



Article

Genome-Wide Identification, Expression Analysis, and Potential Roles under Abiotic Stress of the *YUCCA* Gene Family in Mungbean (*Vigna radiata* L.)

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Abstract: *YUCCA*, belonging to the class B flavin-dependent monooxygenases, catalyzes the rate-limiting step for endogenous auxin synthesis and is implicated in plant-growth regulation and stress response. Systematic analysis of the *YUCCA* gene family and its stress response benefits the dissection of regulation mechanisms and breeding applications. In this study, 12 *YUCCA* genes were identified from the mungbean (*Vigna radiata* L.) genome and were named based on their similarity to *AtYUCCAs*. Phylogenetic analysis revealed that the 12 *VrYUCCAs* could be divided into 4 subfamilies. The evidence from enzymatic assays in vitro and transgenic *Arabidopsis* in vivo indicated that all the isolated *VrYUCCAs* had biological activity in response to IAA synthesis. Expression pattern analysis showed that functional redundancy and divergence existed in the *VrYUCCA* gene family. Four *VrYUCCAs* were expressed in most tissues, and five *VrYUCCAs* were specifically highly expressed in the floral organs. The response toward five stresses, namely, auxin (indole-3-acetic acid, IAA), salinity, drought, high temperatures, and cold, was also investigated here. Five *VrYUCCAs* responded to IAA in the root, while only *VrYUCCA8a* was induced in the leaf. *VrYUCCA2a*, *VrYUCCA6a*, *VrYUCCA8a*, *VrYUCCA8b*, and *VrYUCCA10* seemed to dominate under abiotic stresses, due to their sensitivity to the other four treatments. However, the response modes of the *VrYUCCAs* varied, indicating that they may regulate different stresses in distinct ways to finely adjust IAA content. The comprehensive analysis of the *VrYUCCAs* in this study lays a solid foundation for further investigation of *VrYUCCA* genes' mechanisms and applications in breeding.

Keywords: mungbean; *YUCCA* gene family; identification; expression profiles; abiotic stress; transgene



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1. Introduction

Auxin/indole-3-acetic acid (IAA) is one of the major endogenous phytohormones, playing a critical role in regulating plant growth and development as well as the stress response [1], and it results from the coordinated actions of auxin biosynthesis, metabolism, transport, and signaling [2]. During the plant life cycle, IAA adjusts almost all aspects of biological processes, including embryogenesis [3], shoot and root formation [4], leaf development [5], floral development and fertility [6,7], and fruit initiation [8]. Additionally, auxin homeostasis is essential for plants to adapt to biotic and abiotic stresses, such as pathogens, herbivory, temperature, salt, and drought [1,9,10].

In plants, both tryptophan (Trp)-dependent and -independent pathways contribute to the auxin de novo synthesis, and the former is very well understood [11]. There are four distinct routes that involve different metabolic intermediates, namely, tryptamine (TAM), indole-3-pyruvic acid (IPA), indole-3-acetaldoxime (IAOx), and indole-3-acetamide (IAM) [12]. The IPA pathway (two steps) is mainly responsible for IAA biosynthesis in plants, in which

Trp is first converted into IPA by tryptophan aminotransferase (TAA), and then YUCCA catalyzes the rate-limiting irreversible reaction, producing IAA from IPA [11,13].

YUCCA belongs to class B flavin-dependent monooxygenases (FMOs; EC 1.14.13.8), the only class of FMOs found in plants based on the current status of available sequencing data [12]. It has been demonstrated that YUCCA proteins share several conserved motifs, including the FAD-binding motif (GXGXXG), FMO-identifying sequence (FXGXXXHXXXY/F), NADPH-binding motif (GXGXXG), ATG-containing motif1 ($Y_{(X)7}ATGEN_{(X)5}P$), and ATG-containing motif2 ($D_{xxxx}ATGY$) [12,14]. Among these motifs, the FAD- and NADPH-binding motifs are central to YUCCA activity and have the same characteristic structure in amino acid sequences [11]. The FMO-identifying sequence, a key sequence in all known plant FMOs, contributes to NADPH binding [15].

To date, genome-wide identification of YUCCA family genes has been performed in more than 30 plants species, such as *Arabidopsis thaliana* [16], *Oryza sativa* [17], *Zea mays* [18], *Cucumis sativus* [2], *Glycine max* [19], *medicago truncatula* [20], *Populus trichocarpa* [9], *Lilium* [21], *malus domestica* [22], *Triticum aestivum* [14], *Gossypium* spp. [23], and *Brassica napus* [24]. However, knowledge about the YUCCA gene family and its potential roles in mungbean is limited. The biological functions of YUCCA genes have been demonstrated thoroughly in some species, especially in model plants. In *Arabidopsis*, the YUCCA(YUC) gene family includes 11 members with overlapping functions in plant development. In 2001, *AtYUC1* was first identified from a dominant *Arabidopsis* mutant *yucca* involved in the production of IAA [25]. Subsequent functional studies on the loss-of-function or gain-of-function of YUCCA genes in *Arabidopsis* indicated that they played a vital role in developmental processes, including floral patterning and vascular formation (e.g., *AtYUC1,2,4,6*) [16], embryogenesis and leaf formation (e.g., *AtYUC1,4,10,11*) [26], hypocotyl elongation (e.g., *AtYUC5*) [27], and the regeneration of root systems (e.g., *AtYUC9*) [28]. *AtYUC6* and *AtYUC7* were reported to confer drought tolerance depending on distinct mechanisms [10,29]. *AtYUC6* played a dual role in regulating plant growth as an auxin biosynthetic enzyme and conveying drought response as a thiol-reductase (TR)-like protein, which functioned through ROS regulation [29]. While root *AtYUC7* could be induced by drought in an abscisic acid (ABA)-dependent manner, the auxin level elevation promoted lateral root growth to protect plants from drought [10]. It was reported that *AtYUC8* and *AtYUC9* were upregulated under high temperatures [1,30], and constitutively overexpressing these two genes showed less damage after spider mite infestation [31]. Under high temperatures, the transcriptional regulator phytochrome-interacting factor 4 (*PIF4*) directly induced *AtYUC8* expression to elevate endogenous free IAA levels, and the plant exhibited hypocotyl elongation [30]. In rice, 14 YUCCA genes have been identified, with a crucial role in crown root development (e.g., *OsYUCCA1*) [32], leaf morphogenesis (e.g., *OsNAL7/OsYUCCA8*) [33], maintaining water homeostasis and appropriate root-to-shoot ratios (e.g., *OsCOW1*) [34], and auxin-mediated anther dehiscence (e.g., *OsYUCCA4*) [35]. Two research teams have reported that 22 YUCCA genes were isolated from soybean, though with different naming rules [19,20]. There were, therefore, four controversial sequences. *GmYUCCA2* (*Glyma.03G208900*) and *GmYUCCA21* (*Glyma.19G206200*), identified in 2017 [19], were not included in the 2019 research, replaced by *GmYUC5-8-9a* (*Glyma.10G041800*) and *GmYUC5-8-9b* (*Glyma.13G128800*) [20]. Therefore, the soybean genome contains at least 24 members of the YUCCA gene family. Overexpression of *GmYUCCA5* (*Glyma.04G070100*) in *Arabidopsis* displayed downward curling of the leaf-blade margin, evident apical dominance, higher plant height, and shorter length of siliques [19]. It was also proven that *GmYUC2a* (*Glyma.08g038600*) acted as a vital part in regulating both root growth and nodulation by modulating the auxin balance in soybean [20].

Mungbean is an important warm-season grain legume with rich nutrition and short duration, facilitating environmental sustainability [36]. Our previous study showed that a YUC4-like protein regulated the chasmogamous flower trait in mungbean [37], indicating the crucial roles of YUCCA genes in developmental regulation. In this study, we systematically identified 12 YUCCA family genes (named as *VrYUCCA*) in the mungbean genome

and investigated their gene structures, expression patterns, biological activity in vitro and in vivo, and potential roles under abiotic stresses. This research can provide valuable information about the functional explanation of the *YUCCA* gene family in mungbean, contributing to the application of these genes in mungbean genetic improvement.

2. Results

2.1. Identification of the *YUCCA* Gene Family in Mungbean and Structure Analysis

Based on the output of the Gcorn plant, BLAST homology search, and conserved domain analysis, 12 *VrYUCCA* genes were isolated from the mungbean genome (Table S1), named according to their similarity to *Arabidopsis YUCCA* genes [16]. No homologs for *AtYUC1*, *AtYUC5*, *AtYUC7*, or *AtYUC9* were found in mungbean. Meanwhile, there were three homologs for *AtYUC8* and two each for *AtYUC2*, *AtYUC4*, and *AtYUC6*. The deduced amino acid lengths of the 12 *VrYUCCA* proteins ranged between 385 and 440 aa, with the pI between 7.96 and 9.54 and the molecular weight between 43.14 and 49.89 kD.

To investigate the diversity of the gene structures of the 12 *VrYUCCAs*, the exon and intron configurations were determined through visualized analysis using the GSDS tool. *VrYUCCA3*, *VrYUCCA8a*, *VrYUCCA8b*, and *VrYUCCA8c*, which were clustered in the same group, each contained three exons and two introns. The other *VrYUCCAs* all had four exons and three introns (Figure 1A). The conserved protein motifs were analyzed by MEME, and a total of 12 motifs are exhibited here (Figure 1A). All six of the recognized conserved motifs, including the FAD-binding motif, GC motif, ATG-containing motif1, FMO-identifying sequence, NADPH-binding motif, and ATG-containing motif2, are shown in a both schematic diagram and sequence alignment (Figure 1B and S1).

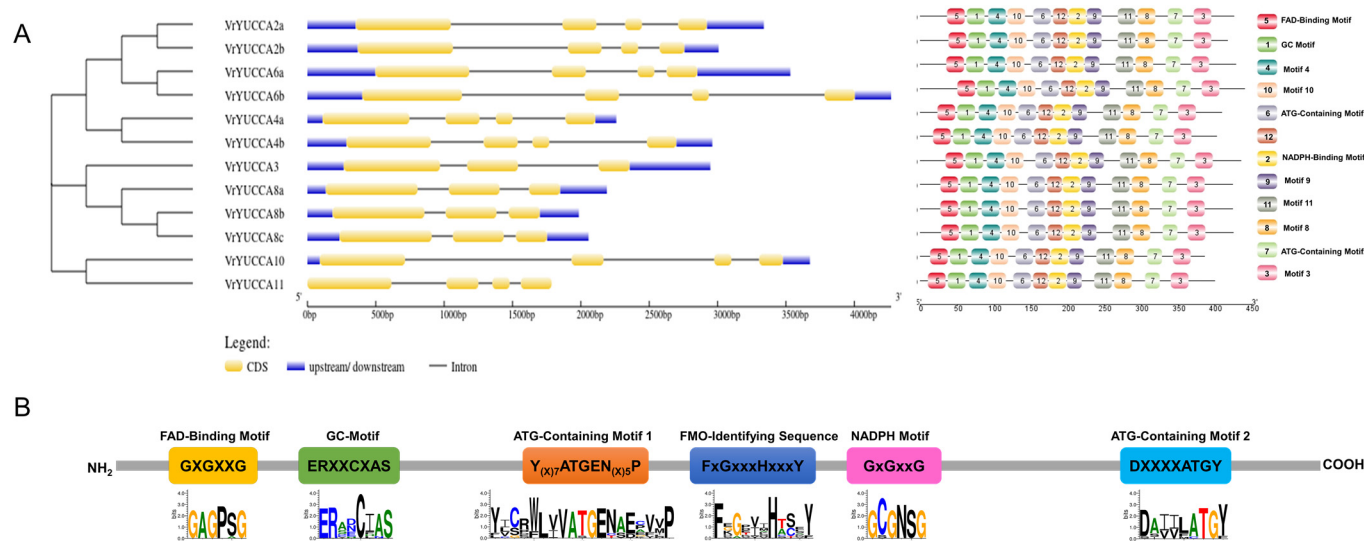


Figure 1. The gene structure and conserved motifs of the *YUCCA* gene family in mungbean. (A) Phylogenetic tree (left), gene structure (middle), and conserved motifs (right) of 12 *VrYUCCAs*. (B) The schematic diagram of six conserved protein motifs.

2.2. Phylogenetic Analysis of *YUCCAs* from Multiple Species

To further describe the evolutionary relationship, we performed an unrooted phylogenetic tree of *YUCCA* proteins from mungbean, *Arabidopsis*, soybean, rice, and Medicago (Figure 2). A total of 75 *YUCCA* proteins from five plant species were classified into four subfamilies (I to IV), which was consistent with previous research on *Arabidopsis* [16] and soybean [19]. *VrYUCCA2a*, *VrYUCCA2b*, *VrYUCCA6a*, and *VrYUCCA6b* were grouped together in subfamily I; *VrYUCCA4a* and *VrYUCCA4b* were placed in subfamily II; *VrYUCCA3*, *VrYUCCA8a*, *VrYUCCA8b*, and *VrYUCCA8c* were located in subfamily III; and *VrYUCCA10* and *VrYUCCA11* were placed in subfamily IV. At the same time, four rice *YUCCA* proteins (*OsYUCCA1*, *OsYUCCA4*, *OsYUCCA5*, and *OsYUCCA8*) did not cluster

well with the YUCCA proteins in subfamily II. Therefore, these four proteins could also be classified into another new subfamily, subfamily V. Among these five analyzed plant species, the evolutionary relationship of VrYUCCA proteins was closest to GmYUCCAs. The sequences information of all the YUCCAs is listed in Supplementary Data S1.

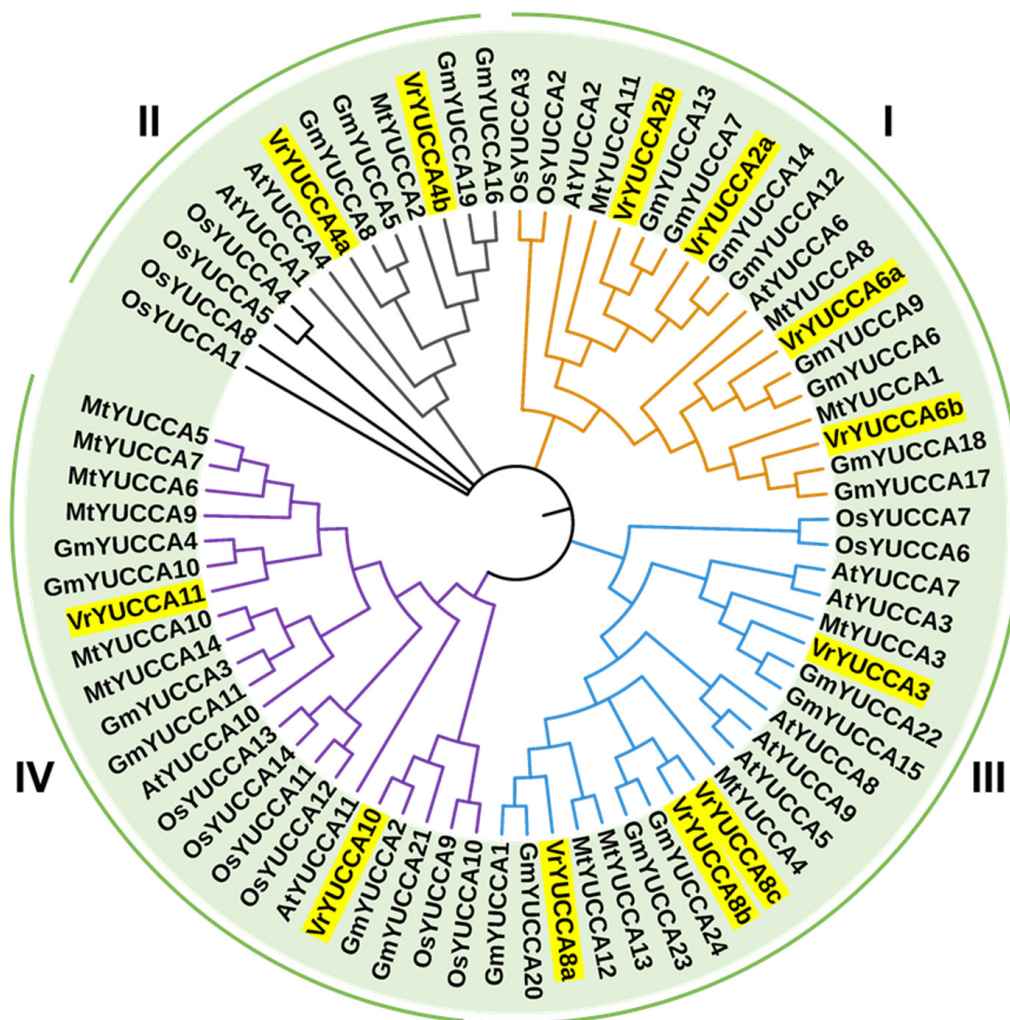


Figure 2. Phylogenetic tree of 75 YUCCA proteins from mungbean, *Arabidopsis*, soybean, rice, and Medicago. Vr, *Vigna radiata*; At, *Arabidopsis thaliana*; Gm, *Glycine max*; Os, *Oryza sativa*; Mt, *Medicago truncatula*. Each color represents a branch, and 12 VrYUCCAs are highlighted.

2.3. Expression Pattern of VrYUCCAs in Mungbean

To explore the potential biological functions of *VrYUCCA* genes during mungbean development, we analyzed their expression patterns in different tissues, including seedling root (SR), seedling true leaf (STL), compound leaf (CL), stem (St), young inflorescence (YI), flower bud (FB), opening flower (OF), 7-day pod (P7), and seed (Se), using real-time PCR (Figure 3). Several *VrYUCCAs* were constitutively expressed in all tissues examined, including *VrYUCCA2a*, *VrYUCCA6b*, *VrYUCCA8a*, and *VrYUCCA10*. Most *YUCCAs* had an extremely low expression in the stems except for *VrYUCCA2a*, *VrYUCCA4a*, and *VrYUCCA8a*; among them, *VrYUCCA4a* was specifically expressed. *VrYUCCA3* was preferentially expressed in seed. There were five *VrYUCCAs* specifically enriched in flower organs, including *VrYUCCA4b* (YI), *VrYUCCA6a* (OF), *VrYUCCA8b* (OF), *VrYUCCA8c* (OF), and *VrYUCCA11* (FB and OF). These results suggest that functional redundancy and divergence exist in the *VrYUCCA* gene family, and during different stages of growth or organs, distinct *VrYUCCAs* might play a major role.

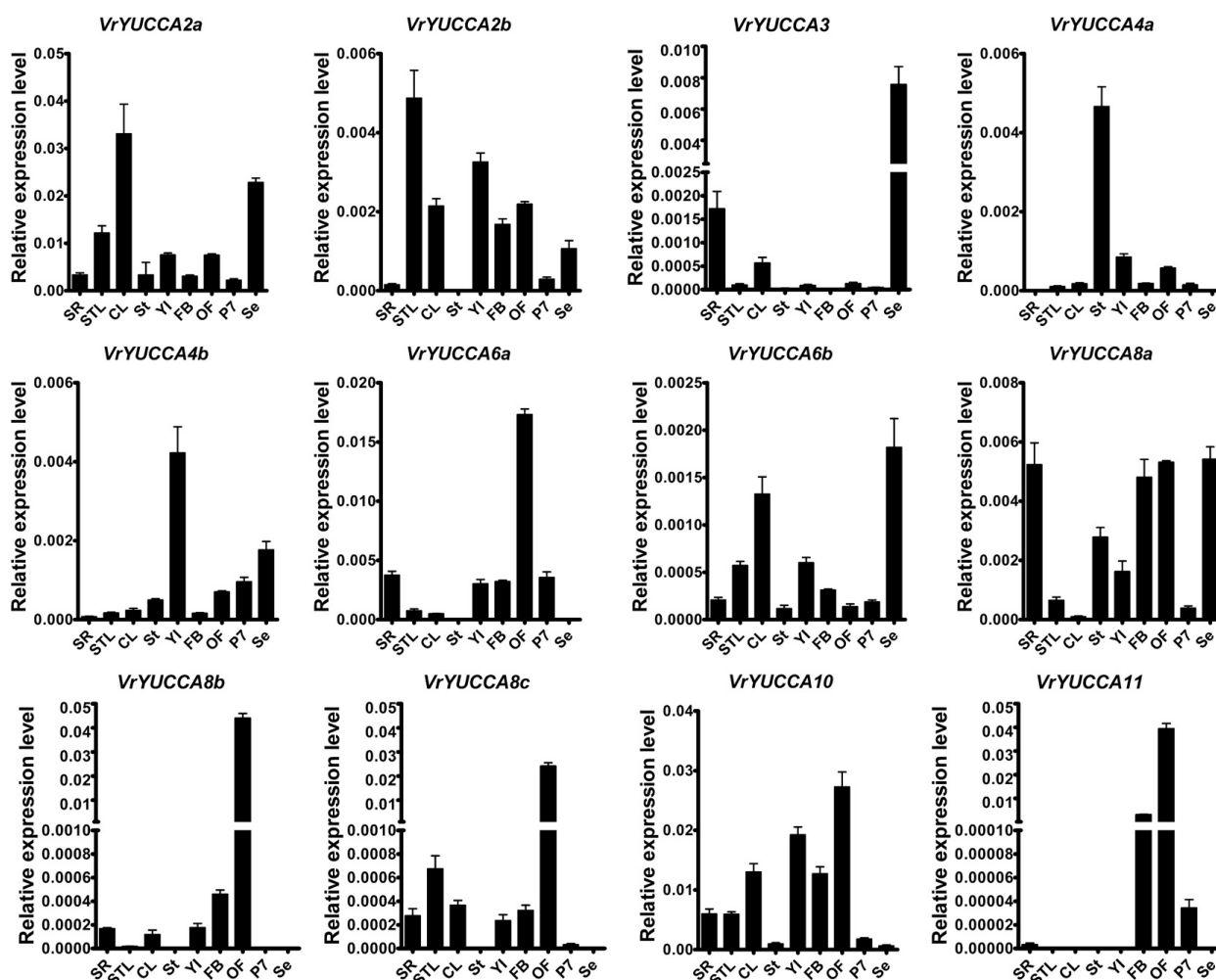


Figure 3. Expression pattern of VrYUCCAs in different tissues. SR, seeding root; STL, seedling true leaf; CL, compound leaf; St, stem; YI, young inflorescence; FB, flower bud; OF, opening flower; P7, 7-day pod; Se, seed. The VrACTIN3 gene (Vradi03g00210) was used as the internal control to normalize the real-time PCR data. The $2^{-\Delta CT}$ method was used to analyze relative expression level. Error bars indicated SDs (standard deviations) from three biological repetitions.

2.4. VrYUCCA Proteins Showed Biological Activity Both In Vitro and In Vivo

It was reported that YUCCA proteins might function in two possible routes to produce IAA, with IPA or TAM as the substrates, respectively [13,25]. To investigate the catalytic substrate of VrYUCCA proteins, enzyme activity detection was conducted in vitro. The results indicated that all 12 VrYUCCAs could catalyze IPA to produce IAA (Figure 4 and S2), but they showed no activity toward TAM (Figure S3). Thus, in mungbean, VrYUCCAs participated in the IPA pathway to synthesize IAA. Further, to examine their biological activity in vivo, VrYUCCAs were overexpressed in *Arabidopsis* (Col-0, wild type; WT) and driven by the 35S strong promoter. The T₁ generation of most VrYUCCAs transgenic plants showed typical auxin-related phenotypes (Figure 5), such as narrow rosette leaves, downward curling of the leaf-blade margin, twisted stem, and shorter or no siliques. What was noteworthy was that 35S::VrYUCCA10-transgenic plants showed a distinct phenotype, as their leaves were broader and slightly curled, with denser siliques on the top of the stem. These results indicated that all 12 of the isolated VrYUCCA genes had a veritable role in IAA synthesis.

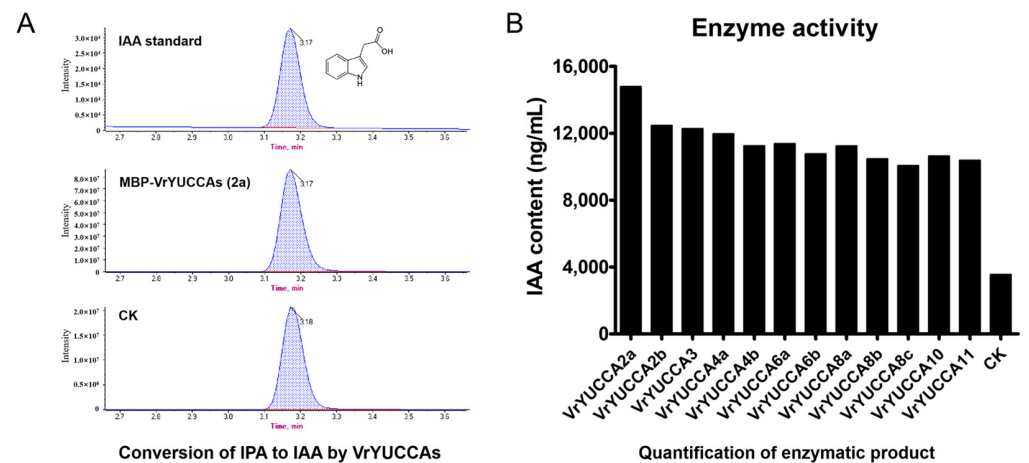


Figure 4. Enzymatic assay of VrYUCCAs with the substrate IPA analyzed by ESI-HPLC-MS/MS. (A) The HPLC profile for IAA standard and the enzymatic products. The profile of VrYUCCA2a reaction was selected as a representative here, and the results of other VrYUCCAs are shown in Figure S2. CK: the enzyme reaction mixture containing a boiled protein. The relatively lower HPLC profile in CK was due to spontaneous conversion of IPA to IAA at room temperature. (B) The statistical results of the IAA content in enzymatic reaction mixture of VrYUCCAs.

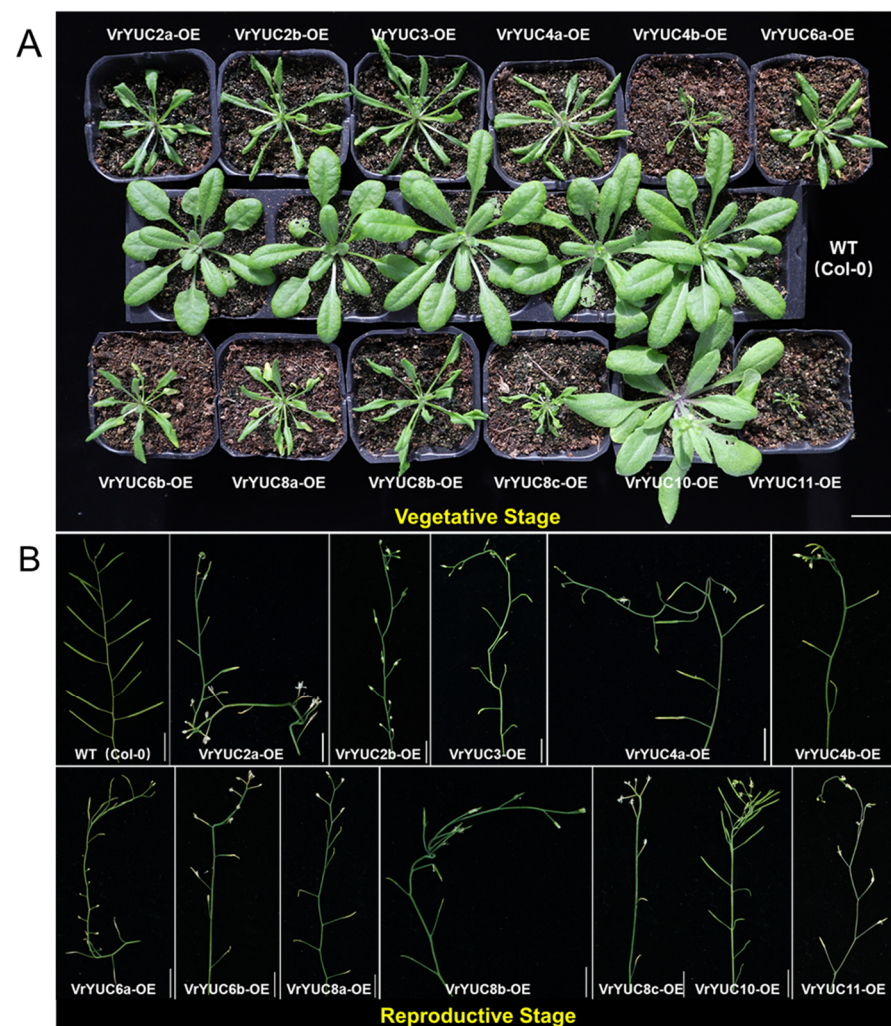


Figure 5. The phenotypic analysis of 35S::VrYUCCAs transgenic *Arabidopsis* during vegetative period (A) and reproductive growth stage (B). OE: overexpression line. Bar = 1 cm.

2.5. VrYUCCAs Expression in Response to Abiotic Stress Treatments

To investigate the response of VrYUCCAs toward surroundings stresses, 12-day-old mungbean seedlings were exposed to IAA (1 μ M), high salinity (200 mM NaCl), drought (15% PEG6000), heat (35 $^{\circ}$ C), and chilling (4 $^{\circ}$ C) for some time. The expression of VrYUCCA11 could not be detected well in the root and leaf, even under these stresses, due to its specific expression in flower organ. As shown in Figure 6, VrYUCCA2b, VrYUCCA6b, VrYUCCA8a, and VrYUCCA10 were inhibited markedly in the root by IAA, while VrYUCCA8b was induced. In the leaf, only the expression of VrYUCCA8a showed a highly significant increase.

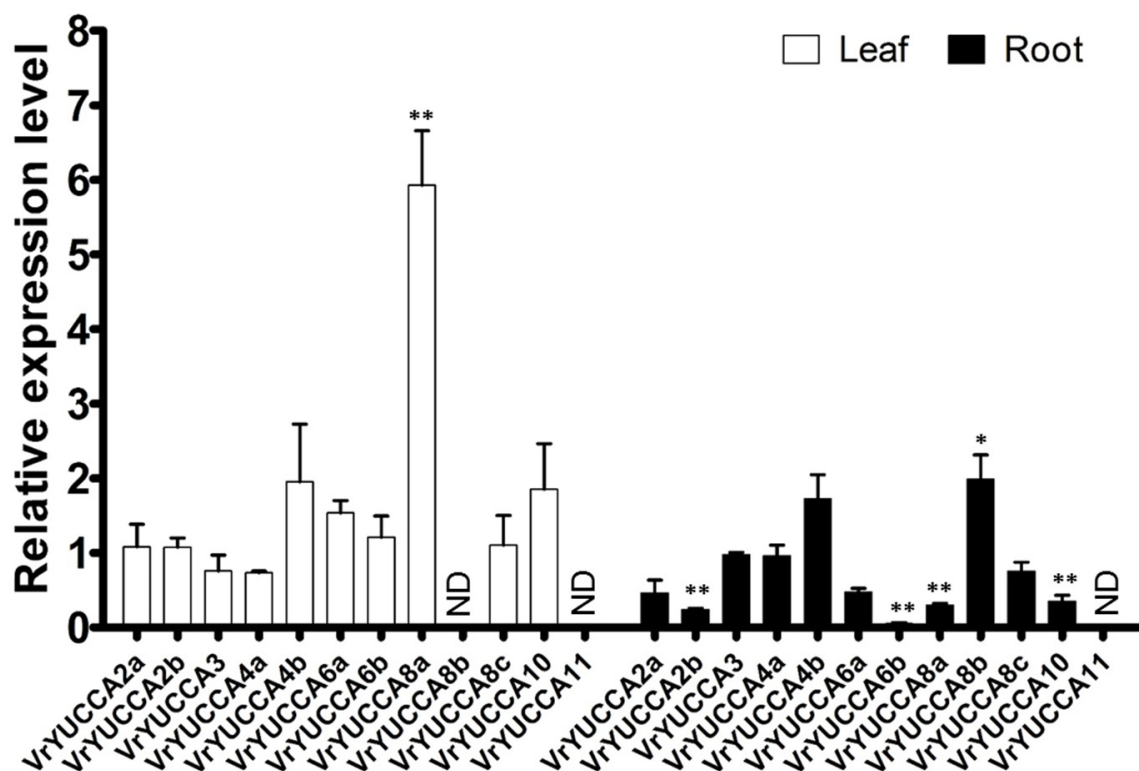


Figure 6. Effect of IAA on the expression of VrYUCCAs. The asterisks indicate statistical significance (*, $p < 0.05$; **, $p < 0.01$) compared with each CK (standardized as 1). Error bars indicate SDs from 3 biological repetitions. ND: not detectable.

As for the other abiotic stresses, all the analyzed VrYUCCA genes showed a visible increase and decrease under high-salinity treatment (Figure 7 and S4). Both at 6 h and 24 h after NaCl treatment, VrYUCCA4b and VrYUCCA8a had high expression in the leaf; meanwhile, the expression of VrYUCCA2b and VrYUCCA6a was inhibited significantly, indicating their main roles under salt stress. Several genes seemed to only function under long-term stress, such as VrYUCCA4a and VrYUCCA8b in the leaf. Additionally, VrYUCCA2a, VrYUCCA6b, VrYUCCA8c, and VrYUCCA10 mainly responded to salinity in the root. Under drought stress (Figure 7 and S5), VrYUCCA6a was inhibited, while VrYUCCA8a and VrYUCCA8b were induced in the leaf. In the root, however, VrYUCCA2a, VrYUCCA8a, VrYUCCA8c, and VrYUCCA10 might play a crucial part. When the mungbean seedlings were exposed to high temperatures, the expression of VrYUCCA2a, VrYUCCA6a, and VrYUCCA6b markedly increased, while that of VrYUCCA8b and VrYUCCA8c was inhibited. Cold prominently induced the expression of VrYUCCA8a and VrYUCCA8b. Meanwhile the expression of VrYUCCA2a and VrYUCCA10 declined (Figure 8 and S6). Overall, VrYUCCA2a, VrYUCCA6a, VrYUCCA8a, VrYUCCA8b, and VrYUCCA10 proved to be sensitive to all four treatments and might play a major role under abiotic stresses.

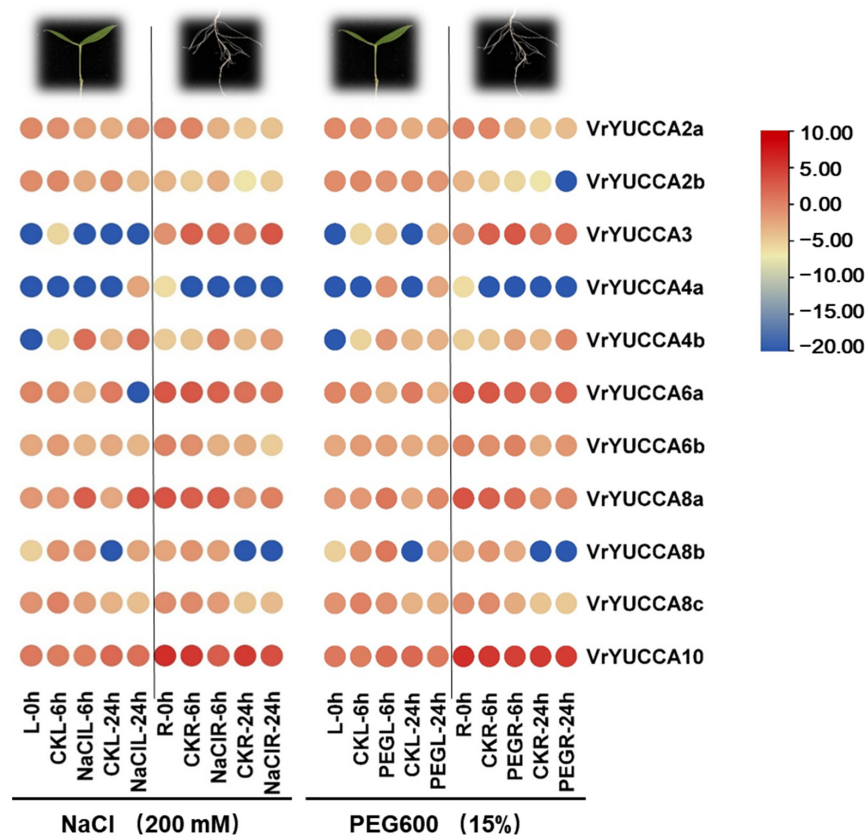


Figure 7. *VrYUCCA* genes expression during salt and drought stress. Values (RNA-seq data), in fragments per kilobase of transcript per million reads mapped (FPKM), used logarithm (LOG 2) for the heat map. L: leaf; R: root.

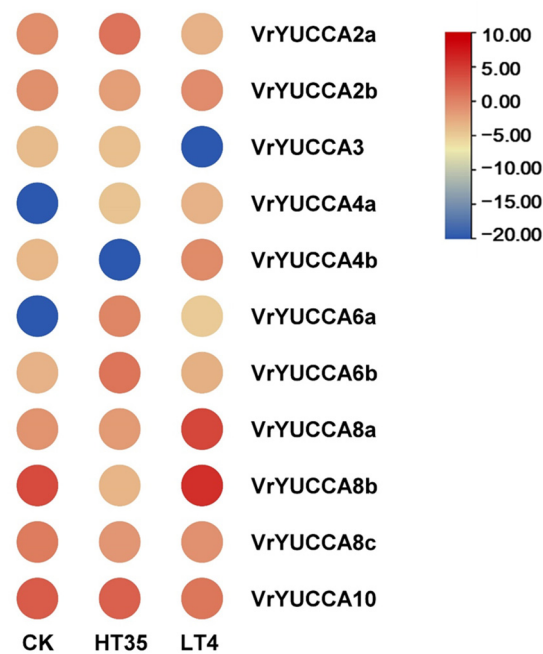


Figure 8. *VrYUCCA* genes expression during high and low temperatures. Values (RNA-seq data), in fragments per kilobase of transcript per million reads mapped (FPKM), used logarithm (LOG 2) for the heat map. CK: seedlings grown under 25 °C; HT35: seedlings grown under high temperatures, 35 °C; LT4, seedlings grown under low temperatures, 4 °C.

3. Discussion

YUCCA, a key rate-limiting enzyme for IAA synthesis, has been widely characterized in quite a few higher plants [11], existing in forms of families in different organisms, with numbers ranging from a few (e.g., 6 in tomato [38]) to dozens (e.g., 57 in *Brassica napus* [24] and 63 in wheat [14]), except for petunia, which only has one YUCCA gene in its genome [39]. Reports about YUCCA genes in mungbean are limited. Previously, a YUC4-like protein (*Vradi06g12650*) was reported to be responsible for the mungbean chasmogamous mutant (CM) [37], indicating the vital role of *VrYUCCAs* during plant growth and development. In this study, we identified 12 *VrYUCCAs* in the mungbean genome, named based on their similarity to *AtYUCCAs*. Homologous genes of *AtYUC1*, *AtYUC5*, *AtYUC7*, and *AtYUC9* were absent, but two each for *AtYUC2*, *AtYUC4*, and *AtYUC6* and three for *AtYUC8* were isolated in mungbean (Figure 1). This phenomenon also exists in apple and cucumber [2,22], which seems normal for gene family studies, and chromosomal fragment deletion and genetic duplication might be the causative factors of this phenomenon. Further, multiple copies of the *VrYUCCAs* displayed distinct expression patterns (Figure 3), indicating their functional differentiation during evolution.

The 12 *VrYUCCA* proteins could be divided into four groups (Figure 2), possessing the conserved motifs of the YUCCA gene family. Subfamily I (YUC2–YUC6 group) and subfamily II (YUC1–YUC4 group) were reported to function in the inflorescence apex and flower in *Arabidopsis*, and *AtYUC4* was specifically expressed in the floral meristems [16]. We previously obtained one mungbean mutant related to *VrYUCCA4b* that had developmental defects in its flower organs [37]. *VrYUCCA4b* (*Vradi06g12650*) seems to have a similar role in floral development, which is also highly expressed in young inflorescence (Figure 3). In *Arabidopsis*, the *Atyuc4* single mutant displayed the normal phenotype, but *Atyuc1yuc4* showed severe defects in its floral organs and fertility [16]; in mungbean, the homologous genes of *AtYUC1* were vacant, and other *VrYUCCAs* including *VrYUCCA4a* seemed not to complement the function of *VrYUCCA4b*, resulting in the petal-loss trait of CM (the *Vryucca4b* mutant) [37]. Thus, YUCCA paralogous genes have functional redundancy to ensure normal plant development. Additionally, in *Isatis indigotica* Fort, *liYUCCA1*, and *liYUCCA4* were also mainly detected in reproductive organs [40]. In *Brassica napus*, *BnYUC4* was also highly expressed in early flower buds [24]. Thus, the expression pattern and function of YUCCAs seem to be conserved. It was reported that subfamily III (YUC3-7-8-5-9 group) showed distinct expression patterns during root development in *Arabidopsis* [41,42]. Interestingly, in the root defects of these five YUCs (YUC3-7-8-5-9), a multiple mutant (*yucQ*) could be rescued by expressing a YUCCA gene in subfamily III rather than in other subfamily members [41]. At the same time, in mungbean, a high transcript of *VrYUCCA3* was detected in its seeds (Figure 3), implying a likely vital role during seed formation. *VrYUCCA8b* and *VrYUCCA8c* seemed like gene clusters located adjacently on chromosome 8, and both were expressed extremely highly during flower opening. However, *VrYUCCA8a* had a relatively high expression in the root. *VrYUCCA10* and *VrYUCCA11* were found in subfamily IV (YUC10-11 group), which was assumed to be the most conserved group of YUCCAs in the plant kingdom [42]. *AtYUC10* and *AtYUC11* mainly expressed in siliques and apices and were suggested to have overlapping functions with *AtYUC1* and *AtYUC4* during embryogenesis [26]. In mungbean, the relative transcript level of *VrYUCCA10* was the highest in all tissues (Figure 3), hinting at its essential role during the whole life cycle. At the same time, the transcript of *VrYUCCA11* was examined only in the floral organs and pods (Figure 3). In plants, YUCCA11 seemed to be specifically involved in the floral organs, embryogenesis, and fruit development. In cucumber, *CsYUC11* was specifically enriched in the opened male flower (mainly detected in the tapetum cell layer and the microspores in the anthers), and transgenic *Arabidopsis* lines of 35S::CsYCU11 displayed defective pollens and reduced fertility [2]. In peach, the transcript of *PpYUC11* increased during the late ripening stage in melting flesh peaches, implying its vital function during fruit ripening [43]. The number of YUCCA11 homologous genes increased in several *Rosaceae* species, such as apple (six YUCCA11) and white pear (seven YUCCA11). *MdYUCCA11a*, *MdYUCCA11b*,

and *MdYUCCA11d* showed high expression in young fruit, the receptacle, and the pistil, respectively [22]. Thus, like *YUCCA* in other species, distinct *VrYUCCA* participated in specific biological processes with redundancy and differential functions.

In this study, we verified 12 *VrYUCCA* proteins' enzyme activity both in vivo and in vitro. The substrate of *YUCCA* was controversial. Initially, it was demonstrated that the *Arabidopsis* *YUCCA1* protein could catalyze the *N*-hydroxylation of TAM to form *N*-hydroxy-TAM in vitro [25]. Meanwhile, later in vivo research showed that *YUCCA1* also catalyzed the oxidative decarboxylation of IPA to form IAA [44,45]. Subsequently, a growing number of studies have suggested that the real substrate of *YUCCA* was IPA rather than TAM [13,46,47]. Here, *VrYUCCA* exhibited explicit activity toward IPA, but no novel compound was produced toward TAM in vitro (Figure 4 and S3). The 35S::*VrYUCCAs* transgenic *Arabidopsis* lines all showed typical IAA over-accumulation defects (Figure 5), indicating the authentic function of the *VrYUCCAs* for IAA synthesis in vivo. No seeds were harvested from the 35S::*VrYUCCA11* line, which displayed the most severe abnormality during T₁ generation. Notable was, the 35S::*VrYUCCA10* line displayed less of an effect in the vegetative growth stage (even stronger than WT), but the siliques showed apex density. Whether this line has special potential advantages during developmental processes or under stress need to be studied further.

Auxin, a central regulator involved in almost all aspects of the plant life cycle, helps plants to adaptive external stresses [1,48]. *YUCCA* protein catalyzes IPA to produce IAA, and IAA can also feedback modulate *YUCCA* expression. Here, five *VrYUCCA* genes responded to IAA stimulation, which mainly functions in the root. Notably, only *VrYUCCA8a* was induced in the leaf, but it was significantly inhibited in the root (Figure 6), indicating that it might control gene expression by regulating hormone signaling through complex crosstalk, as reported in soybean [19]. In apple and soybean, the homologous gene of *VrYUCCA8a* also displayed sensitivity to hormone treatments [19,22], indicating its functional conservation in the plant kingdom. Additionally, *YUCCA* was also proven to be involved in IAA-mediated stress response [2,9,31]. In this study, we investigated the potential roles of *VrYUCCAs* in stress response. More *VrYUCCAs* were sensitive under salt stress than drought treatment (Figure 7, S5 and S6). *VrYUCCAs* showed different expression patterns to respond to the same stress; for example, leaf *VrYUCCA2b* and *VrYUCCA6a* were inhibited markedly under high salinity, while the expression of *VrYUCCA4b* and *VrYUCCA8a* increased significantly. These results hinted that the regulation mechanism of the stress response by *YUCCA*-mediated IAA synthesis is complex and can even antagonize to create a buffering system for endogenous auxin synthesis, as reported in cucumber [2]. Regarding heat and chilling, *VrYUCCA2a*, *VrYUCCA6a*, *VrYUCCA6b*, *VrYUCCA8a*, and *VrYUCCA8b* might act as the main regulators. In *Arabidopsis* and apple, *PIF4* could specifically bind to the G-box motif (CACGTG) in the promoter of *YUCCA8* to activate its expression, which was deemed a conserved molecular mechanism [22,30]. In cucumber, *CsYUC8* and *CsYUC9* were selectively upregulated after being exposed to 38 °C for 3 h [2]. Remarkably, *VrYUCCA8a* and *VrYUCCA8b* were markedly induced by low temperatures rather than heat, similar to that in *Populus* [9], suggesting that high-temperature regulatory mechanisms might be diverse in mungbean. It is possible that several *VrYUCCAs* have a dual role, like *AtYUC6* [29], and transmit stress signals through another functional domain mediated by ROS or hormone crosstalk, such as ABA and SA, which needs further exploration.

Overall, the *YUCCA* gene family is relatively conservative among plants, and the homologous genes in different species tend to act similarly in their roles during plant growth and stress responses. The crucial role of *YUCCAs* in the auxin pathway is beyond doubt, so further in-depth research should be conducted to investigate its functional mechanism and application value.

4. Materials and Methods

4.1. Identification, Structure, and Motif Analysis of VrYUCCA Genes

Multiple methods were used to identify mungbean YUCCAs. Firstly, Gcorn plant (<http://www.plant.osakafu-u.ac.jp/~kagiana/gcorn/p/index18.html>) (accessed on 1 September 2020) [49], a database of plant gene phylogeny, was used to search YUCCA information in mungbean genome. Second, the amino acid sequences of the 11 *Arabidopsis* YUCCA (AtYUCs) were downloaded from TAIR (<https://www.arabidopsis.org/index.jsp>) (accessed on 1 September 2020) and then used as BlastP queries against mungbean genome database (http://plantgenomics.snu.ac.kr/mediawiki-1.21.3/index.php/Main_Page) (accessed on 1 September 2020) to reconfirm the VrYUCCAs. Then, the online tool SMART (<http://smart.embl-heidelberg.de/>) (accessed on 1 October 2020) was used to confirm whether the predicted VrYUCCA proteins had the FMO-like domain. Finally, the sequences information of VrYUCCAs was downloaded from NCBI (<https://www.ncbi.nlm.nih.gov>) (accessed on 1 October 2020). The online tool GSDS (<http://gsds.gao-lab.org/>) (accessed on 1 October 2020) was used to determine the gene structure. The online MEME suite (<https://meme-suite.org/>) (accessed on 1 October 2020) was used to identify the conserved motifs shared among VrYUCCA proteins, which were then visualized with TBtools, and the repeats logos were obtained through WEBLogo (<http://weblogo.threeplusone.com/create.cgi>) (accessed on 1 October 2020). The theoretical pI (isoelectric point) and Mw (molecular weight) of VrYUCCAs were predicted using ExPASy (<https://www.expasy.org/>) (accessed on 1 October 2020). BaCelLo (<http://gpcr.bio-comp.unibo.it/bacello/index.htm>) (accessed on 1 October 2020) was used to predict the subcellular localization. Multiple sequence alignment of the identified VrYUCCA sequences was performed using MEGA-X and ClustalX2 and then visualized using ESPript 3.0 (<https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi>) (accessed on 1 October 2020).

4.2. Phylogenetic Analysis of YUCCA Genes from Multiple Species

The amino acid sequences of YUCCA from *Arabidopsis* (11), *Oryza sativa* (14), *Glycine max* (24), and *Medicago truncatula* (14) were downloaded from TAIR, RAP-DB (The Rice Annotation Project Database), NCBI, and phytozome (<https://phytozome-next.jgi.doe.gov/>) (accessed on 1 November 2020), respectively. The phylogenetic tree was constructed using MEGAX software with the neighbor-joining (NJ) method with 1000 bootstrap replicates. Additionally, it was optimized on the website iTOL (<https://itol.embl.de/>) (accessed on 1 November 2020).

4.3. Plant Materials and Treatment

The seedlings of mungbean cultivar ‘Sulyu 1’ were grown in the illumination incubator at 25 °C with 16 h light/8 h dark photoperiod. Adult mungbean was planted in experimental plots at Jiangsu Academy of Agricultural Sciences, Nanjing, China (32°21′ N, 118°53′ E).

Seedling root, seedling true leaf, stem, compound leaf, young inflorescence, flower bud, opening flower, pod (7 d), and dry seed were sampled from mungbean seedlings (12 d) or adult plants. All the tissues were immediately frozen in liquid nitrogen, and each sample was collected independently three times for further VrYUCCA expression pattern analysis using real-time PCR.

For IAA treatment, 12-day-old mungbean seedlings were soaked in liquid MS (Murashige and Skoog) medium with 1 µM IAA and liquid MS medium only (as the control) for 4 h. The treated roots and leaves were sampled separately after flushing with ddH₂O and immediate freezing in liquid nitrogen. Treatment was repeated three times. The expression of VrYUCCA genes was then examined by real-time PCR.

For high-salinity and drought treatments, 12-day-old mungbean seedlings were soaked in liquid MS medium with 200 mM NaCl and 15% (*w/v*) polyethylene glycol (PEG) 6000 for 6 h and 24 h, respectively. The seedlings soaked in liquid MS medium served as the control, and the treated roots and leaves were sampled separately after flushing with ddH₂O and immediate freezing in liquid nitrogen. Treatment was repeated three times. Then, the expression of VrYUCCA genes was analyzed using RNA-seq (BGI, Shenzhen, China).

For high- and low-temperature treatments, 12-day-old mungbean seedlings were placed in an incubator at 35 °C and 4 °C, respectively, for 24 h. The seedlings grown under 25 °C were taken as a control. Additionally, the treated leaves were sampled and immediately frozen in liquid nitrogen. The expression of *VrYUCCA* genes was detected using RNA-seq (BGI, Shenzhen, China).

4.4. Expression Analysis Using Real-Time PCR

Total RNA was extracted using Polysaccharide polyphenol Plant total RNA Extraction Kit (PD Biotech, Shanghai, China), and cDNAs were synthesized using Goldenstar® RT6 cDNA Synthesis Kit Ver.2 (TSINGKE, Beijing, China). Real-time PCR was performed using ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China) on an ABI prism 7500 real-time PCR System. Mungbean *VrACTIN3* (*Vradi03g00210*) and *Arabidopsis ACTIN2* (*AT3G18780*) were selected as reference genes to normalize the expression data. The $2^{-\Delta CT}$ or $2^{-\Delta\Delta CT}$ methods were used to analyze relative expression levels. The results were calculated from three biological replicates and three technical replicates. Statistical analysis and plot drawing were conducted using Excel and GraphPad Prism 5 software. Heat maps were drawing by TBtools after the FPKM value taken logarithm (LOG 2). The primers used in this study are listed in Table S2.

4.5. Prokaryotic Expression, Purification, and Enzymatic Assays of *VrYUCCA*

The full-length CDS sequence of each *VrYUCCA* gene was ligated into a pMAL-c2x expression vector (NEB, MBP-tag). Then, the recombinant *VrYUCCA* proteins were expressed in *E. coli* (BL21, DE3) and purified by dextrin Sepharose affinity chromatography (Amylose resin, NEB), in accordance with the instructions described previously [50]. The protein concentration was determined using the Bradford method with BSA as the standard.

The enzymatic assays were conducted by referring to a previous study [47]. A total 40 µL reaction system containing 1–2 µg *VrYUCCA* protein, 50 mM Tris-HCl (pH 7.4), 10 mM NADPH/NADP, and 2 mM of the substrates IPA or TAM was incubated at 30 °C for 4 h. A mixture containing one boiled *VrYUCCA* protein was served as the control. The products were analyzed on silica-gel-coated TLC plates with chloroform/methanol/acetic acid (75:20:5) as the mobile phase. The TLC plates were stained using Ehrlich's reagent (Sigma, 02560-500ML, St. Louis, MO, USA). Meanwhile, the products with IPA as the substrate were also detected by ESI-HPLC-MS/MS conducted by Nanjing WEBiolotech Biotechnology Co., Ltd. (Nanjing, China).

4.6. *Arabidopsis* Transformation

The CDS sequence of each *VrYUCCA* gene was cloned into a pCambia1305.1-GFP vector through XbaI and BamHI sites, to create a fusion construct under the control of the CaMV 35S promoter. The fusion vectors 35S::*VrYUCCAs*-GFP were transformed into *Agrobacterium tumefaciens* strain GV3101 and then were introduced into the *Arabidopsis thaliana* (Col-0) wild type (WT) using the floral dipping method described previously [51]. The seeds of transgenic *Arabidopsis* were screened on 1/2 MS culture media containing 15 mg/L hygromycin antibiotics. Phenotypic analysis was performed in the progeny-positive T₁ lines.

5. Conclusions

In this study, 12 *VrYUCCAs* were systematically identified in mungbean, containing the six conserved motifs of the *YUCCA* gene family. The 12 *VrYUCCA* genes could be divided into four subfamilies according to the phylogenetic analysis. The *VrYUCCAs* showed different tissue expression patterns, and five had a highly specific transcript in the floral organs, including *VrYUCCA4b*, *VrYUCCA6a*, *VrYUCCA8b*, *VrYUCCA8c*, and *VrYUCCA11*. *VrYUCCA2a*, *VrYUCCA6b*, *VrYUCCA8a*, and *VrYUCCA10* seemed to regulate the IAA content throughout the whole growing period, due to the constitutive expression pattern in all the tissues. The *VrYUCCA* proteins participated in the IPA pathway for IAA synthesis in vitro and exhibited veritable biological activity to the IAA product in vivo,

which was certified by 35S::VrYUCCAs transgenic *Arabidopsis*. Five YUCCAs in the root responded to IAA, but only VrYUCCA8a responded in the leaf. Regarding the other four stresses, VrYUCCA2a, VrYUCCA6a, VrYUCCA8a, VrYUCCA8b, and VrYUCCA10 might be mainly responsible for auxin regulation, due to their sensitivity to all four treatments used in this study.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms24021603/s1>.

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References

1. Blakeslee, J.J.; Rossi, T.S.; Kriechbaumer, V. Auxin biosynthesis: Spatial regulation and adaptation to stress. *J. Exp. Bot.* **2019**, *70*, 5041–5049. [CrossRef] [PubMed]
2. Yan, S.S.; Che, G.; Ding, L.; Chen, Z.J.; Liu, X.F.; Wang, H.Y.; Zhao, W.S.; Ning, K.; Zhao, J.Y.; Tesfamichael, K.; et al. Different cucumber CsYUC genes regulate response to abiotic stresses and flower development. *Sci. Rep.* **2016**, *6*, 20760. [CrossRef] [PubMed]
3. Wojcikowska, B.; Jaskola, K.; Gasiorek, P.; Meus, M.; Nowak, K.; Gaj, M.D. LEAFY COTYLEDON2 (LEC2) promotes embryogenic induction in somatic tissues of *Arabidopsis*, via YUCCA-mediated auxin biosynthesis. *Planta* **2013**, *238*, 425–440. [CrossRef] [PubMed]
4. Vanneste, S.; Friml, J. Auxin: A trigger for change in plant development. *Cell* **2009**, *136*, 1005–1016. [CrossRef]
5. Wang, W.; Xu, B.; Wang, H.; Li, J.Q.; Huang, H.; Xu, L. YUCCA genes are expressed in response to leaf adaxial-abaxial juxtaposition and are required for leaf margin development. *Plant Physiol.* **2011**, *157*, 1805–1819. [CrossRef] [PubMed]
6. Cardarelli, M.; Cecchetti, V. Auxin polar transport in stamen formation and development: How many actors? *Front. Plant Sci.* **2014**, *5*, 333. [CrossRef] [PubMed]
7. Yao, X.; Tian, L.; Yang, J.; Zhao, Y.N.; Zhu, Y.X.; Dai, X.; Zhao, Y.; Yang, Z.N. Auxin production in diploid microsporocytes is necessary and sufficient for early stages of pollen development. *PLoS Genet.* **2018**, *14*, e1007397. [CrossRef] [PubMed]
8. Pandolfini, T.; Molesini, B.; Spena, A. Molecular dissection of the role of auxin in fruit initiation. *Trends Plant Sci.* **2007**, *12*, 327–329. [CrossRef]
9. Ye, X.; Kang, B.G.; Osburn, L.D.; Li, Y.; Zong-Ming, C. Identification of the flavin-dependent monooxygenase-encoding YUCCA gene family in *Populus trichocarpa* and their expression in vegetative tissues and in response to hormone and environmental stresses. *Plant Cell Tiss. Org.* **2009**, *97*, 271–283. [CrossRef]
10. Lee, M.; Jung, J.H.; Han, D.Y.; Seo, P.J.; Park, W.J.; Park, C.M. Activation of a flavin monooxygenase gene YUCCA7 enhances drought resistance in *Arabidopsis*. *Planta* **2012**, *235*, 923–938. [CrossRef]
11. Cao, X.; Yang, H.L.; Shang, C.Q.; Ma, S.; Liu, L.; Cheng, J.L. The roles of auxin biosynthesis YUCCA gene family in plants. *Int. J. Mol. Sci.* **2019**, *20*, 6343. [CrossRef] [PubMed]
12. Thodberg, S.; Neilson, E.J.H. The “Green” FMOs: Diversity, functionality and application of plant flavoproteins. *Catalysts* **2020**, *10*, 329. [CrossRef]
13. Di, D.W.; Zhang, C.; Luo, P.; An, C.W.; Guo, G.Q. The biosynthesis of auxin: How many paths truly lead to IAA? *Plant Growth Regul.* **2016**, *78*, 275–285. [CrossRef]

14. Yang, Y.L.; Xu, T.; Wang, H.G.; Feng, D.S. Genome-wide identification and expression analysis of the *TaYUCCA* gene family in wheat. *Mol. Biol. Rep.* **2021**, *48*, 1269–1279. [[CrossRef](#)] [[PubMed](#)]
15. Krueger, S.K.; Williams, D.E. Mammalian flavin-containing monooxygenases: Structure/function, genetic polymorphisms and role in drug metabolism. *Pharmacol. Ther.* **2005**, *106*, 357–387. [[CrossRef](#)] [[PubMed](#)]
16. Cheng, Y.; Dai, X.; Zhao, Y. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. *Genes Dev.* **2006**, *20*, 1790–1799. [[CrossRef](#)]
17. Yamamoto, Y.; Kamiya, N.; Morinaka, Y.; Matsuoka, M.; Sazuka, T. Auxin biosynthesis by the YUCCA genes in rice. *Plant Physiol.* **2007**, *143*, 1362–1371. [[CrossRef](#)] [[PubMed](#)]
18. Li, W.L.; Zhao, X.Y.; Zhang, X.S. Genome-wide analysis and expression patterns of the YUCCA genes in maize. *J. Genet. Genom.* **2015**, *42*, 707–710. [[CrossRef](#)]
19. Wang, Y.G.; Liu, H.H.; Wang, S.P.; Li, H.J. Genome-wide identification and expression analysis of the YUCCA gene family in soybean (*Glycine max* L.). *Plant Growth Regul.* **2017**, *81*, 265–275. [[CrossRef](#)]
20. Wang, Y.N.; Yang, W.; Zuo, Y.Y.; Zhu, L.; Hastwell, A.H.; Chen, L.; Tian, Y.P.; Su, C.; Ferguson, B.J.; Li, X. *GmYUC2a* mediates auxin biosynthesis during root development and nodulation in soybean. *J. Exp. Bot.* **2019**, *70*, 3165–3176. [[CrossRef](#)]
21. Chen, M.M.; Li, X.; Cai, Y.M.; Zhang, Y.C.; Gu, J.J.; Yang, L.Y. Identification and expression pattern analysis of YUCCA and ARF gene families during somatic embryogenesis of *Lilium* spp. *Biol. Plant.* **2020**, *64*, 385–394. [[CrossRef](#)]
22. Song, C.H.; Zhang, D.; Zheng, L.W.; Shen, Y.W.; Zuo, X.Y.; Mao, J.P.; Meng, Y.A.; Wu, H.Q.; Zhang, Y.K.; Liu, X.Y.; et al. Genome-wide identification and expression profiling of the YUCCA gene family in *Malus domestica*. *Sci. Rep.* **2020**, *10*, 10866. [[CrossRef](#)]
23. Wang, X.Y.; Chen, B.Z.; Ma, C.K.; Qiao, K.K.; Li, Z.S.; Wang, J.S.; Peng, R.H.; Fan, S.L.; Ma, Q.F. Systematical characterization of YUCCA gene family in five cotton species, and potential functions of YUCCA22 gene in drought resistance of cotton. *Ind. Crop. Prod.* **2021**, *162*, 113290. [[CrossRef](#)]
24. Zhang, K.; Zhang, J.; Cui, C.; Chai, L.; Zheng, B.; Jiang, J.; Li, H.; Tu, J. Genome-wide identification and expression profiling of the YUCCA gene family in *Brassica napus*. *Oil Crop Sci.* **2022**, *7*, 103–111. [[CrossRef](#)]
25. Zhao, Y.; Christensen, S.K.; Fankhauser, C.; Cashman, J.R.; Cohen, J.D.; Weigel, D.; Chory, J. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* **2001**, *291*, 306–309. [[CrossRef](#)]
26. Cheng, Y.F.; Dai, X.H.; Zhao, Y.D. Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in *Arabidopsis*. *Plant Cell* **2007**, *19*, 2430–2439. [[CrossRef](#)]
27. Challa, K.R.; Aggarwal, P.; Nath, U. Activation of YUCCA5 by the transcription factor TCP4 integrates developmental and environmental signals to promote hypocotyl elongation in *Arabidopsis*. *Plant Cell* **2016**, *28*, 2117–2130. [[CrossRef](#)] [[PubMed](#)]
28. Xu, D.Y.; Miao, J.H.; Yumoto, E.; Yokota, T.; Asahina, M.; Watahiki, M. YUCCA9-mediated auxin biosynthesis and polar auxin transport synergistically regulate regeneration of root systems following root cutting. *Plant Cell Physiol.* **2017**, *58*, 1710–1723. [[CrossRef](#)]
29. Cha, J.-Y.; Kim, W.-Y.; Kang, S.B.; Kim, J.I.; Baek, D.; Jung, I.J.; Kim, M.R.; Li, N.; Kim, H.-J.; Nakajima, M.; et al. A novel thiol-reductase activity of *Arabidopsis* YUC6 confers drought tolerance independently of auxin biosynthesis. *Nat. Commun.* **2015**, *6*, 8041. [[CrossRef](#)] [[PubMed](#)]
30. Sun, J.Q.; Qi, L.L.; Li, Y.N.; Chu, J.F.; Li, C.Y. PIF4-mediated activation of YUCCA8 expression integrates temperature into the auxin pathway in regulating *Arabidopsis* hypocotyl growth. *PLoS Genet.* **2012**, *8*, e1002594. [[CrossRef](#)] [[PubMed](#)]
31. Perez-Alonso, M.M.; Sanchez-Parra, B.; Ortiz-Garcia, P.; Santamaria, M.E.; Diaz, I.; Pollmann, S. Jasmonic acid-dependent MYC transcription factors bind to a tandem G-box motif in the YUCCA8 and YUCCA9 promoters to regulate biotic stress responses. *Int. J. Mol. Sci.* **2021**, *22*, 9768. [[CrossRef](#)]
32. Zhang, T.; Li, R.N.; Xing, J.L.; Yan, L.; Wang, R.C.; Zhao, Y.D. The YUCCA-Auxin-WOX11 module controls crown root development in rice. *Front. Plant Sci.* **2018**, *9*, 523. [[CrossRef](#)]
33. Fujino, K.; Matsuda, Y.; Ozawa, K.; Nishimura, T.; Koshiba, T.; Fraaije, M.W.; Sekiguchi, H. NARROW LEAF 7 controls leaf shape mediated by auxin in rice. *Mol. Genet. Genom.* **2008**, *279*, 499–507. [[CrossRef](#)]
34. Woo, Y.M.; Park, H.J.; Su'udi, M.; Yang, J.I.; Park, J.J.; Back, K.; Park, Y.M.; An, G. Constitutively wilted 1, a member of the rice YUCCA gene family, is required for maintaining water homeostasis and an appropriate root to shoot ratio. *Plant Mol. Biol.* **2007**, *65*, 125–136. [[CrossRef](#)]
35. Song, S.; Chen, Y.; Liu, L.; Benjamin, S.Y.H.; Mao, C.; Gan, Y.; Yu, H. OsFTIP7 determines auxin-mediated anther dehiscence in rice. *Nat. Plants* **2018**, *4*, 495–504. [[CrossRef](#)]
36. Lambrides, C.J.; Godwin, I.D. Mungbean. In *Pulses, Sugar and Tuber Crops. Genome Mapping & Molecular Breeding in Plants*; Kole, C., Ed.; Springer: Berlin/Heidelberg, Germany, 2007; Volume 3, pp. 69–90.
37. Chen, J.; Somta, P.; Chen, X.; Cui, X.; Yuan, X.; Srinives, P. Gene mapping of a mutant mungbean (*Vigna radiata* L.) using new molecular markers suggests a gene encoding a YUC4-like protein regulates the chasmogamous flower trait. *Front. Plant Sci.* **2016**, *7*, 830. [[CrossRef](#)] [[PubMed](#)]
38. Exposito-Rodriguez, M.; Borges, A.A.; Borges-Perez, A.; Perez, J.A. Gene structure and spatiotemporal expression profile of tomato genes encoding YUCCA-like flavin monooxygenases: The ToFZY gene family. *Plant Physiol. Biochem.* **2011**, *49*, 782–791. [[CrossRef](#)] [[PubMed](#)]

39. Tobena-Santamaria, R.; Bliet, M.; Ljung, K.; Sandberg, G.; Mol, J.N.M.; Souer, E.; Koes, R. FLOOZY of petunia is a flavin monooxygenase-like protein required for the specification of leaf and flower architecture. *Genes Dev.* **2002**, *16*, 753–763. [[CrossRef](#)] [[PubMed](#)]
40. Qin, M.M.; Wang, J.; Zhang, T.Y.; Hu, X.Y.; Liu, R.; Gao, T.E.; Zhao, S.J.; Yuan, Y.L.; Zheng, J.Y.; Wang, Z.R.; et al. Genome-wide identification and analysis on YUCCA gene family in *Isatis indigotica* Fort. and *liYUCCA6-1* functional exploration. *Int. J. Mol. Sci.* **2020**, *21*, 2188. [[CrossRef](#)]
41. Chen, Q.G.; Dai, X.H.; De-Paoli, H.; Cheng, Y.F.; Takebayashi, Y.; Kasahara, H.; Kamiya, Y.; Zhao, Y.D. Auxin overproduction in shoots cannot rescue auxin deficiencies in *Arabidopsis* roots. *Plant Cell Physiol.* **2014**, *55*, 1072–1079. [[CrossRef](#)]
42. Poulet, A.; Kriechbaumer, V. Bioinformatics analysis of phylogeny and transcription of TAA/YUC auxin biosynthetic genes. *Int. J. Mol. Sci.* **2017**, *18*, 1791. [[CrossRef](#)]
43. Pan, L.; Zeng, W.; Niu, L.; Lu, Z.; Liu, H.; Cui, G.; Zhu, Y.; Chu, J.; Li, W.; Fang, W.; et al. *PpYUC11*, a strong candidate gene for the stony hard phenotype in peach (*Prunus persica* L. Batsch), participates in IAA biosynthesis during fruit ripening. *J. Exp. Bot.* **2015**, *66*, 7031–7044. [[CrossRef](#)]
44. Stepanova, A.N.; Yun, J.; Robles, L.M.; Novak, O.; He, W.; Guo, H.; Ljung, K.; Alonso, J.M. The *Arabidopsis* YUCCA1 flavin monooxygenase functions in the indole-3-pyruvic acid branch of auxin biosynthesis. *Plant Cell* **2011**, *23*, 3961–3973. [[CrossRef](#)]
45. Won, C.; Shen, X.L.; Mashiguchi, K.; Zheng, Z.Y.; Dai, X.H.; Cheng, Y.F.; Kasahara, H.; Kamiya, Y.; Chory, J.; Zhao, Y.D. Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF *Arabidopsis* and YUCCAs in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 18518–18523. [[CrossRef](#)]
46. Mashiguchi, K.; Tanaka, K.; Sakai, T.; Sugawara, S.; Kawaide, H.; Natsume, M.; Hanada, A.; Yaeno, T.; Shirasu, K.; Yao, H.; et al. The main auxin biosynthesis pathway in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 18512–18517. [[CrossRef](#)]
47. Kriechbaumer, V.; Wang, P.W.; Hawes, C.; Abell, B.M. Alternative splicing of the auxin biosynthesis gene YUCCA4 determines its subcellular compartmentation. *Plant J.* **2012**, *70*, 292–302. [[CrossRef](#)] [[PubMed](#)]
48. Park, C.M. Auxin homeostasis in plant stress adaptation response. *Plant Signal. Behav.* **2007**, *2*, 306–307. [[CrossRef](#)]
49. Ogata, Y.; Kimura, N.; Sano, R. Gcorn Plant: A database for retrieving functional and evolutionary traits of plant genes. *Plant Physiol.* **2019**, *180*, 732–742. [[CrossRef](#)] [[PubMed](#)]
50. Zhang, Q.X.; Yan, Q.; Yuan, X.X.; Lin, Y.; Chen, J.B.; Wu, R.R.; Xue, C.C.; Zhu, Y.L.; Chen, X. Two polygalacturonase-inhibiting proteins (VrPGIP) of *Vigna radiata* confer resistance to bruchids (*Callosobruchus* spp.). *J. Plant Physiol.* **2021**, *258–259*, 153376. [[CrossRef](#)] [[PubMed](#)]
51. Zhang, X.R.; Henriques, R.; Lin, S.S.; Niu, Q.W.; Chua, N.H. Agrobacterium-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nat. Protoc.* **2006**, *1*, 641–646. [[CrossRef](#)]

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