PvB3s: Genome-wide and Transcriptome-wide Identification and Analysis Reveals the B3 Family Regulate Auxin to Resist Salt Stress at the Sprout Stage in Common Bean(*P. Vulgaris* L.) .

Qi Zhang
Heilongjiang Bayi Agricultural University
Wei-jia Li
Heilongjiang Bayi Agricultural University
Wen-jing Zhang
Heilongjiang Bayi Agricultural University
Zhen-gong Yin
Heilongjiang Academy of Agricultural Sciences
Yu-xin Wang
Heilongjiang Bayi Agricultural University
Ming-yu Zhang
Heilongjiang Bayi Agricultural University
Shuo Zhang
Heilongjiang Bayi Agricultural University
Yang-cheng Liu
Heilongjiang Bayi Agricultural University
Hao-hao Zhao
Heilongjiang Bayi Agricultural University
Wen-hui Zhang
Heilongjiang Bayi Agricultural University
Ji-Dao Du (djdbynd@163.com)
Heilongjiang Bayi Agricultural University

Research article

Keywords: Common bean, B3 family, auxin, salt stress, transcriptome

Posted Date: July 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-36056/v1

Abstract

Background:

B3 gene family is a transcription factor family unique to plants, which play an important role in plant growth and development by binding specific DNA sequences. However, data on the *B3* genes in the common bean and participate in many abiotic stresses especially salt stress are limited.

Result:

A total of encoding 100 proteins were identified in common bean. Phylogenetic analysis showed that *PvB3s* were classified into 4 subgroups, and these clusters were supported by several group-specific features, including exon/intron structure, MEME motifs, and predicted binding site structure. Collinearity analysis showed the connection of *PvB3s* in the same species and different species. The genes expression pattern showed that *PvB3s* expressed with a tissue-specific manner during sprout stage. Through RNA-seq and qRT-PCR analysis, it was found that there were differences in expression in extreme materials under salt stress. The determination of auxin content and the analysis of *PvB3s* expression in the enriched pathway showed that *PvB3s* would respond to auxin to enhance salt tolerance in common bean sprouting stage.

Conclusion:

The results provided useful and rich resources of *PvB3s* for the functional characterization and understanding of *B3* transcription factors (TFs) in common bean, which further provides insights that *PvB3s* may respond to auxin to enhance salt tolerance of common bean.

Background

B3 gene family is a transcription factor family [1] unique to plants. The B3 family contains B3-DNA binding domains, which play an important role in plant growth and development by binding specific DNA sequences. It is reported that B3-TF from different families regulates different aspects of plant growth and development [2]. According to the domain structure and phylogenetic analysis, the B3 superfamily can be divided into four main families, namely, LAV (LEAFY COTYLE-DON2-ABAINSENSITIVE3-VAL), RAV (related to ABI3/VP1), ARF (auxin response factor) and REM (reproductive meristem) family. The B3 superfamily has found many model plants and crops in Arabidopsis, rice, poplar, rape, castor beans, cocoa, soybeans, corn, tobacco, grapes, moss, algae, citrus [3–7], but not foundings in common beans.

The earliest *B3* gene is the *VP1* gene obtained from maize. Studies have shown that this gene has transcriptional activity and encodes a protein with three basic domains, which are named B1, B2 and B3 domains [8, 9] respectively. Among them, the B3 domain has specific DNA binding activity, which can play a regulatory role in plant growth and development by binding specific DNA. The B3 family is named because of its B3-DNA domain. The *ABI3* gene obtained in *Arabidopsis thaliana* is similar to the domain

of jade *VP1* [10], but also has a B3-DNA domain. *ABI3* genes regulate abscisic acid response genes during seed germination; *LEC2* and *FUS3* genes promote embryonic development, *LEC2* can also regulate hormone synthesis, and play a regulatory role in embryonic development by stimulating auxin biosynthesis genes; these three genes are only heavily expressed in seeds. *VAL* genes of LAV subfamily can inhibit embryonic development and are highly expressed in apical meristems and seeds [11–14]. ARF subfamily genes *AtARF1* and *AtARF2* with B3 domain are involved in the process of flower organ senescence and abscission [15]. Genes *AtARF6* and *AtARF8* play an important role in stress regulation and enhance stress resistance by regulating the synthesis of jasmonic acid [16]. In cotton (*Gossypium Hirsutum*), over expression of *AtRAV1/2* can improve the drought resistance of cotton [17]. Pepper *CARAV1* is induced by abiotic stress and acts as a transcriptional activator to trigger tolerance to osmotic stress [18]. It was also reported that *Arabidopsis thaliana* expressing pepper *CaraV1* improved its tolerance to high salt stress [19], and soybean gene *GmRAV-03*, which contains B3 domain was involved in salt and drought stress [20].

P. vulgaris L. is a valuable legume crop and a source of livelihood for many people [21, 22]. Besides, the crop is rich in proteins and micro-nutrients, which are essential for human health. There has been a slow improvement in the yield of the crop, particularly in tropical regions. However, favorable yields have been realized in temperate countries as a result of the development of new cultivars [23]. Studies have shown that *PvB3s* participate in auxin response, which may have influence related to various abiotic stresses in several plant species. However, the B3 family in *P. vulgaris* has not been characterized. To screen the potential salt tolerance genes in common bean sprouting stage, we identified the members of B3 gene family at the genome level. Here in, we identified 110 PvB3 members according to *P. vulgaris* L. genome sequence. Furthermore, we determined their gene distribution on chromosome and studied their conserved domain and collinearity. We evaluated *PvB3s* expression in multiple varieties of the plant. Also, we analyzed the expression pattern of *PvB3s* in various tissues at sprout stage and explored the possible regulation of specifically expressed *PvB3s* under salt stress and other stress conditions. These findings offer crucial information for studying the evolutionary relationship and functional differentiation of B3 gene family in common bean.

Results

Identification of B3 Family in Common bean

In total, 118 protein sequences with B3 domains were identified by HMM profile analysis from the common bean genome. Furthermore, InterPro and SMART analyses indicated that 110 members could be assigned as common bean B3 family, which were named PvB3-001 ~ PvB3-110 accordingly (Table S1). Among them, 110 PvB3 members were located on the 11 chromosomes (Chrs) of common bean (Fig. 1).

Specifically, most of the PvB3s were located in Chr 3,which 15 members on, followed by Chr 8 with 14 members on, while minimum genes were distributed on Chr 4, Chr 5, which only have 3 and 6 members.

The position of PvB3 members can be mapped to each chromosome can be seen in Fig. 1. The denser the blue, the higher the gene density in the middle of that area.

Phylogenetic Analysis of PvB3s

A comprehensive phylogenetic tree comprising 110 PvB3 protein sequences of common bean (Fig. 2).The phylogenetic tree was generated using the Maximun Likelihoodphy using WAG + G model in MEGA software (MEGAX) with the B3 sequences. Based on the phylogenetic tree, the predicted PvB3 family clustered into 4 subgroups, which is □-□□. Subgroups □ and □□ have the most PvB3 members, compared to subgroups □ and □.

The motif and gene structure conserved PvB3 family members

We employed the MEME software to analyze the conservative motifs of 110 PvB3s in 4 subgroups in celery. Figure 3b shows the symbols of the motifs and the composition of every B3 family members in *P.vulgaris*.L. Each sub-group contains some differences in the motif, but the members within each sub-family have similar motif structures. For example, in the □, □, and □ subgroups all have motif 4, only members of subgroup 4 and a few members of subgroup 1 own motif 8, and the □ subgroup contains many different motifs, but every member of the □ subgroup owns these motifs.

The gene structure of exon and intron of the *PvB3s* were examined to reveal a certain difference in the gene structure among the 4 subfamilies, regardless of the length of their genetic structure, the number of introns and exons, including the number of CDS, but the structure of *PvB3* in each subfamily is relatively consistent No matter the number of their CDS and introns, they are even similar to the position of B3 domain in their gene structure.

Cis-regulatory elements analysis of PvB3s

Cis-regulatory elements was used to be analyzed by PlantCARE software, according to the promoters of *PvB3s* according to the celery genome database, and the related hormone formation seed germination related elements are represented in Fig. 4b. Red boxes are hormone-related elements. These elements revealed that the *PvB3s* may respond to hormones in response to stress; The blue elements represent elements that respectively encounter low-temperature and drought, indicating that the *PvB3s* have a very important role in abiotic stress; yellow represents elements that are related to plant germination or sprout stage.

Collinearity analysis and tandem replication of PvB3s

The results of collinear analysis within common bean B3 family gene species and with legume crops and Arabidopsis thaliana are shown in Fig. 5. Only 14 pairs of bean B3 family genes were collinear and distributed in 11 LGs, of which 9 had the largest number of *PvB3* with 4 members, indicating that *PvB3s*

were not randomly and uniformly distributed on the linkage group (Fig. 5a). In legume species, there was collinearity between *PvB3s* and soybean 88 genes (*G. max*.L), adzuki bean 35 genes (*V. angularis*) and mung bean 50 genes (*V. radiata*.L) (Fig. 5b). However, only 23 genes in the B3 family of common beans are collinear with *Arabidopsis thaliana*,and only 5 genes in rice (*O. sativa* L.) (Fig. 5c). In the selection pressure analysis of *PvB3s* (Table S3), only 3 pairs of tandem replication genes in PvB3 had Ka/Ks < 1, while the Ka/Ks > 1 of 9 pairs of *PvB3s.*

Analysis of PvB3 members and the genes containing B3 domain in Arabidopsis

The same Pfam identified proteins and genes containing B3 conserved domains in *Arabidopsis*, and conducted evolutionary analysis of mixture species with PvB3 members, as well as motif and gene structure analysis (Fig. 6). After analysis, it was found that the B3 family genes of the two species are also divided into 4 subgroups, as PvB3s in. The genetic structure and motif of each subfamily are relatively similar, indicating that each subfamily is traveling The functions are relatively consistent.

Expression analysis of PvB3s in Different Tissues of Common bean in sprout stage

Successively, we examined the transcriptional patterns of *PvB3s* in many tissues using high-throughput sequencing information from Phytozome database, that included flower buds, flowers, leaves, root, trifoliates, nodules, stem, pod, seed, and other tissues (Fig. 7a), *PvB3s* are expressed in various tissues, and the expression differences are relatively large. To elucidate the functions of the *PvB3* genes in *P. vulgaris* at sprout stage, we choose 6 *PvB3* genes randomly, used qRT-PCR for three tissues (cotyledon, hypocotyl, radicle) in common bean R sprout stage to assess the expression of the *PvB3* genes in sprout stage diverse tissues. As shown (Fig. 7b), among the 6 *PvB3* genes randomly selected, The relative expression of some *B3* family genes in the radicle is relatively high, and some *PvB3s* have relatively high expression in the hypocotyl.

Expression analysis of PvB3s in extreme materials under salt stress

From a laboratory basis, we tested two kinds of extreme common bean materials under water treatment and salt stress treatment (R is salt-tolerant variety, and N is salt-sensitive variety). The assembled unigene dataset was used as a reference for further analyses, and it can be found at the National Centre for Biotechnology Information under the accession number PRJNA558376 (http://www.ncbi.nlm.nih.gov/bioproject/). Through the determination of transcriptome, we found that there was a significant difference in *PvB3s'* expression, but many *PvB3s* of the fourth subfamily are not expressed(Fig. 8a). 9 *PvB3* genes were randomly selected for qRT-PCR analysis, and it was found that under W (water treated) and S (salt stress treated), the transcriptome results of *PvB3* genes expression in extreme materials R and N were consistent (Fig. 8b). It is suggested that under salt stress, the differential expression of *PvB3*s in extreme materials may affect the salt tolerance of plants.

PvB3s expression analysis of IAA enrichment pathway in extreme materials under salt stress

The KEGG analysis of *PvB3s* showed that *PvB3s* were enriched into the pathway “Plant hormone signal transduction” (pvu04075) in common bean, and its Corrected P-Value was $1.81e^{-14}$(Fig. 9a). The result showed that *PvB3s* could adjust plant stomata in response to IAA. The IAA content of extreme materials was determined, and it was found that the IAA content of salt-resistant variety R itself was higher and increased sharply under salt stress, while the change of IAA content of salt-sensitive variety N was relatively small (Fig. 9b). The results of qRT-PCR analysis of radicles of two varieties showed that, compared with S treatment, some *PvB3s* the change of gene expression of R variety was larger than that of N variety, which indicated that the change of IAA content did affect the change of *PvB3s* under salt stress, which may further affect the salt tolerance of common bean at sprout stage (Fig. 9c).

The Relationship between IAA, salt stress and PvB3s

After IAA and salt treatment, the sensitive salt variety N has been alleviated a lot compared with S treatment, such as the number of lateral roots of bean sprouts increased significantly (Fig. 10a). Exogenous IAA could significantly increase the content of endogenous IAA in N varieties (Fig. 10b), and 8 *PvB3s*, were randomly selected and verified by qRT-PCR analysis (Fig. 10c). It was found that the variable of these genes increased or decreased significantly in N, indicating that *PvB3s* will respond to the changes of IAA to resist salt stress in plants.

Discussion

Characterization of B3 Family Members in Common bean

In previous studies, the genes of the B3 family have been reported in citrus (*C. sinensis* and *C. grandis*) [36], Savoy cabbage (*B. oleracea*)[37], and grapes (*V. vinifera* L.) [38], but no related articles have been reported in common beans. Through evolutionary tree analysis, the B3 transcription factor family has been divided into 4 Subgroups, and the motifs, *cis* elements, and gene structures within each sub-group are very similar, indicating that each sub-group has *PvB3s* with similar functions; but the *PvB3s* among the four subgroups are very large In the analysis of the B3 transcription factors of *Arabidopsis* and the common bean (*P. vulgaris* L.) two species, they are divided into 4 subgroups according to the genetic relationship, and the members in each subgroup are similar in terms of motif or gene structure. This conclusion is consistent among citrus (*C. sinensis* and *C. grandis*)[36] and grapes (*V. vinifera* L.) [6].In tissue-specific analysis, more *PvB3s* are expressed in the bud stage (whether flower buds, plant buds, or young pods) compared to other tissues. The same conclusion is found in grapes (*V. vinifera* L.) [37], citrus (*C. sinensis* and *C. grandis*) [36] and tobacco [38] (*Nicotiana tabacum* L.).

Analysis collinear genes of PvB3s

In order to determine the relationship between *PvB3s* and potential repetitive events in evolution, the genomic information of less-studied biological genome taxonomic units was transferred to the biological taxonomic units that have been deeply studied [39]. Another use of collinear analysis provides theoretical

and practical value for studying loci that control common traits among species. In 2013, Chen [40] identified the Rice (*O. brachyantha*.L) local duplications, insertions, and inversions by constructing a collinearity map of the whole genome comparison of rice and rice. The analysis further revealed the mechanism of rice genome changes, gene family expansion, gene migration and transformation. For common bean *PvB3s* perform collinear analysis between *PvB3s* and multiple species.By analyzing the collinear genes of family genes within species, LG 9 contains the most collinear genes, which can be inferred that in the process of evolution, gene replication events are most likely to occur in LG9; In the collinear analysis with three legume crops, the collinear genes in soybeans are significantly higher than that in adzuki bean (*V. angularis*) and mung bean (*V. radiata*.L), indicating that there may be more collinear genes due to soybean containing a larger genome; In the analysis of collinearity with *Arabidopsis* and Rice (*O. sativa* L.), the limit of collinear genes is reduced. Monocotyledonous Rice even has fewer collinear genes than dicotyledonous *Arabidopsis*, with only 5 paired genes, which may cause few collinear genes due to differences between species. Among 23 *Arabidopsis* genes which are collinear with *PvB3s*, as shown in Table 1. 14 of these genes have clearly stated that they are related to auxin or respond to IAA, 8 genes have been reported to be related to sprout stage or seedlings; the other 4 genes have been described to be related to stress resistance.These results predict that *PvB3s* is likely to be closely related to auxin response, plant seedling stage and stress resistance. In selective pressure analysis, this study found that most Ka/Ks are greater than 1, and only 3 pairs are less than 1, which indicated that 3 groups of genes chose pure selection in the face of evolution, and they maintained the changes of their ancestors under the requirements of evolution, but most *PvB3s* in common bean chose adaptive forward evolution in the process of evolution, thus deriving new functions.

Table 1
Collinear gene analysis of *PvB3s* in *Arabidopsis*.

***PVB3s* ID**	**Collinear gene in *Arabidopsis***	**Function**	**Pumbed**
PVB3-001	*AT1G19850*	Response to auxin, plant seedling growth.	[41]
PVB3-099	*AT1G01030*		
PVB3-070	*AT1G77850*	Auxin-activated signaling pathway.	[42]
PVB3-078	*AT1G77850*	Auxin-activated signaling pathway.	[42]
PVB3-009	*AT2G36080*	During Seedling Development.	[43]
PVB3-099	*AT2G46870*	Resistance to abiotic stress.	[44]
PVB3-110	*AT2G28350*	Response to auxin, plant seedling.	[45]
PVB3-040	*AT2G28350*	Response to auxin, plant seedling.	[45]
PVB3-047	*AT2G46530*	Response to auxin.	[46]
PVB3-048	*AT2G33860*	Response to auxin, resistance to abiotic stress.	[47, 48]
PVB3-076	*AT2G30470*	Resistance to abiotic stress.	[49]
PVB3-009	*AT3G11580*	Resistance to abiotic stress.	[50]
PVB3-007	*AT3G26790*	Seed development.	[51]
PVB3-099	*AT3G61970*	Response to auxin.	[52]
PVB3-047	*AT3G61830*	Response to auxin.	[52]
PVB3-066	*AT3G24650*	Resistance to abiotic stress, plant seedling.	[53, 54]
PVB3-049	*AT4G21550*		

PVB3s ID	Collinear gene in *Arabidopsis*	Function	Pumbed
PVB3-108	*AT5G60450*	Response to auxin.	[55]
PVB3-030	*AT5G20730*	Response to auxin.	[56]
PVB3-041	*AT5G60450*	Response to auxin, plant seedling.	[52]
PVB3-074	*AT5G37020*	Response to auxin, plant seedling.	[57]
PVB3-079	*AT5G42700*		
PVB3-088	*AT5G20730*	Response to auxin.	[58]

The relationship between PvB3s, **IAA and salt stress in common bean sprouts**

The previous paragraph has shown that the B3 family gene and auxin response is very large, whether in the bud stage or other growth periods. Both in grapes and citrus have been reported to identify the relationship with auxin. But it also appears to be related to plant stress resistance in collinear genes. There are also many auxin-responsive genes, especially the *B3* family genes of certain species, and their relationship with plant stress, especially salt stress; Rhizobacteria Pseudomonas Putida and Novosphingobium Sp can enhance the secretion of IAA to enhance the salt tolerance of citrus[59] ;*MicroRNA390/ TRANS-ACTING SHORT INTERFERING RNA3* in poplar (*Populus spp.*), which can regulate the auxin pathway can be used to enhance the stress resistance of plants, especially the salt tolerance[60];The auxin pathway can play a role in maintaining the morphology and physiology of the Brassica napus seedlings in response to stress[61];IAA-Asp, a combination of IAA and amino acids, can directly and specifically affect pea (*P. Sativum* L) response to abiotic stress[62].These all indicate that IAA has a very close relationship with salt stress, and auxin responsive gene *IbARF5* has also been reported to enhance the salt tolerance of sweetpotato[63]. In this experiment, through identification and analysis of the common bean B3 gene family, *PvB3s* and auxin regulation pathways were found to be closely related by KEGG enrichment. Auxin was applied externally to observe the growth of common bean sprouts and the response of *PvB3s*, and further reveal *PvB3s* with the relationship between auxin and coercion (Fig. 11).

Conclusion

Despite the importance of *B3* transcription factors for plant growth, development, and abiotic stress responses, little is known about the *PvB3s* in common bean. We used the previously published common bean reference genome to perform a comprehensive analysis of common bean *B3s*. The *PvB3s* and

genes which have B3 domain in *Arabidopsis* were clearly divided into phylogenetic clades. These clades were supported by various group-specific sequence characteristics, including exon/intron structure, MEME motif composition, Intra-species collinearity and inter-species collinearity. By analyzing expression of different tissues in *PvB3s* expression during sprout stage and extremely varieties in response to salt stress, we characterized the overall expression of *PvB3s* in sprouts.Transcriptomic, qRT-PCR analysis and KEGG enrichment revealed some *PvB3s* that may be important for the salt stress response by responding to IAA, and the application of IAA exogenously determined that the growth of common beans under salt stress could be changed, and the expression level of *PvB3s* could also be changed.. These informations generated in this study could facilitate further research on *B3* family and other gene families in common bean.

Methods

Identification and Phylogenetic Analysis of the B3 Gene Family in common bean

The HMMER software (http://hmmer.janelia.org/) and the Pfam protein family database (http://pfam.sanger.ac.uk/) [24] were employed to identify the candidate B3 proteins at the B3 domain (PF02362). The protein annotation file was retrieved from the website of Esembl plants (http://plants.ensembl.org/index). Subsequently, InterPro (http://www.ebi.ac.uk/interpro/) [25] and SMART (http://smart.embl-heidelberg.de/)[26, 27] programs were applied to verify the integrity of the B3 domain. Lastly, Interpro (http://prosite.expasy.org/),WoLF PSORT (https://wolfpsort.hgc.jp/), P3DB (http://www.p3db.org/) and ExPASy Proteomics Server (http://prosite.expasy.org/) were employed to verify the integrity of B3 domain in candidate genes. Each *PvB3* was named according to their precise position on the chromosomes.All of the other species like *Arabidopsis* B3 protein sequences were obtained from Esembl plants, also used B3 domain. All these protein sequences of common bean and other species. Pv*B3s* were imported into MEGA X [28], and multiple sequence alignments were performed Maximun Likelihoodphy using WAG + G model, which MEGA predicted, 1000 replicates were used to produce bootstrap values. MEGA X was utilized to edit and construct the phylogenetic tree.

Analyses of Gene Structure, Conserved Motif, Promoter

The exon/intron structure of *PvB3s* was analyzed and displayed using the GSDS platform (http://gsds.cbi.pku.edu.cn/) [29]. Gene wise [30] was used to determine the correspondence on coordinates between DNA (containing exon and intron together) and protein sequences. Then, the coordinates of B3 domain in protein sequence were transformed to that in gene sequence using in-house perl scripts. The intron splicing phase within the basic and hinge regions of B3 domains from all *PvB3s* were characterized and divided into different types.The MEME tool (http://meme.nbcr.net/meme/) [31] was employed to detect the additional motifs outside the B3 domain of protein sequences. The motifs with 10–50 amino acids in length and E-value less than $1e^{-20}$ were characterized. All the motifs were compared among *PvB3s* to identify the group-conserved or group specific signatures. These motifs were numbered according to their order in the protein sequences.

Collinearity analysis of PvB3s

The *PvB3s* were mapped to the chromosome based on the chromosomal location provided in the Esembl plants. Gene duplication events were analyzed using the Multiple Collinearity Scan toolkit (MCScanX) using default parameters [32]. Finally, the visualization was generated by the circos (version 0.69) (http://circos.ca/) [33].

Transcriptomic identification of Extreme materials in different Treatments

Seeds of extreme common bean salt stress varieties "R and N" were provided by the National Cereals Center. The stress concentration of NaCl solution was 70 mmol/L, exogenously applied auxin(IAA) with a concentration of 100 μmol/L, which was the basis for the preliminary research of the National Cereals and Seeds Breeding Laboratory. They were protected without light for 5 d in a 28 °C incubator. The transcriptome sequencing work is determined by Novogene (China). The KEGG annotation came from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg) and the database from phytozome.

qRT-PCR Analysis of PvB3s

Processing the extremely common bean sprouts at different sprout stages into different tissues, the samples RNA was extracted by TRIzol (Invitrogen), and the quality of the RNA was detected using 1% agarose gel electrophoresis and NanoDrop instrumentation. The concentration and purity of the RNA were determined by NanoDrop. The OD260/280 was required to be between 1.8 and 2.2, and 28S/18S was not less than 1. Quantitative Real-time PCR (qRT-PCR) primers were designed using Primer 5.0 (http://www.premierbiosoft.com/primerdesign/index.html). We selected many genes from KEGG pathway, and also selected some *PvB3s* as candidate genes for verification.The *PvACTIN-11* gene of common bean was selected as the internal reference gene [34]. The primers for genes used for real-time PCR are listed in Table S1. The material RNA was extracted by TRIzol, and the genomic DNA was then removed by a 4 × gDNA wiper mix (Vazyme, R223, Vazyme biotech, China). The RNA was retrieved as a single-strand cDNA by 5 × HiScript II qRT SuperMix II (Vazyme, R223, Vazyme biotech, China). The qRT-PCR program uses a Light Cycler 480 system (Roche, Roche Diagnostics, Switzerland) and the 2 × ChamQ Universal SYBR qPCR Master Mix Kit (Vazyme, Q711, Vazyme biotech, China). The qRT-PCR analysis was carried out based on 3 biological replicates. The relative expression of the candidate genes was calculated according to the following formula [35]:

Relative expression = $2^{\Delta\Delta}Ct$, $\{\Delta\Delta Ct = [Ct_2(Pv \text{ target genes}) - Ct_2(ACTIN11)] - [Ct_1(Pv \text{ target genes}) - Ct_1(ACTIN11)]\}$

Abbreviations

LSD	Least Significant Difference
qRT-PCR	Quantitative Real-time PCR
KEGG	Kyoto Encyclopedia of Genes and Genomes
IAA	Indolyl-3-acetic acid

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The raw RNA-seq data are available at the National Centre for Biotechnology Information under the accession number PRJNA558376 (http://www.ncbi.nlm.nih.gov/bioproject/) . The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Financial support was received from the Research Project of Heilongjiang Bayi Agricultural University (XDB2011-02).

Authors' contributions

Q.Z., and J.D. conceived the study and designed and managed the experiments. W.L., W.Z., provided the plant lines. Y.W., M.Z., S.Z., Y.L., H.Z., and Q.Z. performed the trials and collected the data. Z.Y., W.Z., and Q.Z. completed the statistical analysis of the phenotypic data and wrote the paper. All authors contributed to writing the paper.

References

1. Yamasaki K, Kigawa T, Seki M, Shinozaki K, Yokoyama S. DNA-binding domains of plant-specific transcription factors: structure, function, and evolution. Trends Plant Sci. 2013;18:267–76.

2. Swaminathan K, Peterson K, Jack T. The plant B3 superfamily. TRENDS PLANT SCI. 2008;13:647–55.
3. Romanel EAC, Schrago CG, Counago RM, Russo CAM, Alves-Ferreira M. Evolution of the B3 DNA binding superfamily: new insights into REM family gene diversification. PLoS One. 2009;4:e5791.
4. Peng FY, Weselake RJ. Genome-wide identification and analysis of the B3 superfamily of transcription factors in Brassicaceae and major crop plants. Theor Appl Genet. 2013;126:1305–19.
5. Xia F, Sun T, Yang S, Wang X, Chao J, Li X, Cui JHu,M, Liu G, Wang D. Yuhe Sun. Insight into the B3Transcription Factor Superfamily and Expression Profiling of *B3* Genes in Axillary Buds after Topping in Tobacco (*Nicotiana tabacum* L.). Gene. 2019;10:164.
6. Ahmad B. Genomic organization of the B3-domain transcription factor family in Grapevine (*Vitis vinifera* L.) and expression during seed development in seedless and seeded cultivars. Int J Mol Sci. 2019;20:4553.
7. Zheng Liu X-X, Ge X-M, Wu Q, Xu RG, Atkinson. Wen-Wu Guo. Genome-wide analysis of the citrus B3 superfamily and their association with somatic embryogenesis. BMC Genom. 2020;21:305.
8. McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M, Vasil IK. The Viviparous-1 developmental gene of maize encodes a novel transcriptional activator. Cell. 1991;66:895–905.
9. Suzuki M, Kao CY, McCarty DR. The conserved B3 domain of VIVIPAROUS$_1$ has a cooperative DNA binding activity. Plant Cell. 1997;9:799–807.
10. Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F. Isolation of the Arabidopsis *ABI3* gene by positional cloning. Plant Cell. 1992;4:1251–61.
11. Matteo Sugliani V, Brambilla, Emile JM, Clerkx M, Koornneef, Wim JJ, Soppe. The conserved splicing factor SUA controls alternative splicing of the developmental regulator *ABI3* in *Arabidopsis*. Plant Cell. 2010;22:1936–46.
12. Chiu RS, Nahal H, Provart NJ, Sonia Gazzarrini. The role of the Arabidopsis *FUSCA3*transcription factor during inhibition of seed germination at high temperature. BMC Plant Biol. 2012;12:1186–471.
13. Sandra L, Stone L, WKwong KM, Yee J, Pelletier L, Lepiniec RL, Fischer RB, Goldberg, John J, Harada. *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. Proc Natl Acad Sci USA. 2001;98:11806–11.
14. Luerssen H, Kirik V, Herrmann P, Miséra S. *FUSCA3* encodes a protein with a conserved VP1/AB13-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. Plant J. 1998;15:755–64.
15. Mun JH, Yu HJ, Shin JY, Oh M, Hwang HJ, Chung H. Auxin response factor gene family in *Brassica rapa*: genomic organization, divergence, expression, and evolution. Molecular Genetics Genomics. 2012;287:765–84.
16. Suzuki M, Wang HH, McCarty DR. Repression of the *LEAFY COTYLEDON$_1$/B3* regulatory network in plant embryo development by *VP$_1$/ABSCISIC ACID INSENSITIVE$_3$-LIKE* B3 genes. Plant Physiol. 2007;143:902–11.

17. Matías-Hernández L, Aguilar-Jaramillo AE, Marin-Gonzalez E, Suarez-Lopez P, Pelaz S. *RAV* genes: regulation of floral induction and beyond. Ann Bot. 2014;114:1459–70.
18. Sohn KH, Lee SC, Jung HW, Hong JK, Hwang BK. Expression and functional roles of the pepper pathogen-induced transcription factor RAV_1 in bacterial disease resistance, and drought and salt stress tolerance. Plant Mol Biol. 2006;61:897–915.
19. Lee SC, Choi DS, Hwang IS, Hwang BK. The pepper oxidoreductase CaOXR1 interacts with the transcription factor CaRAV1 and is required for salt and osmotic stress tolerance. Plant Mol Biol. 2010;73:409–24.
20. Zhao S-P, Xu Z-S, Zheng W-J, Zhao W, Wang Y-X, Yu T-F, Chen M, Zhou Y-B, Min D-H, Ma Y-Z, Chai S-C, Zhang X-H. Genome-Wide Analysis of the RAV Family in Soybean and Functional Identification of *GmRAV-03* Involvement in Salt and Drought Stresses and Exogenous ABA Treatment. Front. Plant Sci. 2017;00905.
21. Broughton WJ, Hernández G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (*Phaseolus* spp.): model food legumes. Plant Soil. 2003;252:55–128.
22. Jing Wu L, Wang J, Fu J, Chen S, Wei S, Zhang J, Zhang Y, Tang M, Chen J, Zhu L, Lei Q, Geng C, Liu L, Wu X, Li X, Wang Q, Wang Z, Wang S, Xing H, Zhang MW, Blair. Shumin Wang. Resequencing of 683 Common Bean Genotypes Identifies Yield Component Trait Associations Across a North-South Cline. Nat Genet. 2019;52:118–25.
23. Rosenzweig C, Liverman D. Global climate change: Implications, challenges and mitigation measures (ed. Majumdar, S. K.). Pennsylvania Academy of Sciences. 1992;342–361.
24. Robert D, Finn P, Coggill, Ruth Y, Eberhardt SR, Eddy J, Mistry AL, Mitchell SC, Potter, Marco Punta, Matloob Qureshi, Amaia Sangrador-Vegas, Gustavo A, Salazar, John Tate, Alex Bateman. The Pfam protein families database: Towards a more sustainable future. Nucleic Acids Res. 2016;44:D279–D285.
25. Robert Finn TK, Attwood PC, Babbitt A, Bateman P, Bork AJ, Bridge H-Y, Chang Z, Dosztányi S, El-Gebali M, Fraser J, Gough D, Haft G, Holliday H, Huang X, Huang I, Letunic R, Lopez S, Lu, Aron Marchler-Bauer, Huaiyu Mi, Jaina Mistry, Darren Natale, Marco Necci, Gift Nuka, Christine Orengo, Youngmi Park, Sebastien Pesseat, Damiano Piovesan, Simon C, Potter, Neil D, Rawlings, Nicole Redaschi, Lorna Richardson, Catherine Rivoire, Amaia Sangrador-Vegas, Christian Sigrist, Ian Sillitoe, Ben Smithers, Silvano Squizzato, Granger Sutton, Narmada Thanki, PD, Thomas S, Tosatto CH, Wu, Ioannis Xenarios, Lai-Su Yeh, Siew-Yit Yong, Alex L Mitchell. InterPro in 2017-beyond protein family and domain annotations. Nucleic Acids Res. 2017;45:D190–D199.
26. Han Z, Liu Y, Deng X, Liu D, Liu Y, Hu Y, Yan Y. Genome-wide identification andex pression analysis of expansin gene family in common wheat (*Triticum aestivum* L.). BMC Genom. 2019;20:101.
27. Letunic I, Doerks T, Bork P. Smart. Recent updates, new developments and status in 2015. NUCLEIC ACIDS RES. 2015;43:D257–60.
28. Kumar S, Stecher G, Tamura K. MEGA7:molecular evolutionary genetics analysis version 7.0 for bigger datasets. MOL BIOL EVOL. 2016;33:1870–4.

29. Guo AY, Zhu QH, Chen X, Luo JC. GSDS: a gene structure display server. Yi Chuan. 2007;29:1023–6.
30. Birney E, Clamp M, Durbin R. GeneWise. and Genomewise. GENOME RES. 2004;14:988–95.
31. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. MEME SUITE: tools for motif discovery and searching. NUCLEIC ACIDS RES. 2009;37:W202–8.
32. Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res. 2012;40:e49–9.
33. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19:1639–45.
34. Borges A, Tsai SM, Caldas DG. Validation of reference genes for RT-qPCR normalization in common bean during biotic and abiotic stresses. Plant Cell Rep. 2012;31:827–38.
35. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\triangle\triangle CT}$method. METHODS. 2001;25:402–8.
36. Liu Z, Ge XX, Wu XM, Xu Q, Guo WW. Genome-wide analysis of the citrus B3 superfamily and their association with somatic embryogenesis. BMC Genomics. 2020;21(1).
37. Wen J, Guo P, Ke Y, Liu M, Du H. The auxin response factor gene family in allopolyploid *Brassica napus*. Plos One. 2019;14(4).
38. Xia F, Sun T, Yang S, Wang X, Chao J, Li X, Hu J, Cui M, Liu G, Wang D, Sun Y. Insight into the B3transcription factor superfamily and expression profiling of *B3* genes in axillary buds after topping in tobacco(*Nicotiana tabacum* L.). Genes. 2019;10(2).
39. Lyons E, Pedersen B, Kane J, Alam M, Ming R, Tang H, Wang X, Bowers J, Paterson A, Lisch D, Freeling M. Finding and comparing syntenic regions among Arabidopsis and the outgroups papaya, poplar, and grape: CoGe with rosids. Plant Physiol. 2008;148:1772–81.
40. Chen J, Huang Q, Gao D, Wang J, Lang Y, Liu T, Li B,Zetao Bai JL, Goicoechea C, Liang C, Chen W, Zhang SSun,Yi, Liao X, Zhang Lu, Yang C, Song M, Wang J, Shi G, Liu, Junjie Liu,Heling Zhou, Weili Zhou, Qiulin Yu, Na An, Y, Chen Q, Cai B, Wang, Binghang Liu, Jiumeng Min,Ying Huang, Honglong Wu, Z, Li Y, Zhang Y, Yin W, Song J, Jiang, Scott A, Jackson RA, Wing J, Wang M Chen. Whole-genome sequencing of *Oryza brachyantha* reveals mechanisms underlying *Oryza* genome evolution. Nat. Commun. 2013;4:67–88.
41. Fendrych M, Leung J, Friml J. TIR1/AFB-Aux/IAA auxin perception mediates rapid cell wall acidification and growth of Arabidopsis hypocotyls. Elife. 2016;5:e19048.
42. Mallory AC, Bartel DP, Bartel B. MicroRNA-directed regulation of Arabidopsis *AUXIN RESPONSE FACTOR17* is essential for proper development and modulates expression of early auxin response genes. Plant Cell. 2005;17(5):1360–75.
43. Nelson DC, Flematti GR, Riseborough JA, Ghisalberti EL, Dixon KW, Smith SM. Karrikins enhance light responses during germination and seedling development in *Arabidopsis thaliana*. P NATL ACAD SCI USA. 2010;107(15):7095–100.

44. Hikaru Sato H, Takasaki F, Takahashi T, Suzuki S, Iuchi, Nobutaka Mitsuda, Masaru Ohme-Takagi, Miho Ikeda, Mitsunori Seo, Kazuko Yamaguchi-Shinozaki, Kazuo Shinozaki. *Arabidopsis thaliana* NGATHA1 transcription factor induces ABA biosynthesis by activating *NCED3* gene during dehydration stress. P NATL ACAD SCI USA. 2018; 115(47):E11178-E11187.
45. Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, Luan S, Li L, He ZH. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing arf-mediated *ABI3* activation in *Arabidopsis*. P NATL ACAD SCI USA. 2013;110(38):15485–90.
46. Emilie V, Florian B, Judit, Szécsi, Julie, Leroux, Spencer, Brown, Catherine, P-R, Mohammed, Bendahmane. AUXIN RESPONSE FACTOR8 regulates *Arabidopsis* petal growth by interacting with the bHLH transcription factor BIGPETALp. The Plant cell. 2011;23(3):973 – 83.
47. Zheng Y, Zhang K, Guo L, Zhang Z. Auxin response factor3 plays distinct role during early flower development. Plant Signal Behav. 2018;13(5):e1467690.
48. Ulmasov T, Hagen G, Guilfoyle TJ. Dimerization and dna binding of auxin response factors. Plant J. 1999;19(3):309–19.
49. Sharma N, Bender Y, Boyle K, Fobert PR. *High-level expression of sugar inducible gene2 (HSI2)* is a negative regulator of drought stress tolerance in *Arabidopsis*. BMC Plant Biol. 2013;13(1):170.
50. Li B, Gaudinier A, Tang M, Taylor-Teeples M, Ngoc T, Nham Cyrus, Ghaffari D, Scott B, Margaret S, Jennifer A, Gray,Siobhan M, Brady,Daniel J, Kliebenstein. Promoter-based integration in plant defense regulation. Plant physiology. 2014;166(4):1803–20.
51. Wang F, Perry SE. Identification of Direct Targets of FUSCA3, a Key Regulator of Arabidopsis Seed Development. Plant Physiol. 2013;161(3):1251–64.
52. Vale´rie Morin1
Teva Vernoux G, Brunoud E, Farcot, Vale´rie Morin1, Van den Daele H, Legr J, Oliva M, Das P, Larrieu A, Wells D, Gue´don Y, Lynne Armitage, Franck Picard, Soazig Guyomarc'h, Coralie Cellier, Geraint Parry, Rachil Koumproglou, Doonan JH, Estelle M, Christophe Godin, Stefan Kepinski, Malcolm Bennett, Lieven De Veylder, Jan Traas. The Auxin Signalling Network Translates Dynamic Input Into Robust Patterning at the Shoot Apex. Mol Syst Biol. 2014;7(1):508–508.
53. Bedi S, Sengupta S, Ray A, Nag Chaudhuri R. ABI3 mediates dehydration stress recovery response in *Arabidopsis thaliana* by regulating expression of downstream genes. Plant Sci. 2016;250:125–40.
54. Bassel GW, Bewley RTMD. *ABI3* expression ceases following, but not during, germination of tomato and *Arabidopsis* seeds. J Exp Bot. 2006;57(6):1291–7.
55. Irena P, Paul AJ, Yuval E. Auxin response factors mediate Arabidopsis organ asymmetry via modulation of kanadi activity. Plant Cell. 2005;17(11):2899–910.
56. Yoko O, Overvoorde PJ, Kazunari A, Jose M, Alonso, April, Chan, Charlie C, Ecker JR. Beth, Hughes, Amy, Lui, Diana, Nguyen, Courtney, Onodera, Hong, Quach, Alison, Smith, Guixia, Yu, Athanasios, Theologis. Functional genomic analysis of the AUXIN RESPONSE FACTOR gene family members in *Arabidopsis thaliana*: unique and overlapping functions of ARF7 and ARF19. Plant Cell. 2005;17(2):444–63.

57. Tian C, Muto H, Higuchi K, Matamura T, Tatematsu K, Koshiba T, Yamamoto KT. Disruption and overexpression of *auxin response* factor 8 gene of *Arabidopsis* affect hypocotyl elongation and root growth habit, indicating its possible involvement in auxin homeostasis in light condition. Plant J. 2004;40(3):333–43.
58. Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M. ARF7 and ARF19 regulate lateral root formation via direct activation of *LBD/ASL* genes in *Arabidopsis*. Plant Cell. 2007;19:118–30.
59. Vicente V-P, Gómez-Cadenas A, Rosa M. Salt stress alleviation in citrus plants by plant growth-promoting rhizobacteria *Pseudomonas putida* and *Novosphingobium* sp. Plant Cell Reports. 2018.
60. He Fu Xu, Changzheng X, Shen. The microrna390/trans-acting short interfering rna3 module mediates lateral root growth under salt stress via the auxin pathway. Plant physiology. 2018.
61. Salopek-Sondi B, Samec D, Snježana Mihaljević Smolko A. Jutta Ludwig-Müller. Influence of stress hormones on the auxin homeostasis in brassica rapa seedlings. Plant Cell Reports. 2013;32(7).
62. Ostrowski M, Ciarkowska A, Jakubowska A. The auxin conjugate indole-3-acetyl-aspartate affects responses to cadmium and salt stress in pisum sativum l. J Plant Physiol. 2015;191:63–72.
63. Chen Kang S, He H, zhai R, Li N, Zhao Q, Liu. A sweetpotato auxin response factor gene (*IbARF5*) is involved in carotenoid biosynthesis and salt and drought tolerance in transgenic *Arabidopsis*. Frontiers in Plant Science. 2018.

Figures

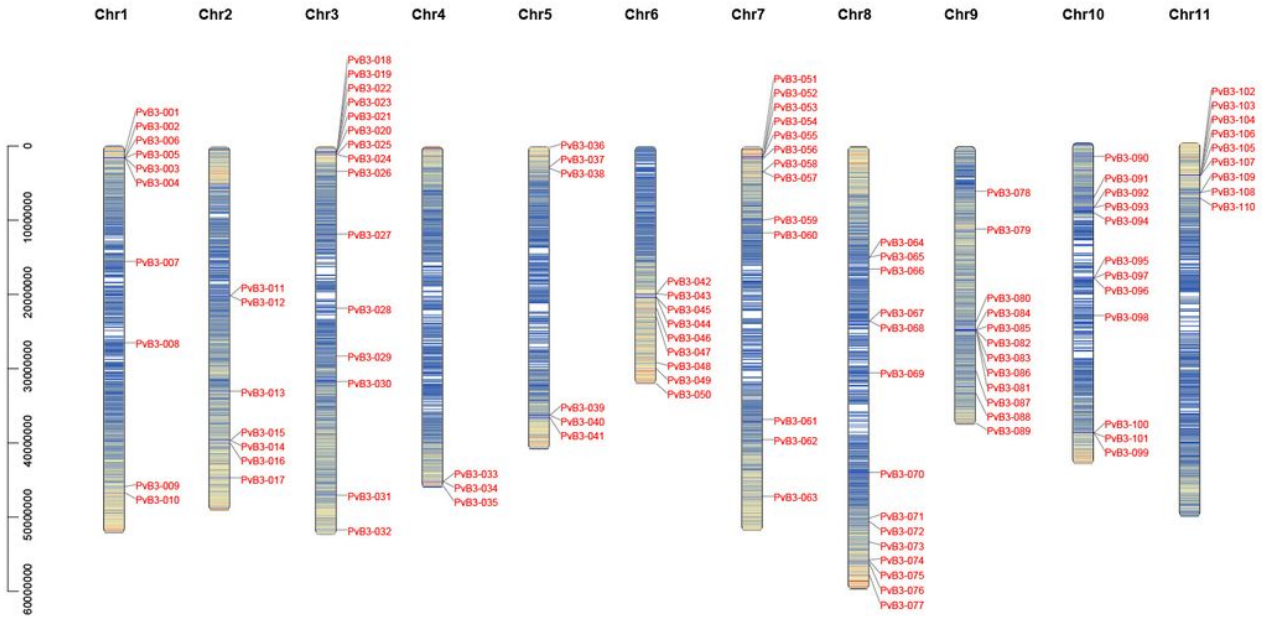

Figure 1

Chromosome localization of PvB3 members. The PvB3 members were localized onto Common bean chromosomes (Chr1-11).

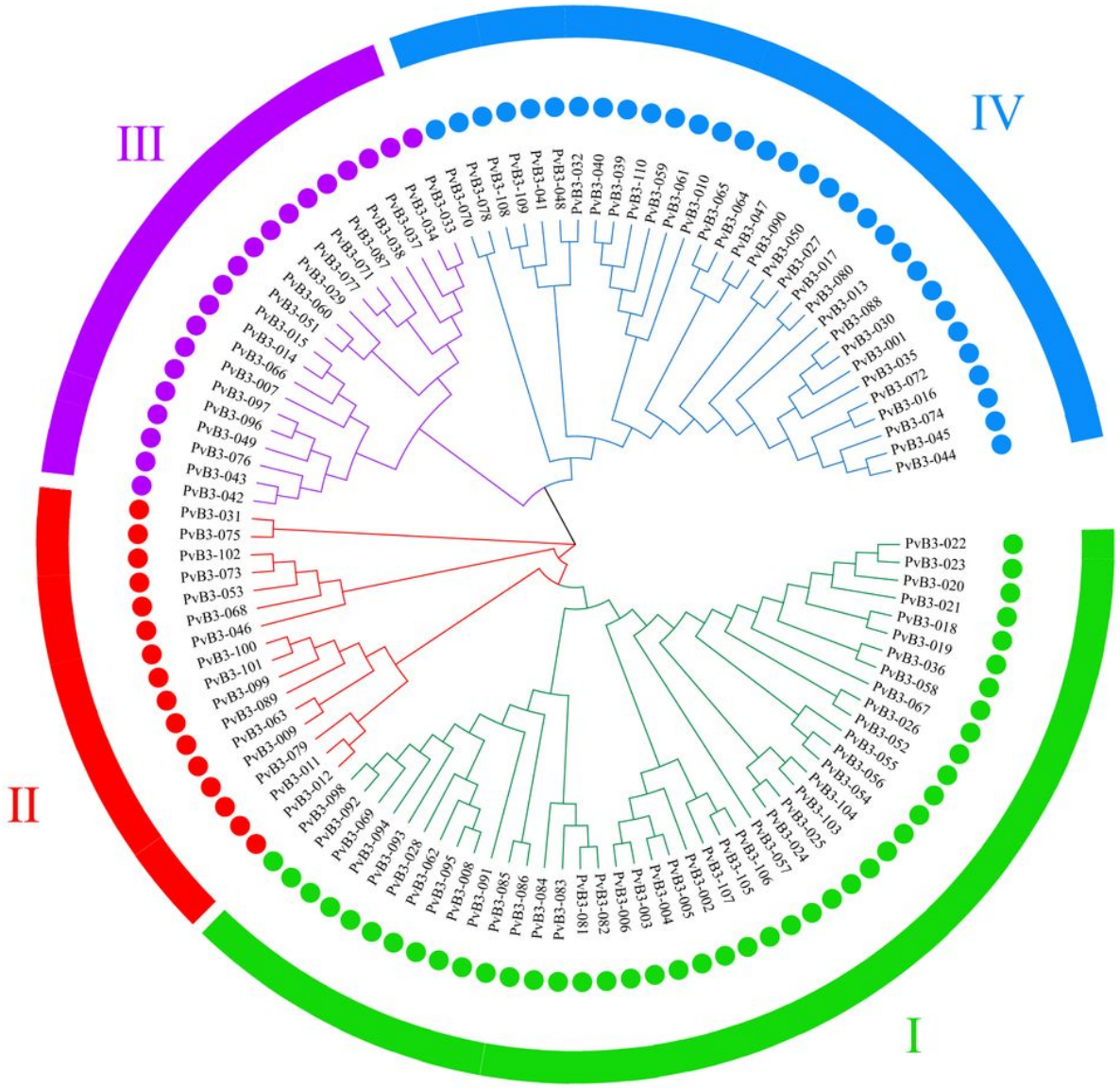

Figure 2

Phylogenetic tree showing the spread of B3 proteins of P. vulgaris.L. The phylogenetic tree was constructed using the ML method WAG+G model as implemented in MEGAX from PvB3 protein sequences alignment. Bootstrap values from 1000 replicates are displayed at each node. The proteins on the tree can be divided into 4 subgroups (□-□□) by different colors.

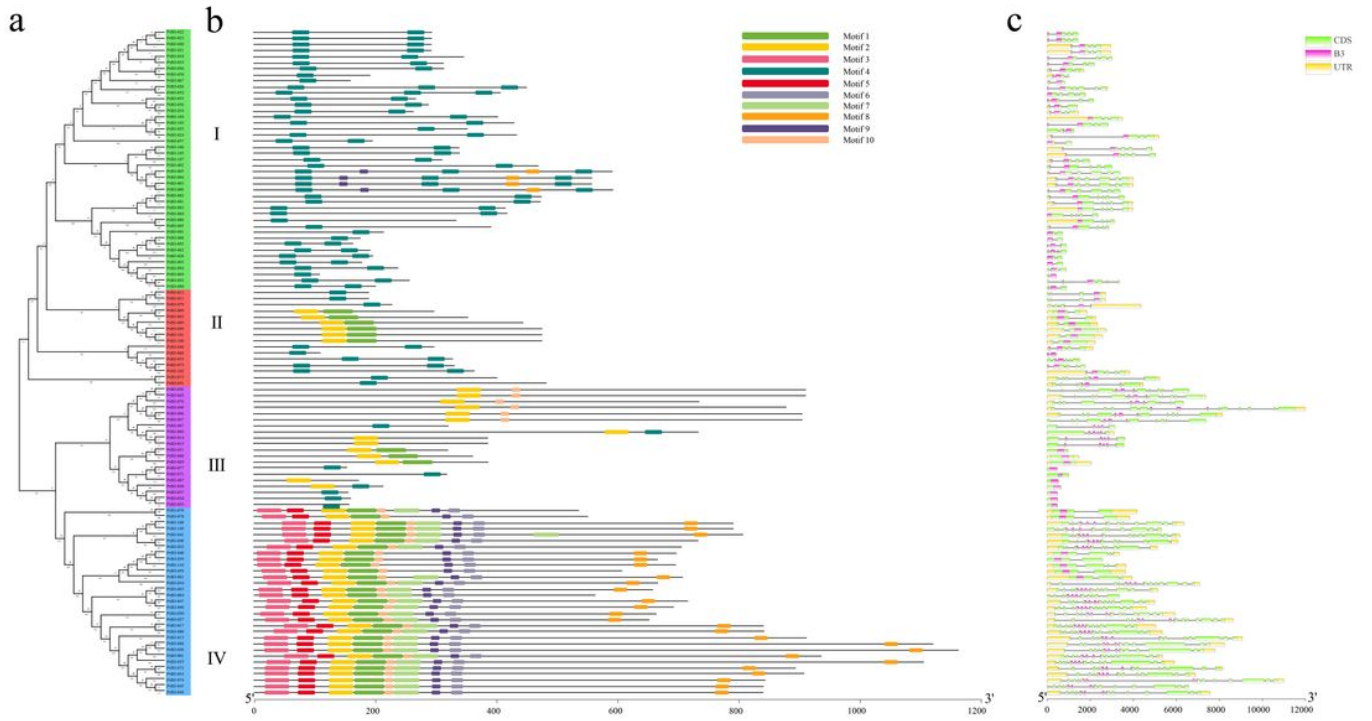

Figure 3

Gene structure and motifs of B3 family. (a) The clustering of PvB3s' protein based on Maximun Likelihoodphy phylogenetic tree. (b) Exon-intron structure of PvB3s, the red boxes are the domain of B3, the green boxes are the UTR region of PvB3s, the yellow boxes are the exon of CDS, and the black line is the intron. (c) Schematic representation of 10 conserved motifs in PvB3s' protein. Motifs of the PvB3s' protein were identified using the MEME online tool. Each motif is indicated by different colored blocks, with respective numbers in the center of the motifs. The number in boxes (1–10) represents motif 1–motif 10. The position and length of each colored box represents the actual motif size.

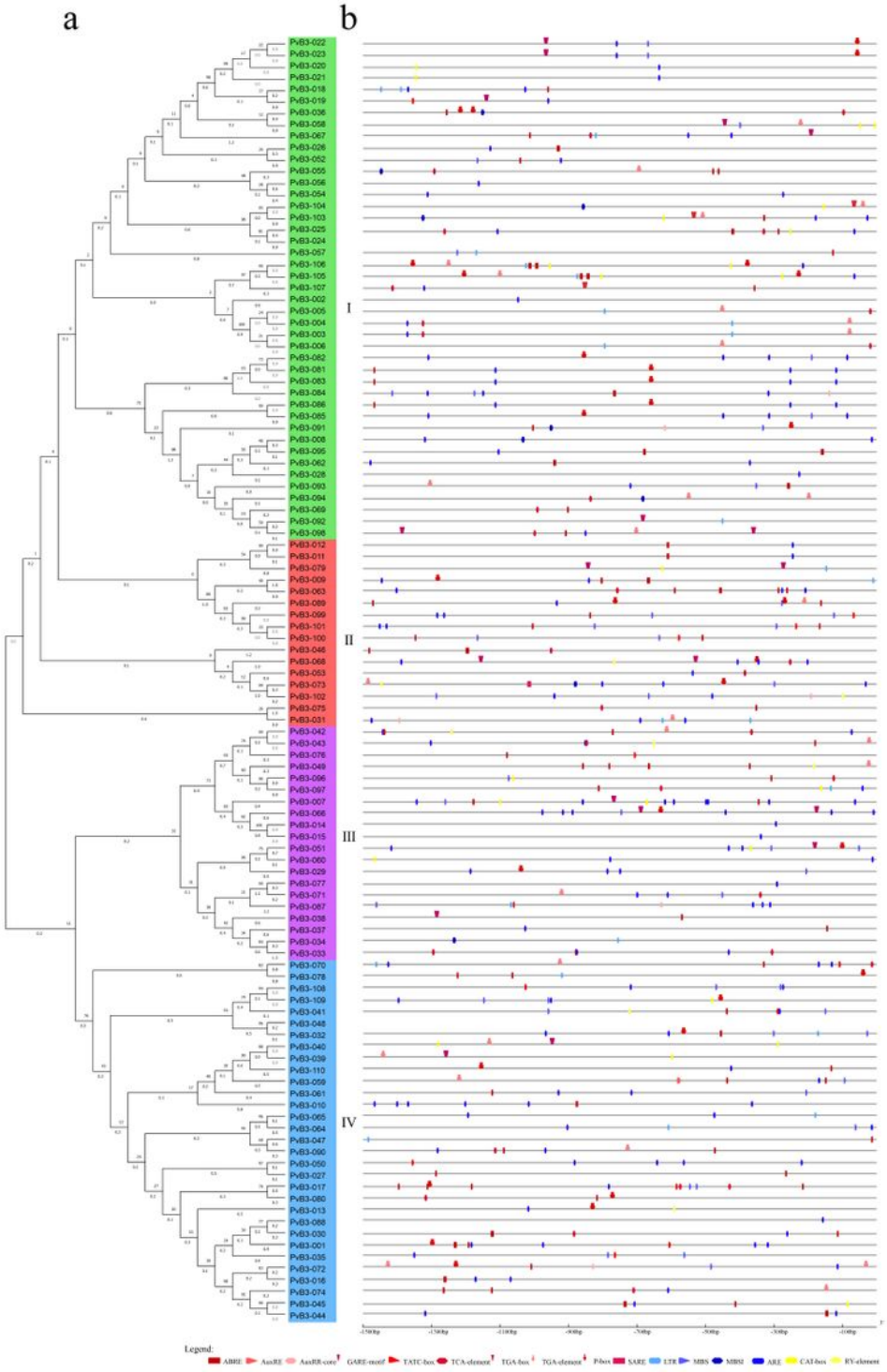

Figure 4

Cis elements analysis of PvB3 family. (a)The clustering of PvB3 proteins according to ML phylogenetic tree. (b) Cis-elements analysis of the promoter regions of common bean B3 genes. Different colored elements show different kinds of cis-regulatory elements. Red are an hormone-related elements, blue are clear and stress-related elements, and yellow ones are element related to seed germination.

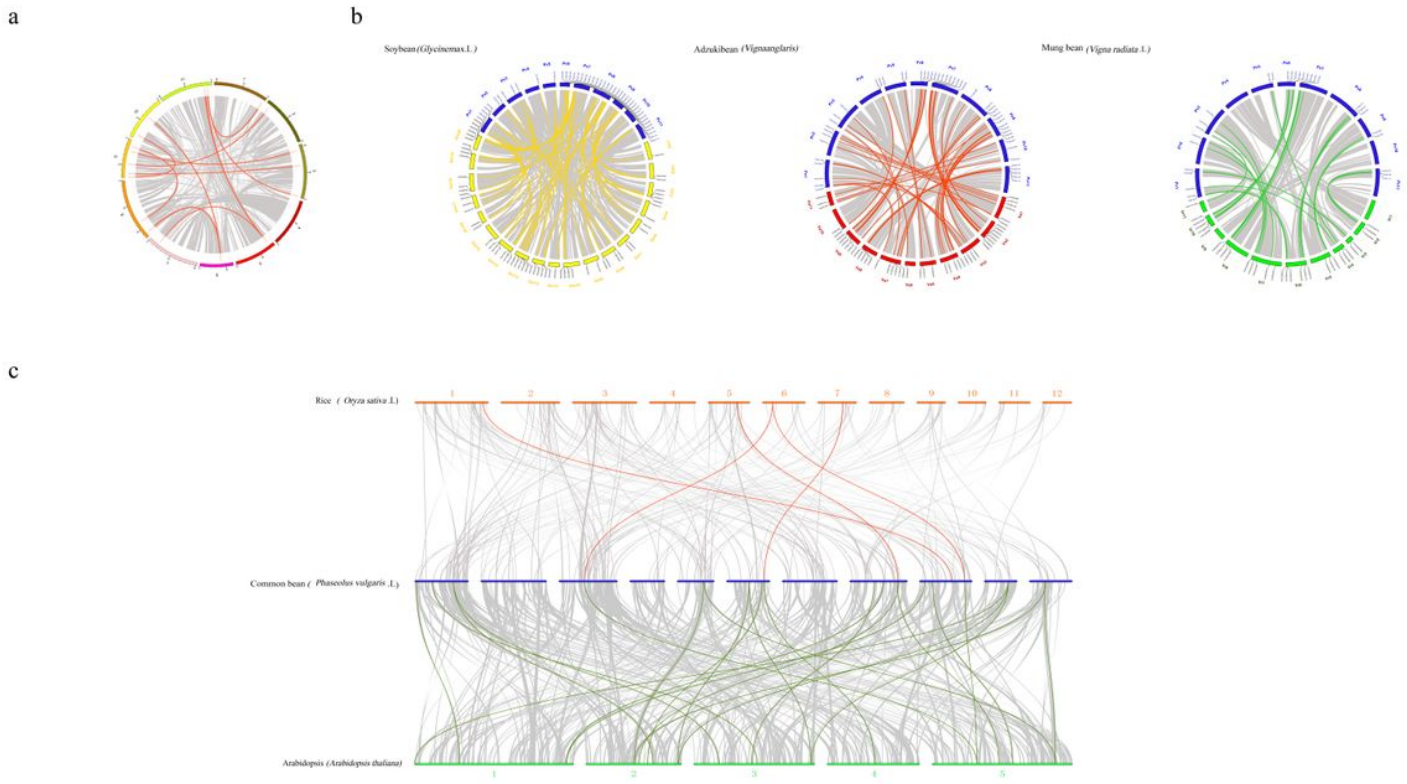

Figure 5

Collinearity analysis and selection pressure analysis of PvB3 family members. (a)Collinearity analysis within the species (P. vulgaris L.) of PvB3s.(b) The collinear relationship between PvB3 members and three legume crops, yellow ones represent soybean, red ones represent adzuki bean(V. angularis), and green ones represent mung bean(V. radiata.), the different colored lines illustrate the collinear relationship between PvB3 members and various legume crops.(c)The collinear relationship between PvB3s and Arabidopsis and Rice, green refer to the chromosomes of Arabidopsis, orange represents the chromosomes of Rice.

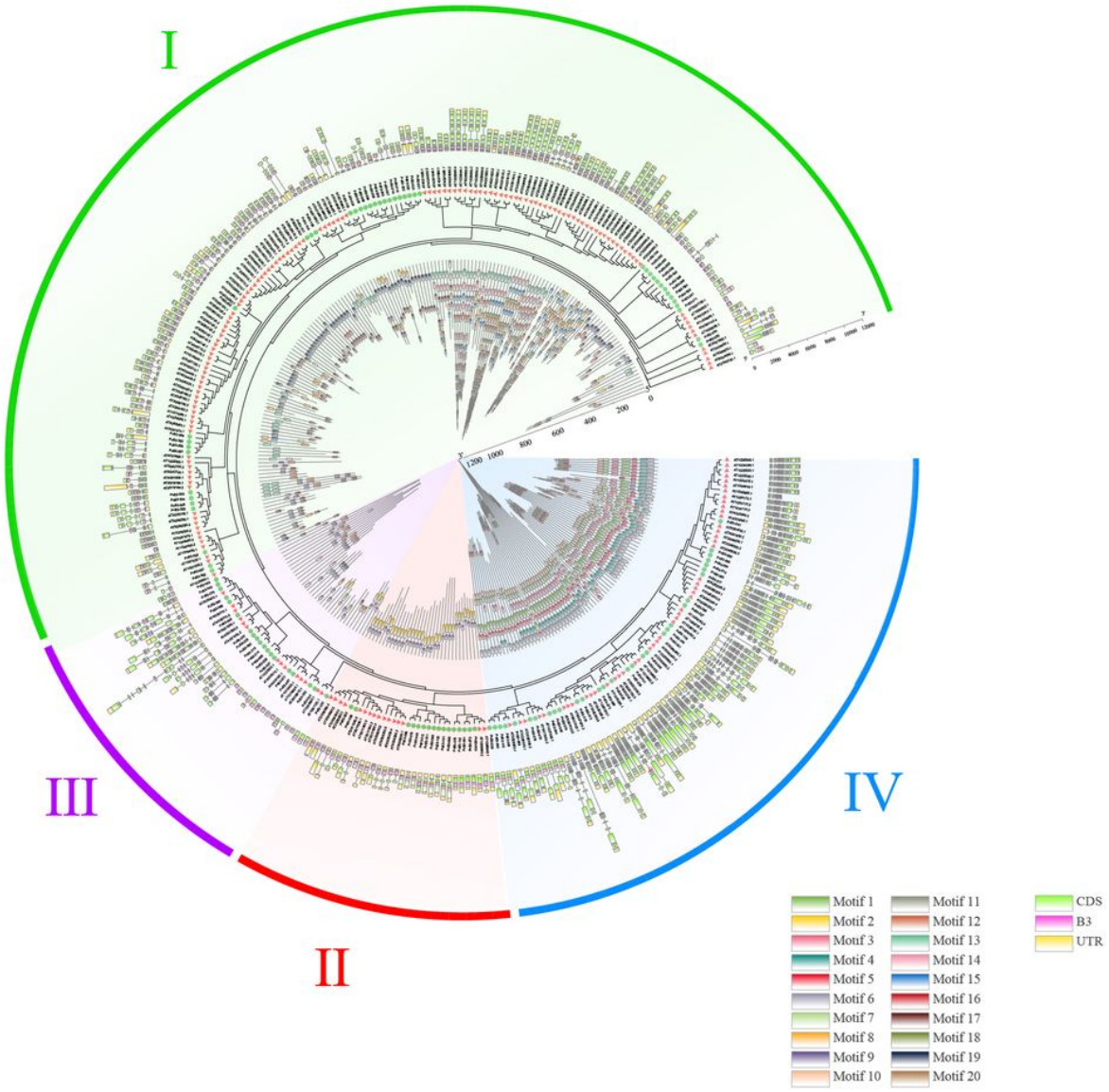

Figure 6

The Arabidopsis members and the common bean members containing the B3 domain, 4 different colors represent 4 subfamilies, the outer column represents the genetic structure of the B3 gene family of Arabidopsis and the common bean, the middle is the B3 protein phylogenetic tree of two species according to ML with WAG+G model, the inner The layer is the motif structure of genes of two species.

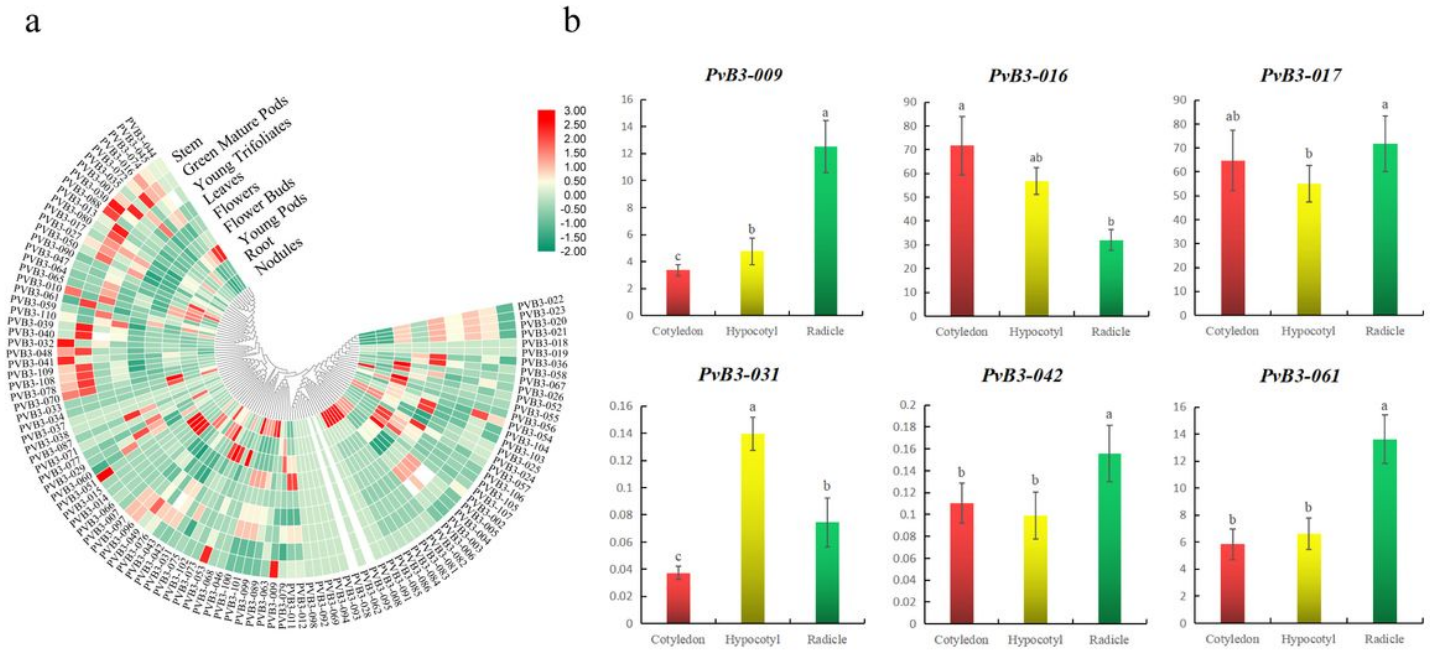

Figure 7

The expression profiles of PvB3s. (a) Cluster analysis of expression patterns of PvB3s in tissue development. PvB3s transcript in various tissues as retrieved from Phytozome database. The data are presented as heat maps. The color scale indicates expression values; red denotes high transcription level, whereas green denotes low transcription level. The circle size is proportional to the level of transcription; a larger circle indicates high-abundance transcripts. (b) The PvB3s' expression during common bean sprout stage in three tissues, including Cotyledon, Hypocotyl, Radicle corresponding PvB3s with specific expression profiles were identified. The Cartoon shows various tissues of common bean in sprout stage: Cotyledon, Hypocotyl, and Radicle. (b) 6 PvB3s were selected randomly, analyzed the expression between different tissues using qRT-PCR method. Letters shown above the bars stands for significant differences (α= 0.05, LSD) among the treatments.

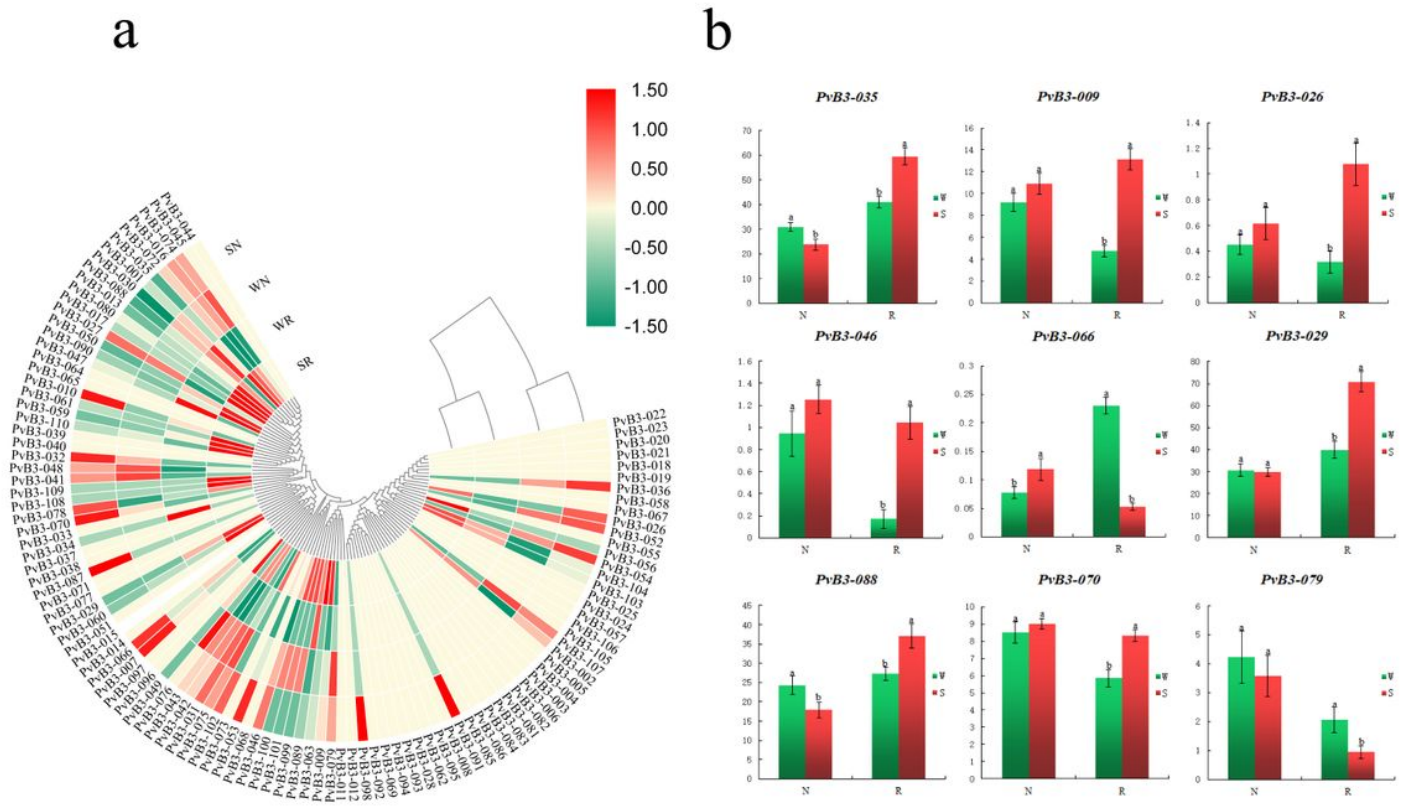

Figure 8

Transcriptome analysis of extremely varieties R and Nunder salt stress. (a) A heat map with clustering is constructed according to PvB3s in different varieties in salt stress. The colored scale varies from blue to red, representing relatively low or high expression. (b) 9 PvB3s were selected and the accuracy of transcriptome was detected by qRT-PCR. Lower case letter(s) on top of the bars show significant differences (α= 0.05, LSD) between the treatments.

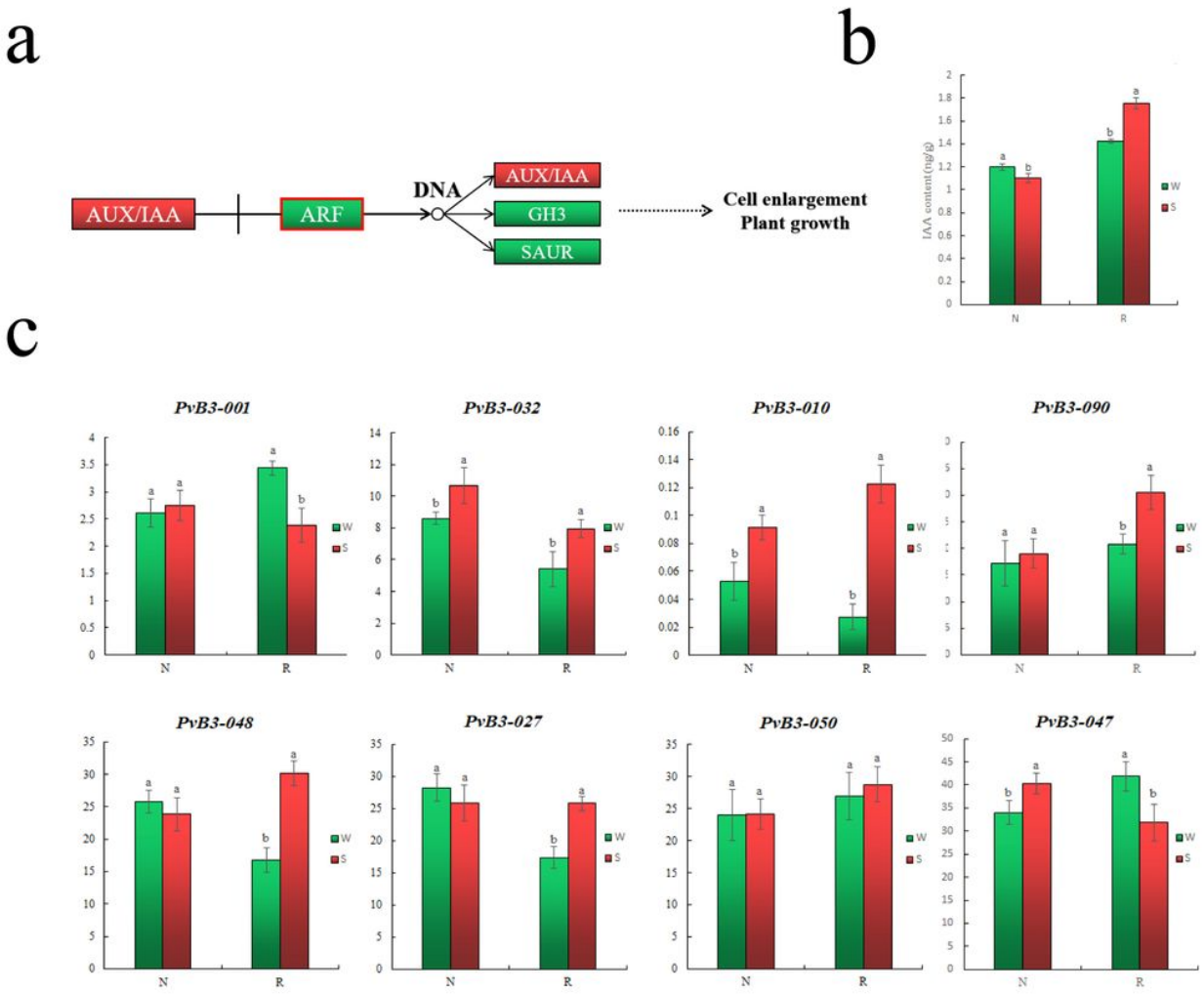

Figure 9

The application of exogenous IAA to the morphological relief of common bean sprouts under salt stress, IAA content response and PvB3s expression changes. (A) Exogenous IAA can alleviate the lateral root number of salt-sensitive variety N under salt stress. (B) Changes in IAA content under three treatments. (C) The expression level of PvB3s under three treatments. Lower case letter(s) above the bars indicate significant differences (α= 0.05, LSD) among the treatments.

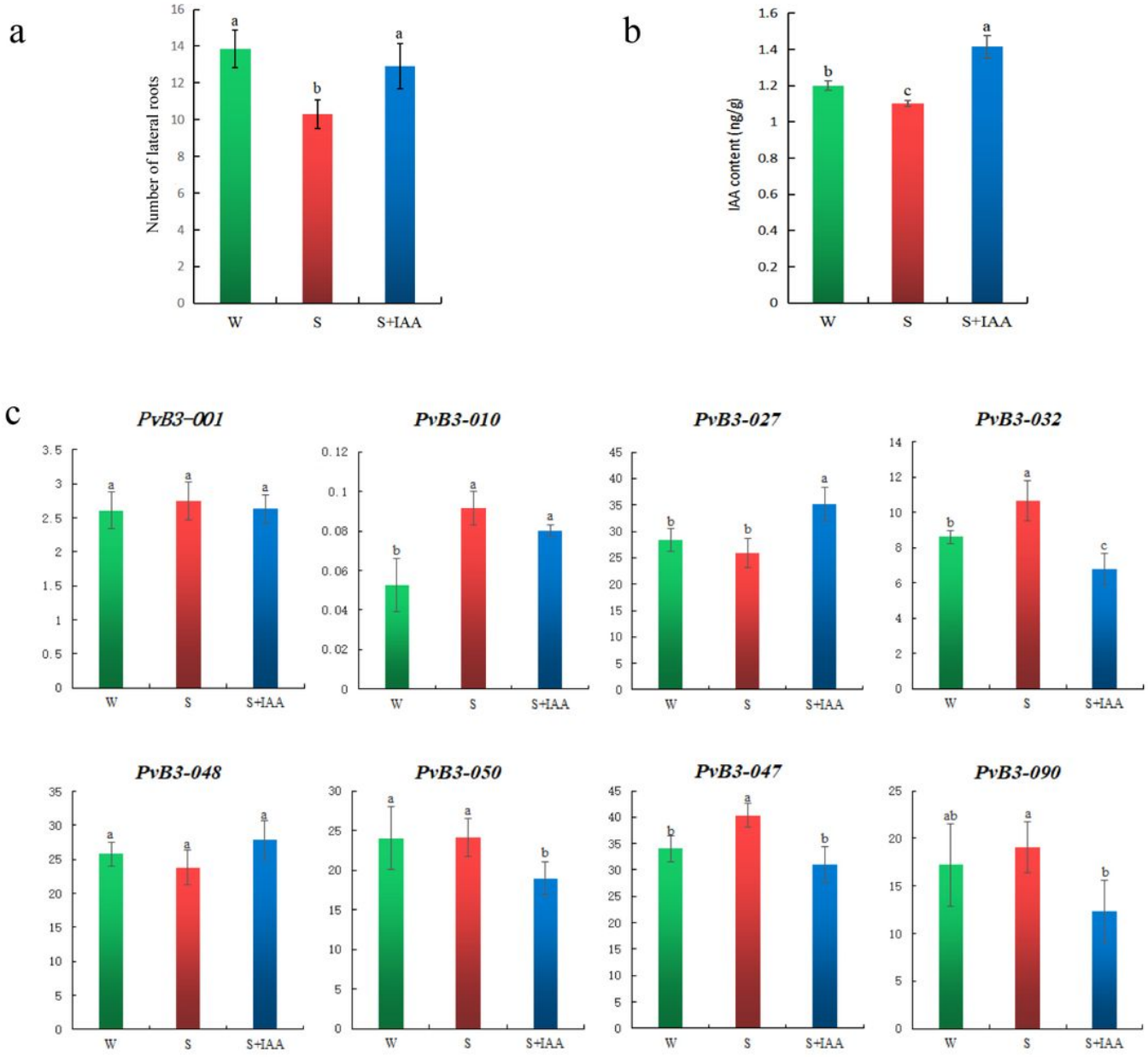

Figure 10

The application of exogenous IAAto the morphological relief of common bean sprouts under salt stress, ABA content response and PvB3s expression changes. (A) Exogenous IAA can alleviate the lateral root number of salt-sensitive variety N under salt stress. (B) Changes in IAA content under three treatments. (C) The expression level of PvB3s under three treatments. Lower case letter(s) above the bars indicate significant differences (α= 0.05, LSD) among the treatments.

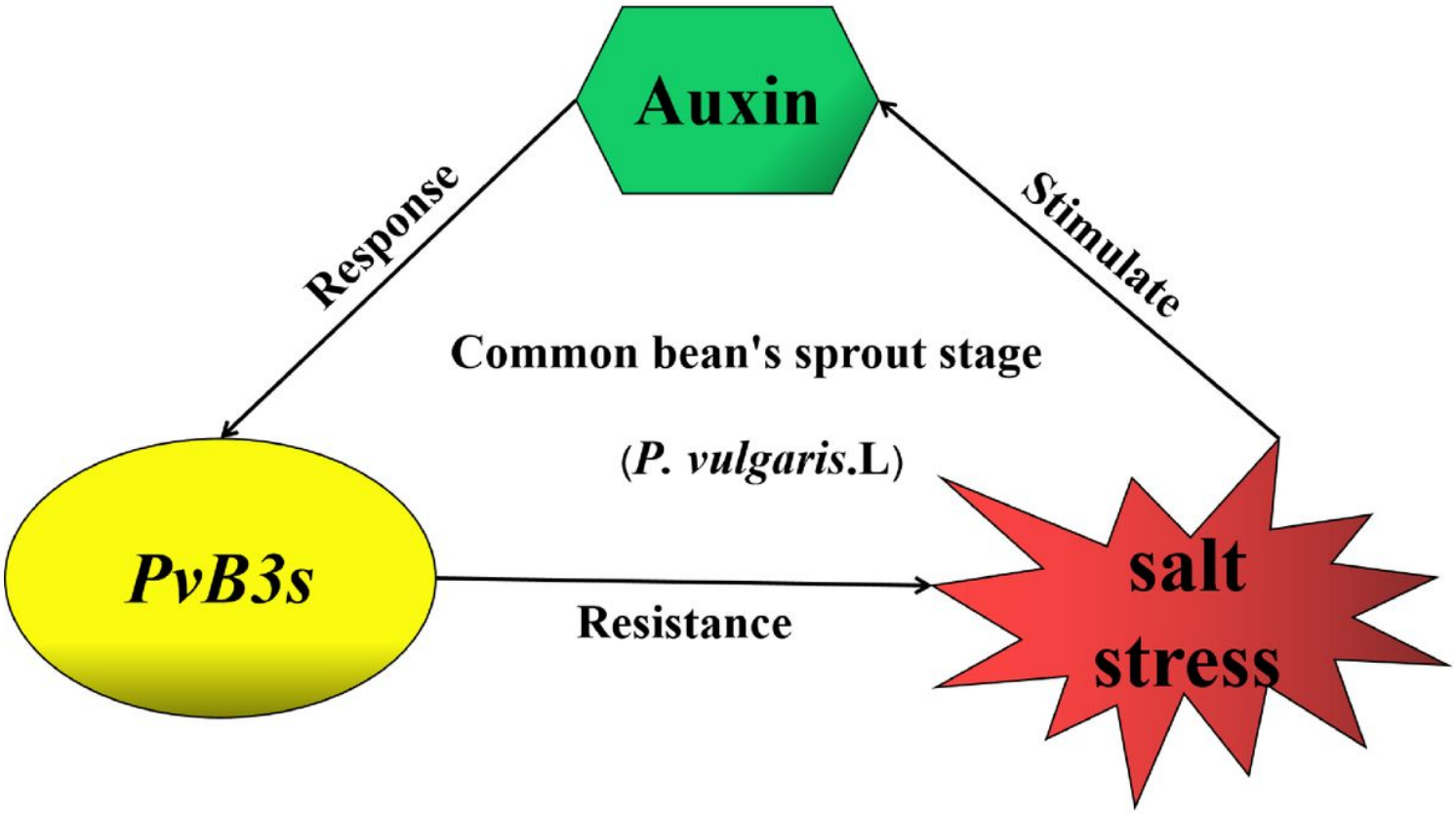

Figure 11

The cartoon shows the relationship between PvB3 and auxin regulation and salt stress.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- B3S3.doc
- B3S2.doc
- B3S1.doc