez *et al*., 2019). Our study demonstrated that overexpression of *GmSQE1* in soybean improved significantly soybean's tolerance to oxidative stress while reducing

oxidative stress-induced cell injury (Figure 4m,n,q). These findings suggested that SQE-mediated sterol synthesis pathway played a crucial role in modulating ROS and cell antioxidants. Furthermore, our results demonstrated that NF-Y transcription factors could also upregulate the expression of genes associated with oxidative stress response, including heat shock proteins (HSPs) (Ul Haq *et al*., 2019), antioxidant enzymes (Mittler *et al*., 2022), and ferritins (Reyt *et al*., 2014) (Figure S13). This highlighted the complexity of NF-Y transcription factors regulation on plant cell antioxidants.

Interestingly, we observed a reversal of SQE-mediated cell antioxidant behaviours between human cells and soybean. While the mutation of SQE in human cells led to strong cell antioxidant activity (Garcia-Bermudez *et al*., 2019), our findings demonstrated that overexpression of *GmSQE1* in soybean enhanced oxidative stress tolerance (Figure 4m,n,q). Further analysis reveals large differences in sterol metabolism process between mammals and plants, as almost all plants steroids are derived from lanosterol and cycloartenol, while only lanosterol serves as the precursor of all animal steroids (Figure S14) (Prashant *et al*., 2016; Yoshioka *et al*., 2020). Both lanosterol and cycloartenol are produced directly from 2,3-OSQ (Prashant *et al*., 2016; Yoshioka *et al*., 2020). In plants, 2,3-OSQ also serves as a substrate for the amyrin synthase (AYSE)-mediated amyrin metabolism pathway for production of triterpenoids such as soyasaponin (Figure S14) (Mertens *et al*., 2016). These substantial disparities indicate that steroids in plants exhibit a greater diversity and complexity compared to those observed in mammals, highlighting the crucial roles of plant-specific cycloartenol synthase (CYSE)-mediated cycloartenol metabolism and amyrin synthase (AYSE)-mediated amyrin metabolism pathways in plant stress tolerance, including oxidative stress tolerance. Indeed, our targeted metabolome analysis revealed significant accumulations of multiple steroids and triterpenoids (including their derivatives) following stress treatments (Figure 5g,h). Spraying these compounds *in vitro* significantly improves drought stress tolerance of wheat, maize, and foxtail millet (Figure 6, Figures S11, S12), suggesting that the effect is also relevant to monocots and the function is crucial on enhancing plant stress tolerance. These findings suggest that these compounds, through genetic modification or selection to enhance their production, or by their chemical application, can be used to improve stress tolerance and quality in crops. However, further elucidation is required regarding the molecular mechanism underlying how plant steroids affect cell viability. Together, our research uncovered an undiscovered NF-Y-SQE module that modulated sterol synthesis and played a crucial role in plant stress tolerance (Figure 7). Application of plant steroids *in vitro* showed potential for enhancing crop tolerance to abiotic stress (Figure 7). Furthermore, we hypothesised that phytosterols may exhibit significant potential in the field of medicine, particularly in enhancing the microenvironment of animal cells through augmenting their antioxidative capacity.

Materials and methods

Generation and detection of transgenic soybean plants

Soybean cultivated variety (*Glycine max*, 'Tiefeng 8') was utilised to amplify *GmNF-YC9* (Glyma.13G189400) and *GmSQE1* (Glyma.08G063700). The open reading fames (ORFs) of *GmNF-YC9* and *GmSQE1* were cloned into the pTF101 vector to generate the recombinant vectors, which were then transformed into *Agrobacterium* strain EHA105. Soybean variety "Williams 82" was used as the recipient material for transformation, and stable transformation was performed following the protocol described by Chen *et al*. (2018). T_3 homozygous generations of *GmNF-YC9* and *GmSQE1* transgenic soybean plants were confirmed by PCR and qPCR (Table S2), which were used for functional analysis.

Analysis of stress tolerance on transgenic soybean plants

For 150 mM NaCl and 200 mM mannitol treatments, T_3 homozygous *GmNF-YC9* and *GmSQE1* transgenic soybean seeds were surface-sterilised with chlorine for 6 h, following the method described by Chen *et al*. (2018) and Cheng *et al*. (2021). The sterilised seeds were then transferred to a B5 vitamin solid medium for growth under normal conditions. After 7 days of growth, the seedlings were transferred into a B5 vitamin solid medium supplemented with 150 mM NaCl and 200 mM mannitol for stress tolerance analysis. After 7 days of treatment, the seedlings were analysed.

For stress tolerance analysis during the soybean seedling stage, T_3 homozygous *GmNF-YC9* and *GmSQE1* transgenic soybean seeds were sown in flowerpots filled with equally weighted nutrient soil for growth under normal conditions. Four-week-old soybean seedlings were treated with either water controlling or 250 mM NaCl. After 14 days of drought or 7 days of salt treatment, the stress tolerance phenotypes of transgenic soybean seedlings were analysed. The method was expounded upon by Wang *et al*. (2020) and Yu *et al*. (2021).

For drought tolerance analysis at soybean adult stage, we utilised rain-proof installations to systematically investigate the effects of water stress on transgenic soybean plants. Every year at the end of May (Beijing, China), we planted transgenic soybean seeds in the field for growth under natural conditions. Around the middle of August, soybean plants began to enter periods of pod filling. We thus stopped irrigation to control water at the beginning of August and utilised rain-proof installations to prevent weather from affecting drought treatment (Figure S15). After 1 month of water controlling treatment, the soil water content was measured using the method described by Yu *et al*. (2021) (Table S3), and the phenotype under drought conditions was recorded. In the middle of October, soybean plants began to be harvested, and agronomic traits were recorded for analysis.

Bimolecular fluorescence complementation (BiFC) assay

The ORFs of bait protein and candidate protein were cloned into the pUC-SPYNE and pUC-SPYCE vectors, respectively. Subsequently, the recombinant vectors were co-transformed into *Arabidopsis* protoplasts through PEG-mediated transfection, followed by incubation in the dark at 25 °C. The detailed method was described by Yoo *et al*. (2007). YFP fluorescence of the protoplasts was observed using a confocal laser scanning microscope (LSM 700; Zeiss, Oberkochen, Germany).

Luciferase complementation imaging (LCI) assay

The bait protein and candidate protein genes were individually inserted into pCAMBIA1300-nLUC and pCAMBIA1300-cLUC vectors. Subsequently, *Agrobacterium* GV3101 harbouring the nLUC and cLUC derivative binary recombinant plasmids were separately introduced via co-injection into leaves of 4-week-old *Nicotiana benthamiana*. The firefly luciferase complementation imaging (LCI) transient expression assay was performed following the protocol established by Fujikawa and Kato (2007).

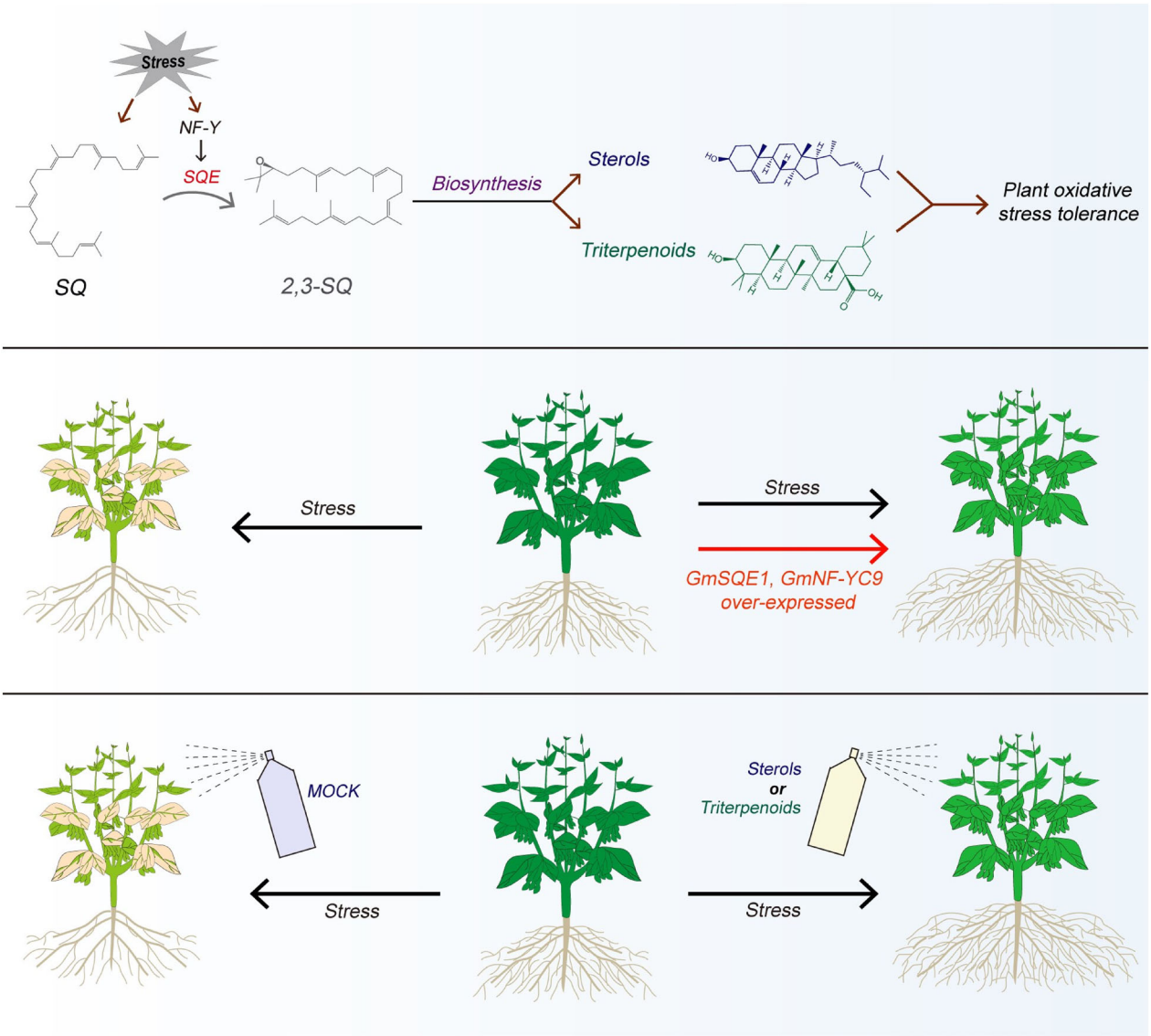


Figure 7 The model of NF-Y-SQE module and soybean steroids improving plant stress tolerance. NF-Y-SQE module participates in regulating plant stress tolerance by enhancing SQE-mediated squalene metabolic pathways. Application of plant steroids or triterpenoids in crops enhanced their tolerance to drought stress.

In vitro GST pull-down assay

The open reading frames (ORFs) of bait protein and candidate protein genes were cloned into the pCold™ TF vector (TaKaRa, Bio) and pGEX-4T-1 vector (GE Healthcare, Chicago, IL), respectively. Subsequently, the recombinant plasmids were introduced into the *E. coli* strain transetta (TransGen, Biotech). Expressions of the corresponding proteins were induced overnight using 0.5 mM isopropyl β-D-thiogalactoside (IPTG) (Inalco SPA, San Luis Obispo, CA) at 16 °C, and then a pull-down assay was performed. The experimental details were described by Yu *et al*. (2021).

In vitro EMSA binding assay

The genes encoding three subunit proteins of the NF-Y transcription factor were cloned into expression vectors harbouring the tag proteins (His, GST, and MBP), respectively, and subsequently transferred into competent cells of the *E. coli* strain *Rosetta*. These recombinant proteins were employed for an EMSA using a biotin end-labelled duplex DNA probe. The method was previously described by Yu *et al*. (2021).

Effect of fucosterol and soyasaponin II on crop growth under different treatments

Three-week-old of soybean, wheat, foxtail millet, and maize seedlings grown under normal conditions were subjected to controlled water treatment. During the treatment, the seedlings were sprayed with aqueous solutions of mock (40 mL distilled water containing 200 μL of 40 mM fatty alcohol polyoxyethylene ether (APE), which was a leaf surfactant), fucosterol (20 μL of fucosterol at a concentration of 600 mg/L was added into 40 mL distilled water containing 200 μL of 40 mM APE), and soyasaponin II (20 μL of soyasaponin II at a concentration of 600 mg/L was added into 40 mL distilled water containing 200 μL of 40 mM APE). The sprays were applied four times each week, with at least 1 day between each spraying. The changes in crop phenotypes

were observed and recorded during controlled water treatment. The leaf samples of crops were collected at the onset of phenotypic variations in order to quantify physiological and biochemical alterations.

Five-week-old of soybean, wheat, foxtail millet, and maize seedlings cultivated under normal conditions were subjected to osmotic stress treatment by irrigating them with 200 mM mannitol aqueous solutions. During osmotic stress treatment, the seedlings were sprayed with mock (mock, 40 mL distilled water containing 200 μL of 40 mM APE), fucosterol (fucosterol aqueous solutions, 20 μL fucosterol (600 mg/L) into 40 mL distilled water containing 200 μL of 40 mM APE), and soyasaponin II aqueous solutions (soyasaponin II aqueous solutions, 20 μL soyasaponin II (600 mg/L) into 40 mL distilled water containing 200 μL of 40 mM APE). The sprays were applied four times each week, with at least 1 day between each spraying. The phenotype changes of the crops were observed and recorded during controlled water treatment. Leaf samples of the crops were taken when differences in the leave phenotype began to appear in order to measure physiological and biochemical changes.

Metabolites extraction, UHPLC–MS–MS analysis, and data processing

The samples of stress induced WT and transgenic soybean seedlings (*GmSQE1*-OE6 and *GmNF-YC9*-OE8 plants) were freeze-dried, and then crushed with a mixer mill. A 50 mg aliquot of each individual samples were precisely weighed and transferred to Eppendorf tubes, which were used for metabolites extraction and UHPLC–MS analysis (Allwegene Technology Co., Ltd, Beijing, China). The methods of plant metabolites extraction were described in detail by Vos *et al*. (2007).

The UHPLC separation was performed using an EXIONLC System (Sciex). The mobile phase A was 0.1% formic acid in water, while mobile phase B was acetonitrile. The column temperature was set at 40 °C, and the auto-sampler temperature was set at 4 °C with an injection volume was 2 μL. A Sciex QTrap 6500+ (Sciex Technologies) was applied for assay development. Typical ion source parameters were as follows: IonSpray Voltage, +5500/−4500 V; Curtain Gas, 35 psi; Temperature, 400 °C; Ion Source Gas, 1:60 psi; Ion Source Gas 2, 60 psi; DP, ± 100 V (Allwegene Technology Co., Ltd, Beijing, China).

SCIEX Analyst Work Station Software (Version 1.6.3) was employed for MRM data acquisition and processing. MS raw data (.wiff) files were converted to the TXT format using MSconverter. An in-house R program and database were applied to peak detection and annotation (Allwegene Technology Co., Ltd, Beijing, China).

Measurement of squalene, cycloartenol, and beta-amyrin by LC–MS

The samples of untreated, 25 mM terbinafine treated, and 100 mM mannitol induced soybean seedlings were freeze-dried, and then crushed with a mixer mill. A 50 mg aliquot of each sample was accurately weighed and transferred to Eppendorf tubes for metabolites extraction. The extracted metabolites were used for squalene, cycloartenol, and beta-amyrin content measurement by UHPLC–MS analysis. The method of extracting plant metabolites was described in detail by Vos *et al*. (2007).

Squalene (5 mg/mL) (Sigma, USA), cycloartenol (5 mg/mL) (Sigma, USA), and beta-amyrin (5 mg/mL) (Sigma, USA) were used in appropriate amounts to form standard solutions. These solutions were then diluted with methyl alcohol to create a suitable standard series of standards at concentrations of 5, 10, 20, 50, 100, 200, and 500 ng/mL. The peak areas of the standard substances at different concentrations were used to draw the standard curves that exhibited a good linear relationship with an R^2 value >0.99. The standard curves were drawn based on the peak areas of the standard substance at different concentrations and their corresponding concentrations. With an R^2 value >0.99, it indicated that the standard curves exhibit a strong linear relationship and could be utilised for calculating the concentrations of squalene, cycloartenol, and beta-amyrin in the samples.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

ZSX coordinated the project, conceived and designed experiments, and edited the manuscript; TFY, HZH, HLW, SYC, and XYS performed the experiments and wrote the first draft; SYC, WJZ, LZ, and JTW generated and analysed LC–MS and cell antioxidant-related data; ZWL, LZ, JC, YBZ, MC, SLS, and LGJ contributed valuable discussions; QYJ reviewed and revised this paper; YZM coordinated the project and edited the manuscript. All authors have read and approved the final manuscript.

Data availability statement

The data that supports the findings of this study are available in the supplementary material of this article.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Generation and stress tolerance identification of *GmNF-YC9*-OE soybean plants.

Figure S2 Functional analysis of GmNF-YB24 and GmNF-YA2 candidate proteins.

Figure S3 Differentially expressed genes of *GmNF-YC9*-OE soybean plants by transcriptome sequencing.

Figure S4 Interaction analysis between the NF-Y transcription factor and the potential target gene *GmSQE1*.

Figure S5 Effect of terbinafine on soybean plant growth and content analysis of squalene, cycloartenol, and β-amyrin under different treatments by LS-MS.

Figure S6 Identification and stress tolerance identification of *GmSQE1* overexpressing soybean plants.

Figure S7 Agronomic trait analysis of *GmNF-YC9*- and *GmSQE1*-OE soybean plants under normal conditions.

Figure S8 Agronomic trait analysis of *GmSQE1*- and *GmNF-YC9*-OE soybean plants under drought conditions.

Figure S9 Oxidative stress tolerance analysis of *GmNF-YC9*- and *GmSQE1*-OE soybean plants.

Figure S10 Effect of terbinafine on soybean oxidative stress tolerance.

Figure S11 Effect of fucosterol and soyasaponin II on crop stress tolerance.

Figure S12 Effect of cycloartenol and beta-amyrin on crop stress tolerance.

Figure S13 Expression level analysis of the genes related with response to ROS in *GmNF-YC9*-OE and WT soybean plants.

Figure S14 Difference analysis of SQE-mediated catalytic pathway in humans and higher plants.

Figure S15 Stress tolerance of soybean materials by application of rain-proof installations in the experimental field.

Table S1 Different types of seeds divided into four groups (Field).

Table S2 *GmNF-YC9* and *GmSQE1* gene-specific primers used in this article.

Table S3 Relative soil water content analysis of the experimental field.

Table S4 Specific primers of the candidate proteins of GmNF-YC9.

Table S5 qPCR primers for detecting DEGs associated with ROS response.

