Soybean F-Box-Like Protein GmFBL144 Interacts With Small Heat Shock Protein and Negatively Regulates Plant Drought Stress Tolerance

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The F-box gene family is one of the largest gene families in plants. These genes regulate plant growth and development, as well as biotic and abiotic stress responses, and they have been extensively researched. Drought stress is one of the major factors limiting the yield and quality of soybean. In this study, bioinformatics analysis of the soybean F-box gene family was performed, and the role of soybean F-box-like gene GmFBL144 in drought stress adaptation was characterized. We identified 507 F-box genes in the soybean genome database, which were classified into 11 subfamilies. The expression profiles showed that GmFBL144 was highly expressed in plant roots. Overexpression of GmFBL144 increased the sensitivity of transgenic Arabidopsis to drought stress. Under drought stress, the hydrogen peroxide (H$_2$O$_2$) and malonaldehyde (MDA) contents of transgenic Arabidopsis were higher than those of the wild type (WT) and empty vector control, and the chlorophyll content was lower than that of the control. Y2H and bimolecular fluorescence complementation (BiFC) assays showed that GmFBL144 can interact with GmsHSP. Furthermore, our results showed that GmFBL144 may negatively regulate plant drought stress tolerance by interacting with shHSP. These findings provide a basis for molecular genetics and breeding of soybean.

Keywords: soybean, F-box protein, drought stress, Skp1-Cullin1-F-box (SCF) complex, segmental duplication, protein interaction

INTRODUCTION

Abiotic stress is the primary factor limiting plant growth and crop yield. Abiotic stresses include drought, saline-alkali, high/low temperature, and metal stress, of which drought stress is the most common stress (Baldoni et al., 2015; Gong et al., 2020). Plants have evolved multiple strategies to deal with drought stress. Common strategies include reducing water loss, maintaining chlorophyll content, and reducing reactive oxygen (Ritchie et al., 1990; Zafari et al., 2020).
Molecular breeding has long been expected to improve crop drought tolerance. Thus far, many genes involved in drought stress regulation have been identified. For instance, *ZmVPP1*, a vacuolar-type H^+^ pyrophosphatase gene, can improve the drought tolerance of transgenic maize by enhancing root development and photosynthetic efficiency (Wang X. et al., 2016). Tubby-like F-box protein 8 (SITLTP8) enhances plant drought tolerance by reducing water loss to improve water-use efficiency (Li et al., 2020b). Additionally to the above positive regulatory genes, some negative regulatory genes have been found. For example, MdSE reduces the expression of *MdNCED3* by negatively regulating the *MdMYB88* and *MdMYB124* transcription factors (*MdMYB88*, *MdMYB124*, *MdNCED3*; positive regulators of drought resistance) to reduce the drought tolerance of apples (Li et al., 2020c). Although many genes responding drought stress have been investigated, the molecular network of plant responses to drought stress is still imperfect.

The ubiquitin-proteasome system (UPS) and molecular chaperone system play important roles in plant responses to drought stress. Protein degradation mediated by UPS is an important post-translation regulation mechanism, which includes ubiquitin, ubiquitin-activating enzymes (E1s), ubiquitin conjugating enzymes (E2s), ubiquitin ligase enzymes (E3s), and 26S proteasomes (Sadanandom et al., 2012; Xia et al., 2020; Ban and Estelle, 2021). Ubiquitin is activated by E1 in the presence of ATP, and is then transferred to the cysteine residue of E2. Then, E3 transfers ubiquitin to the lysine residue of the substrate, and finally 26S proteasome degrades the ubiquitinated substrate. In this pathway, E3s are responsible for substrate recognition and substrate ubiquitination. Research has found that there are thousands of E3s in plants, which can be classified as many different types, of which RING E3s are the most abundant (Zheng and Shabek, 2013; Morreale and Walden, 2016). SCF is a well characterized RING E3 ubiquitin ligase, which contains four subunits: Cullin1 (CUL1), Ring-box protein (Rbx), S-Phase kinase associated protein 1 (Skp1), and F-box protein. Cullin1, Rbx, and Skp1 interact to form a core scaffold with ligase activity, and the F-box proteins are responsible for substrate recognition (Zheng et al., 2016; Zhang S. et al., 2019).

Recently, large numbers of F-box proteins were identified. Especially in plants, there are hundreds of F-box proteins. Their common feature is that the N-terminal contains a relatively conservative F-box domain that interacts with Skp1 and CUL1 to form SCF. Besides the F-box domain, there are other variable domains in the C-terminal. The C-terminal domains are responsible for substrate recognition and also provide a foundation for subfamily classification of F-box proteins. The diversity of F-box proteins can help SCF distinguish and recruit multiple substrates. Therefore, it is not surprising that F-box proteins can regulate many physiological processes. For example, Carbonnel et al. (2020) found that F-box protein MAX2 can inhibit the growth of primary roots and promote the growth of root hairs by increasing the content of ethylene through the karrikins signaling pathway. Another case in point is that ORE9, an F-box protein, can regulate leaf senescence through the ubiquitin-proteasome pathway (Woo et al., 2001; Zhang et al., 2016). In addition, in recent years, a growing number of F-box proteins involved in abiotic stress responses have been studied, e.g., drought, salt, ion, and low temperature stresses (Lim et al., 2019; Zhang Y. et al., 2019; Jie et al., 2020; Venkatesh et al., 2020).

Presently, most research on F-box protein function are from other plants, and research on soybean F-box proteins is relatively limited. Soybean is an important food crop and oil crop, and its yield is seriously affected by drought stress. Therefore, it is necessary to study the function of GmF-box proteins in drought. In this study, we performed functional characterization of GmFBL144 in drought stress adaptation. The results showed that overexpression of GmFBL144 significantly reduced plant drought tolerance. Under drought stress, the overexpression lines had higher hydrogen peroxide (H2O2) and malonaldehyde (MDA) content, lower chlorophyll content, and a higher water loss rate compared to the controls [wild type (WT) and empty vector]. Furthermore, our results showed that GmFBL144 can form SCF/FBL144 (E3 ubiquitin ligase) with GmsSkp1 and GmCulin1, and GmFBL144 can interact with GmsHSP.

**MATERIALS AND METHODS**

**Identification and Bioinformatics Analysis of GmF-Box Genes**

The whole genome protein sequence of soybean (Glycine max Wm82.a2.v1) was downloaded from the plant genome database1. Simultaneously, the hidden Markov model of F-box domain (PF0046) and F-box-like domain (PF12937) were downloaded from the Pfam database2. The proteins sequences were searched by conserved domain using the BLAST method in the protein database. The genes of the two families were merged and then identified using the NCBI database (E-value cutoff 1.0). The genes without the F-box domain and F-box-like domain were deleted.

Phylogenetic analysis was inferred using the Maximum Likelihood method based on the Poisson correction model by MEGA7 (1000 bootstrap replicates for detection reliability). The annotation file GFF3 for soybean was downloaded from the Phytozone database, and visualized using TBtools. Gene duplication analyses were performed with the MscanX (Wang et al., 2013).

A 2,000 bp DNA sequence upstream of GmFBL144 gene was download from the soybean genome database, and the promoter elements were analyzed using the PlantCARE website3 (Lescot et al., 2002).

**Plant Materials and Growth Conditions**

The soybean genotype Williams 82 was cultivated in the field. Roots, stems, leaves, flowers, and embryos of different stages were collected and stored at −80°C for RNA extraction for quantitative RT-PCR assay. *Arabidopsis thaliana* were cultivated

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1http://phytozone.jgi.doe.gov
2http://pfam.xfam.org
3http://bioinformatics.psb.ugent.be/webtools/plantcare/html/
in a controlled-climate room with 16/8 h light/dark cycle at 23°C and 60% relative humidity.

**Generation of Transgenic Arabidopsis**
The full-length coding sequence (CDS) of GmGBL144 was inserted after the CaMV35S promoter, resulting in overexpression of recombinant vectors. After sequencing, the fusion constructs were transformed into Agrobacterium tumefaciens (GV3101) and then transformed into Arabidopsis (Col-0) plants using the floral dip (Clough and Bent, 2010). T1 seedlings were screened with 1% glucosamine and then reconfirmed using genomic PCR. After screening the separation ratio and high expression, three homozygous T3 transgenic lines (L16, L17, and L19) were selected for phenotypic experiments.

**Stress Treatments**
For rapid assay of the function of GmFBL144 in the drought response, the full-length CDS of GmGBL144 was inserted in pYES2 vector. The fusion construct was transformed into INVSc1 yeast competent cells using the PEG/LiAc-mediated method and pYES2 vector. The fusion construct was transformed into Agrobacterium tumefaciens (GV3101, pSoup-p19) and then co-transformed into epidermal Nicotiana benthamiana cell for transient assays (Lu et al., 2009). The fluorescent signals of yellow fluorescent protein (YFP) were observed using a laser confocal microscope (TCS SP8, Leica, Germany).

The full-length GmCullin1 gene (Glyma.17G025200) was inserted into pGADT7 and pGBKTK7. The full-length GmSkp1 gene was inserted into pGADT7. The full-length GmFBL144 and GmSkp1 genes (Glyma.11G079600) were inserted into two distinct multiple cloning sites of the pBridge vector simultaneously. The pairs were co-transformed into Y2H gold yeast competent cells using the PEG/LiAc-mediated method. The interaction between bait and prey proteins were identified on SD/-leu/-Trp and SD/-Leu/-Trp/-His/-Ade/X-α-gal media.

**Determination of Chlorophyll, Malonaldehyde, and Hydrogen Peroxide Content**
Chlorophyll content was assessed following previously used methods (jie et al., 2020). The contents of MDA and H$_2$O$_2$ were determined though the corresponding test kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**Statistical Analysis**
The results were analyzed using GraphPad Prism 5.0 software. The data are expressed as means ± SD. All experiments were repeated thrice. The statistical significance was considered at the $P < 0.05$ or $P < 0.01$ level, as revealed by $t$-tests.

**RESULTS**

**Identification and Bioinformatics Analysis of Gm-F-Box Genes**
The F-box genes belong to a supergene family, which is a cluster of genes produced by duplication and mutation from an ancestor. Gm19G196400 was defined as an F-box-like gene belonging to the F-box gene family. To understand Gm19G196400, we performed bioinformatics analysis of the soybean F-box gene family. We identified 507 F-box genes in the soybean genome database, and we renamed these genes according to the N-terminal domain and chromosomal distribution. Our objective gene, Gm19G196400, is named GmFBL144 (Supplementary Table 2).

F-box proteins contain other domains at C-terminal besides N-terminal relatively conserved F-box domain. In order to
research conveniently, the whole F-box protein family was classified into 11 subfamilies (Supplementary Table 2) and the GmFBL144 belongs to the FBO subfamily (F-box proteins with C-terminal other known domain). The phylogenetic tree analysis showed that most members of the same subfamily tended to cluster in the same evolutionary branch (Supplementary Table 2 and Supplementary Figure 1).

The distribution of GmF-box genes on chromosomes was visualized (Figure 1A). The number of F-box genes on chromosome 8 was the largest (48 F-box genes), and on chromosome 1 was the lowest (10 F-box genes). There was no significant correlation between F-box gene number on a chromosome and chromosome length. Gene duplication is the source of evolutionary innovation and main factor in gene family expansion. In this study, gene duplication analysis was performed. 307 WGD/segmental duplication GmF-box genes, corresponding to 192 duplicated gene pairs in the entire GmF-box gene family, were identified in soybean; 109 dispersed duplication GmF-box genes, 44 proximal duplication GmF-box genes, and 26 tandem duplication GmF-box genes corresponded to 37, 17, and 9 duplicated genes pairs in the GmF-box gene family, respectively (Figure 1B). The results of the gene duplication analysis revealed that the GmF-box gene family expansion was largely a result of WGD/segmental duplication.

In addition, we analyzed the gene structure and promoter regions of GmFBL144 and GmFBL25 that showed the closest genetic relationship with GmFBL144 (Figures 2A,B and Supplementary Figure 1). The results showed that GmFBL144 had a similar structure to GmFBL25 and adds a NleF-casp-inhib domain (Figure 2B). Furthermore, we found that there are four drought response elements (MYB motif, C/TAACNA/G) and a stress response element (TC-rich repeats, ATTCTCTAAG) in the promoter region of GmFBL144, whereas GmFBL25 only had an MYB motif (Figure 2C). These findings suggest that GmFBL144 may be involved in drought regulation. We also found that the relative expression of GmFBL144 was high in roots (Figure 2D). Roots are the main organ of external environment perception. Therefore, we conjecture that GmFBL144 may be involved in the drought stress response.

Overexpression of GmFBL144 Increased Drought Stress Sensitivity in Transgenic Arabidopsis Seedlings

There are multiple drought response elements in the promoter region of GmFBL144 and our transcriptome data of soybean (Zhou et al., 2020) showed that the GmFBL144 was downregulated under drought stress (Supplementary Figure 2). Accordingly, we studied the function of GmFBL144 in drought stress. In this study, the yeast transient expression system was used. The results showed that the heterologous expression of GmFBL144 yeast was undergrown compared with the vector control under drought stress conditions (Figure 3A). Next, we carried out the Arabidopsis plate drought stress study. Mannitol (250 mM) was used to simulate drought stress in 1/2 MS medium. The results showed that after 10 days of simulated drought stress, the primary root length of GmFBL144-overexpression Arabidopsis was shorter and the fresh weight was lighter than that of controls (WT and empty vector) (Figures 3B–D).

Similarly, the transgenic Arabidopsis showed drought sensitivity under soil drought stress. Under normal conditions, there was no significant difference between controls (WT and empty vector) and the GmFBL144-overexpression transgenic lines. After 2 weeks without watering, the foliar loss of GmFBL144-overexpression transgenic lines was more serious than that in the controls (WT and empty vector) (Figure 4A). The chlorophyll content of GmFBL144-overexpression transgenic lines was lower, and the MDA and H2O2 content were higher than those of the controls (WT and empty vector) (Figures 4B–G). After 20 days without watering, the GmFBL144-overexpression transgenic lines were almost dead; however, the controls (WT and empty vector) were still alive. After 3 days of rehydration, the controls (WT and empty vector) recovered but the GmFBL144-overexpression transgenic lines could not (Figure 4A). These results indicate that GmFBL144 increased plant drought sensitivity through damaging the system of scavenging activated oxygen.

Subcellular Localization of GmFBL144

Proteins are the most important biomacromolecules in organisms, and the major performers of life activities. Mature proteins can exert different biological functions in specific subcellular organelles. Thereby, the function of a protein is not only related to its structure but also to its subcellular localization. The subcellular localization prediction websites forecast that the GmFBL144 protein could be located on the nucleus and chloroplast. At the same time, we investigated the subcellular localization of GmFBL144 using the transient transformation system of tobacco leaves. The fluorescence signal of the fusion protein was mainly located on the nucleus, and a small part also exist on the cell membrane (Figure 5).

Identification of the Formation Mechanism of the SCF FBL144 Complex

Studies have shown that most F-box proteins perform functions by forming SCF complexes (Kipreos and Pagano, 2000; Ao et al., 2020). To verify whether GmFBL144 can form SCF complex, we evaluated the interactions among Cullin1 (CUL1), Skp1, and GmFBL144 using Y2H assay. The results showed that GmFBL144 can interact with GmSkp1, cannot interact with GmCUL1; GmCUL1 can interact with GmSkp1 but is weak; the complex of GmFBL144-GmSkp1 can interact with GmCUL1. These results indicated that GmFBL144 is a key subunit of SCF complex, and GmFBL144 can promote the combination of GmSkp1 and GmCUL1 (Figure 6).

Screening and Identification of GmFBL144 Interacting Proteins

In order to further research the mechanism, we screened the interacting proteins through the cDNA library. Because

4https://www.genscript.com/wolf-psort.html
5http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/
FIGURE 1 | Chromosome distribution and gene duplication analysis of GmF-box genes. (A) Chromosome distribution of GmF-box genes. (B) Gene duplication analysis of GmF-box genes. The GmF-box gene family expansion was primarily caused by WGD/segmental duplication. WGD/segmental duplications gene pairs are shown in green lines; tandem duplication gene pairs are shown in red lines; dispersed duplication gene pairs are shown in orange lines; proximal duplication gene pairs are shown in blue lines.
FIGURE 2 | Collinearity analysis and the structural and expression profile analysis of GmFBL144. (A) Collinearity analysis between chromosome 19 and chromosome 3. (B) Gene structure analysis of GmFBL144 and GmFBL25. GmFBL144 protein shares an F-box domain with GmFBL25, and GmFBL144 has an additional NleF_casp_inhib domain. (C) Promoter region analysis of GmFBL144 and GmFBL25. The GmFBL144 promoter contains four drought response elements (MYB motif) and one stress response element (TC-rich repeats). The GmFBL25 promoter contains only one drought response element (MYB motif). (D) Expression profile analysis of GmFBL144. The expression of GmFBL144 in roots, stem, leaves, flowers, embryos of different stages, and seeds. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Data represent means ± SE of three biological replicates.

GmFBL144 was primarily located on the nucleus, we constructed a soybean cDNA library of a yeast nuclear system. The primary library capacity was approximately $1.04 \times 10^7$, the recombination rate was approximately 100%, and the average insert length was > 1,000 bp (Figure 7A). The sub-library capacity was approximately $1.44 \times 10^7$, the recombination rate was approximately 100%, and the average insert length was > 1,000 bp (Figure 7B). After the soybean cDNA library was constructed, we used BD-GmFBL144 as bait for screening interaction proteins with the Y2H assay. Forty-five positive blue clones were obtained and annotated in Supplementary Table 3. Among these clones, small heat shock protein (sHSP) (GenBank: XP_014626034.1), involved in multiple abiotic stress in some plants (Kuang et al., 2017; Guo et al., 2020), was identified for four times, indicating that it might have the strong interaction with GmFBL144.

The Y2H assay results of the one-to-one interaction verification showed that GmFBL144 can interact with GmsHSP (Figure 7C). We further verified the interaction between
FIGURE 3 | Phenotype of transgenic Arabidopsis under simulated drought stress. (A) Phenotype of heterologous expression of GmFBL144 yeast and control under drought stress (1 M mannitol). (B) Phenotype of control (wild type and vector) and GmFBL144-overexpression lines (L16, L17, and L19) under drought stress (250 mM mannitol). (C) Primary root length of control and GmFBL144-overexpression lines under drought stress (250 mM mannitol). (D) Fresh weight of control and GmFBL144-overexpression lines under drought stress (250 mM mannitol). Data represent means ± SE of three biological replicates. Asterisks indicate significant difference applying ANOVA (*P < 0.05; **P < 0.01; ***P < 0.001).

GmFBL144 and GmsHSP using BiFC assay. The fusion constructs of FBL144-nYFP and sHSP-cYFP were co-transferred into epidermal Nicotiana benthamiana cells for transient assays through Agrobacterium infiltration. Yellow fluorescence was observed in cells co-transferred with fusion constructs (Figure 7D), whereas in the negative control, yellow fluorescence was not observed. The results revealed that GmFBL144 can interact with GmsHSP protein.

Small heat shock proteins (sHSPs) are important molecular chaperones, which can prevent damaged protein aggregation caused by stress. sHSP help damaged protein refold to restore biological function by cooperating with other HSPs (HSP100 or HSP70) in the presence of ATP (Lee et al., 2005; Bernfur et al., 2017; Waters and Vierling, 2020). Previous studies have shown sHSP can enhance plant tolerance to external stress (Chauhan et al., 2012; Kuang et al., 2017; Guo et al., 2020). In this study, we identified the function of sHSP through the yeast transient expression system. The results showed that the growth of overexpression of GmsHSP yeast was better than that with vector control under drought stress (Figure 7E). This result shows that GmsHSP is a positive regulator of drought stress. The subcellular localization analysis of GmsHSP found that GmsHSP was mainly localized in nucleus and peroxisome (Supplementary Figure 3).

DISCUSSION

Gene Duplication

In the process of evolution, soybean has experienced two genome duplication or polyploidization events, resulting in a highly duplicated genome with 75% of genes present in the form of paralogous copies (Shoemaker et al., 1996; Gill et al., 2009; Schmutz et al., 2010). In addition, segmental duplication and
FIGURE 4 | Phenotype and physiological index of transgenic Arabidopsis under drought stress. (A) Phenotype of control (wild type and vector) and GmFBL144-overexpression lines (L16, L17, and L19) under drought stress. (B–G) Physiological index of control and GmFBL144-overexpression plants under drought stress, including chlorophyll content (B–D), fresh weight (E), MDA content (F), $\text{H}_2\text{O}_2$ content (G). Data represent means ± SE of three biological replicates. Asterisks indicate significant difference applying ANOVA (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
tandem duplication are important reasons for increasing gene copies and genetic diversity (Zhao et al., 2018). Previous studies have shown that F-box gene family expansion of rice and chickpea mainly caused by tandem duplication (Jain et al., 2007; Gupta et al., 2015). However, in our study, WGD/segmental duplication was the main expansion form of F-box gene in soybean. Similarly, the expansion of the F-box gene family in wheat, pear, and cotton was dominated by WGD/segmental duplication (Wang G. et al., 2016; Zhang S. et al., 2019; Li et al., 2020a). These differences may be caused by variations in the definition of tandem duplication. Previous studies of the F-box gene family have used different definitions on tandem duplication. In contrast, the tandem duplication definition used by MCScanX is more restrictive than that of previous studies (Wang et al., 2012).

The terminal genes of chromosome 19 and chromosome 3 showed collinearity in addition to some F-box genes (Figure 2A), which may have contributed to chromosome rearrangement after polyploidization. Similar results have also reported in other studies (Schmutz et al., 2010; Zhang et al., 2018). GmFBL144 and GmFBL25 have high homology (Supplementary Figure 1 and Figure 2B), which may be the result of evaluation selection of double-copy gene. Evolutionary selection can lead to loss one of the homologous genes or pseudogenization, and the evolution of new function (Moore and Purugganan, 2003; Semon and Wolfe, 2007; Zhao et al., 2018). Future studies will assess whether GmFBL25 has other functions.

GmFBL144 Is a Key Subunit of the SCF^{FBL144} Complex

The SCF complex is the main form of the F-box protein that performs function. Some F-box proteins perform function in a non-SCF form in yeast and human (Galan et al., 2001; Nelson et al., 2013), but this has not been found in plants.
FIGURE 7 | Screening and identification of GmFBL144 interacting proteins. Quality identification of primary library (A) and sub-library (B). Recombination rate = successful recombinant clones (24)/total clones (24) × 100%, library capacity = total clones (1300)/spreading volume (0.05 mL) × dilution multiple (100) × total volume (4 mL). Interaction of GmFBL144 with GmsHSP was verification by Y2H (C) and BiFC (D). nYFP denotes the YFP N-terminal protein. cYFP denotes the YFP C-terminal protein. Scale bars = 25 µm. (E) Phenotype of heterologous expression of GmsHSP in yeast under drought stress (1 M mannitol).

FIGURE 8 | Model for molecular mechanism of GmFBL144. (A) In response to drought stress, GmsHSP was increased through the inhibition of GmFBL144. (B) GmFBL144 can form SCF<sub>FBL144</sub> with Skp1 and Cullin1 to promote the ubiquitinated degradation of GmsHSP by 26S proteasome, which reduces plants drought tolerance.
Interestingly, some F-box proteins can perform functions in both SCF and non-SCF forms in human, for example Fbx07 (Nelson et al., 2013). Presently, research on plant F-box proteins mainly focuses on their SCF-dependent functions, and there are a large number of F-box proteins in plant. Therefore, it is impossible to rule the existence of the SCF-independent functions of F-box proteins. Previous studies suggested that Cullin1 and Rbx1, Skp1 form a core scaffold (Gagne et al., 2002; Qin-xue et al., 2018; Ban and Estelle, 2021). However, in our study, the interaction between Cullin1 and Skp1 was weak, but GmFBL144 can promote the combination of Cullin1 and Skp1 to form SCF<sup>GmFBL144</sup>. This result may be related to the binding of GmFBL144 and GmSkp1, which changes the conformation of GmSkp1 resulting in binding-capacity enhancement of GmCullin1 and GmSkp1.

**GmFBL144 Enhances the Sensitivity of Plants to Drought Stress**

*F-box* genes play an important role in plant growth, development and stress responses. Recently, considerable research has suggested that F-box genes participate in drought stress responses. For example, *Capsicum annuum* Drought-Induced F-box Protein 1 (CaDIF1) is a positive regulator of drought tolerance (Lim et al., 2019). Under drought stress, *F-BOX OF FLOWERING 2* (FOF2) positively regulated ABA-induced stomatal closure, resulting in reduced water loss (Qu et al., 2020). In addition, Li et al. (2020b) found that SITLFP8 (Tubby-like F-box protein 8) can enhance tomato drought tolerance by decreasing water loss via changing stomatal density. In general, previous studies have shown that F-box genes enhance the drought stress tolerance of plants. However, our study showed that GmFBL144 enhanced the sensitivity of plants to drought stress, potentially caused by the interaction between GmFBL144 and GmsHSP. sHSP as an important molecular chaperone can maintain protein stability (Morris et al., 2010; Papsdorf and Richter, 2014). They also associate with membranes. Sakthisel et al. (2009) found that HspA can stabilize membrane proteins such as the photosystems and phycobilisomes from oxidative damage. Balogi et al. (2008) found that a mutant Hsp17 (Q16R) with increased thylakoid association can improve the tolerance of UV-B damage in *Synechocystis*. In our study, GmsHSP was a positive regulator of drought stress (Figure 7E). When plants were subjected to poor environmental conditions, the homeostasis was maintained through the molecular chaperone and proteolytic systems (Li et al., 2019, 2020d). In our study, GmFBL144 was down-regulated and GmsHSP was up-regulated under drought stress (Supplementary Figure 2). These results indicated that soybean can withstand drought stress by enhancing the chaperone system. Previous studies have found that F-box genes positively regulated drought tolerance, likely because F-box proteins maintain intracellular homeostasis via the UPS, or increase the content of positive regulatory factors of drought stress. However, in our study, the GmFBL144 may degrade GmsHSP by SCF<sup>GmFBL144</sup>, destroying the molecular chaperone system and aggravating the protein homeostasis imbalance that leads to drought sensitivity (Figure 8).

**CONCLUSION**

In this study, a total of 507 *GmF-box* genes were identified, and classified into 11 subfamilies. The expansion of the *GmF-box* gene family was primarily caused by WGD/segmental duplication. Under drought stress, the expression of GmFBL144 was down-regulated (Supplementary Figure 2). Overexpression of GmFBL144 enhanced sensitivity to drought stress. GmFBL144 can form SCF with Skp1 and Cullin1, and interact with GmsHSP. GmFBL144 may promote sHSP ubiquitination through forming SCF<sup>GmFBL144</sup>, and then the ubiquitated sHSP is degraded by 26S proteasome (Figure 8B).

**DATA AVAILABILITY STATEMENT**

The original contributions presented in this study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

**AUTHOR CONTRIBUTIONS**

KX and YuZ performed the bioinformatics analysis. YaZ, CF, YIZ, FW, XL, HG, WL, YJ, RS, and XF provided assistance on the experiments. HL and YoZ designed the study and revised the manuscript. All authors reviewed and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.823529/full#supplementary-material
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