Genome-wide Systematic Survey and Analysis of the RNA Helicase Gene Family and their Response to Abiotic Stress in Sweetpotato

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Genome-wide Systematic Survey and Analysis of the RNA Helicase Gene Family and their Response to Abiotic Stress in Sweetpotato

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Abstract

RNA helicase is a large family that plays a significant role in plant evolution and in the abiotic stress response. Sweetpotato is one of the majority essential foods in the world, and their yields are often contrived by environmental stresses. Previously, the RNA helicase gene family has not been described in sweetpotato, hence we went a comprehensive genome-wide examination of the sweetpotato RNA helicase family, containing chromosome distribution, promoter elements, and motif compositions. All of 300 RNA helicase genes were divided into three subfamilies, including IbDEAD, IbDEAH and IbDExDH, and they are unevenly scattered across 15 chromosomes of the sweetpotato. Furthermore, tandem replication and segmental replication events played a key role in the amplification of sweetpotato RNA helicase genes. The collinear relationship amidst sweetpotato RNA helicase genes and 8 other related homologous genes in plants was deeply explored, which supplied a reliable basis for studying the development of sweetpotato RNA helicase gene family. RNA-seq examination and qRT-PCR recognition showed that the expression of eight RNA helicase genes was significantly diverse under four abiotic stresses (cold, drought, heat, salt). At the same time, the expression of these RNA helicases in different tissues of the 10 sweetpotato varieties also differed significantly. The promoters of the RNA helicase genes contain a great deal of cis-acting elements related to temperature, hormone and light response. The results indicated that sweetpotato RNA helicase genes played a key role in development or the abiotic stress response.

Keywords: abiotic tress; genome-wide analysis; RNA helicase; sweetpotato

1. Introduction

The RNA helicases are ubiquitous in prokaryotes and eukaryotes, from viruses to humans, catalyzing the unwinding of duplex RNA

dependent on the energy of NTP (Vashisht and Tuteja, 2006). RNA molecules undergo a series of modifications in the process of RNA metabolism, and their own instability is easy to cause RNA metabolic disorders, which make a difference in the development and different abilities to resist abiotic stress of plants (Nawaz and Kang, 2017;Gc et al., 2020).

The helicases are divided into 6 superfamilies, namely SF-1, -2, -3, -4, -5, and -6. Among them, the most representative and largest family is SF2. According to the change of DEAD (Asp-Glu-Ala-Asp) motif, the sweetpotato RNA helicase superfamily is DEAD, DEAH and DExD / H, respectively (Rocak and Linder, 2004;Xu et al., 2013b). Almost all the helicase proteins contain nine conserved motifs (Tuteja and Tuteja, 2004). Each of these nine motifs plays distinct roles, which are essential for helicase enzymatic activities (Tanner and Linder, 2001;Jiang et al., 2020). Structurally RNA helicases are very similar, but their functions are very different. The RNA helicase is present during RNA splicing in RNA metabolism, ribosome formation, and nuclear cytoplasmic transport (de la Cruz et al., 1999;Lorsch, 2002;Sahoo et al., 2022). These genes have many functions in RNA metabolism, among which RNA helicase is associated with growth and development and resistance to stress.

In *Arabidopsis*, DEAD-box LOS4 is able to participate in the process of cryogenic stress, flowering, vernalization, and other processes (Gong et al., 2002;Gong et al., 2005). Among them, in the cold stress response pathway, LOS4-1 and LOS4-2 can regulate the expression of C repeat binding factors and their downstream target genes. LOS RNA helicase plays a key role in target gene output, maturation and reaction to temperature stress. The transcription of STRS1 and STRS2 was inhibited under salt stress. The salt tolerance of mutants strs1 and strs2 was enhanced, and the expression of RD29A, DREB1A and DREB2A was enhanced (Kant et al., 2007). The development of the germ and leaf of the *Arabidopsis* rh7 mutant was seriously delayed under low temperature stress (Liu et al., 2016). AtRH3 plays a role in ribosome formation and seedling growth and development, the growth of rh3 mutants was severely inhibited under salt or cold stress (Gu et al., 2014). Studies have shown that *Arabidopsis* TEBICHI is necessary for regulating cell division and meristem (Inagaki et al., 2006), ISE2 is involved in the function of the plasmodesmata during *Arabidopsis* embryogenesis (Kobayashi et al., 2007;Carlotto et al., 2016). Our previous findings showed that the tolerance of tomato SlDEAD31 was enhanced in salt and drought stress, and the expression of stress genes was increased such as *Cat1*, *Cat2*, *APX2*, and *ER5* (Zhu et al., 2015). The rice SUV3 protein has DNA and RNA helicase and ATPase activities, and *SUV3*

expression can be induced by salt stress (Tuteja et al., 2013;Sahoo et al., 2015). Low temperature and high salt stress can induce the expression of GmRH in soybeans, and GmRH plays a significant in RNA processing (Chung et al., 2009). Tobacco P68 can improve plant growth, photosynthesis, stimulate antioxidant mechanism, and enhance tolerance to salt stress (Tuteja et al., 2014). AvDH1 increased salt tolerance and played an important role in boll number, boll weight and seed yield (Chen et al., 2015). The *Arabidopsis* RCF1 gene plays an integral role in maintaining normal splicing of mRNA precursors, and some cold stress-induced genes were error spliced in the rcf-1 mutant (Guan et al., 2013). Maize DRH1 can interact with the nucleoprotein fiber MA16, which is involved in ribosomal RNA metabolism (Gendra et al., 2004). DEVH-box RNA helicase AtHELPS play a key role in K^+ deprivation in *Arabidopsis thaliana* (Xu et al., 2011).

The RNA helicase has been nominated in many plant variety namely *Arabidopsis thaliana* (Boudet et al., 2001), *Oryza sativa* (Umate et al., 2010), *Ipomoea trifida* (Wan et al., 2020), *Glycine max* (Xu et al., 2013a), *Zea mays* (Xu et al., 2013a), *Gossypium spp* (Chen et al., 2014), soybean (Karthik et al., 2019), *Gossypium raimondii* (Chen et al., 2014) and *Solanum lycopersicum* (Xu et al., 2013b). A total of 32 DEAD were initially identified in *Arabidopsis thaliana* (Aubourg et al., 1999). Then, 113 and 115 RNA helicase genes were identified in *Arabidopsis* and *Oryza sativa* (Umate et al., 2010). Studies have shown that RNA helicase genes in *Arabidopsis*, *Oryza sativa*, *Gossypium spp*, *Gossypium raimondii* and *Zea mays* are divided into three subfamilies, the numeral of genes in apiece subfamily is as follows: DEAD-box (50, 51, 87, 51, and 57 genes), DEAH-box (40, 33, 48, 52, and 31 genes), and DExDH-box (71, 65, 78, 58, and 50 genes). Sweetpotato (*Ipomoea batatas* (L.) Lam.) is an important food source and industrial raw material with high economic value (Katayama et al., 2017). Sweetpotato is a hexaploid with 90 chromosomes, high heterozygosity, and a large number of repetitive sequences (Isobe et al., 2017;Yan et al., 2022), thus hindering gene identification and functional studies. The RNA helicase is a ubiquitous protein that involved in plant growth and abiotic stress. Sweetpotato is susceptible to abiotic stress, which plays a significant in the growth of potato chips (Ramamoorthy et al., 2022). At present, however, genome-wide identification of sweetpotato RNA helicase genes has not been communicated. To improve the yield of sweetpotato, genomic assisted breeding technology can be used to develop new or improved sweetpotato varieties. To explore the biological basis of cold resistance in sweetpotato, it is consequence to recognize differentially expressed genes in response to low temperature stress

and apply them to production. Therefore, the main molecules are the recognition of proteins and enzymes, it is very important for these molecules to control a large number of metabolic pathways by regulating the occurrence and metabolism of RNA. The RNA helicases are concerned in many molecular functions, including tolerance, and regulation of development. Their identification in sweetpotato and improvement of sweetpotato varieties are of great significance and practical value.

Therefore, in order to comprehend the purpose and participation pathway of the RNA helicase genes in sweetpotato, this study aims to conduct genome-wide confirmation of the RNA helicase genes in sweetpotato, and to analyze the molecular mechanism of sweetpotato participation.

2. Materials and methods

2.1 Identification of the RNA helicase genes in sweetpotato genomes

The whole sweetpotato genome sequence was derivative by Ipomoea genome Hub (https://ipomoea-genome.org) (Yang et al., 2017). To identify members of the RNA helicase gene family in *Ipomoea batatas*, we used BLASTP to search all known *Arabidopsis* and rice RNA helicase gene sequences in multiple databases (Altschul et al., 1990). And all the information about the RNA helicase genes in *Arabidopsis* (https://www.arabidopsis.org/) and rice (http://rice.plantbiology.msu.edu/) was downloaded (Xu et al., 2020). Subsequently, all protein were covered and each member of the RNA helicase gene was verified using the Pfam database (http://pfam.xfam.org/), the CD-search (https://www.ncbi.nlm.nih.gov/cdd/Structure/cdd/wrpsb.cgi), and the PROSITE (https://prosite.expasy.org/), and members lacking typical conserved RNA helicase domains were deleted. The sequence information of all sweetpotato RNA helicase proteins can be found in the **Supplementary File. 1**.

2.2 Phylogenetic relationships of RNA helicase proteins in sweetpotato

The RNA helicase sequence was aligned using the Clustal X program. The MUSCLE program was used for multiple sequence alignment to support Clustal X (http://www.clustal.org/) (Edgar, 2004). Phylogenetic trees were constructed employing the maximum likelihood (ML) method (Guindon et al., 2005).

2.3 Protein property and conserved domain of helicase genes in sweetpotato

The physicochemical properties of the RNA helicase proteins were forecasted by the online ExPASy database (http://expasy.org/). Prediction of subcellular sites

(http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) and phosphorylation sites (http://www.cbs.dtu.dk/services/NetPhos/) of the RNA helicase genes. The gene structure was obtained by comparing the sequence of the RNA helicase genes with the genome sequence. The consequence was realized by Tbtools (Chen et al., 2020). The conserved domain was determined using MEME (https://meme-suite.org/meme/tools/meme) (Bailey et al., 2015). Then, the protein interacting networks were ensured by STRING (https://string-db.org/).

2.4Chromosomal location and collinearity analysis of the RNA helicase genes in sweetpotato

Isoelectric points and molecular weights of these proteins are obtained by ExPASy (http://expasy.org/) (Gasteiger et al., 2003). The structural intelligence of these sweetpotato RNA helicase genes was analyzed together with genomic data. To examine the collinearity both RNA helicase gene and other plant genes, The genome sequence information of sweetpotato, *Arabidopsis thaliana* and rice was downloaded and examined. MCScanX was used to create gene duplication and collinearity relationships through failure parameters (Wang et al., 2012), data results are envisaged by TBtools (Krzywinski et al., 2009;Chen et al., 2020). Default parameters were used in all steps.

2.5qRT-PCR ascertain of cold stress

The experimental materials were XuShu 18 roots, different cold stress treatment methods have been described in our previous reports (Xie et al., 2019). Four abiotic stress treatments and four hormone stress treatments were carried out on XuShu 18, and samples were taken after 0h, 1h, 3h, 6h, 12h, 24h, 48h and 72h, respectively. Ten different varieties of sweetpotato were planted, and their young leaves, leaves, stems and roots were taken respectively. RNA of these samples was extracted for subsequent experiments.

The total RNA was excavated using RNA extraction kit (TianGen, Beijing, China), and reverse transcriptions using TransScript® gDNA removal (TransGen, Beijing, China). All the sweetpotato RNA helicase promoter regions were examined by plantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

2.6Statistical Analysis

Statistical reasoning was achieved by Microsoft Excel 2019, Graphpad Prism 5.0 and SPSS statistical. Considering the biological significance, differential gene expression using two times the cut-off value.

3. Results

3.1Identification of RNA helicase family genes in sweetpotato

To recognize the elements of the sweetpotato RNA helicase gene, we used bioinformatics methods to collect details on many sweetpotato RNA helicases. A total of 300 sweetpotato RNA helicase proteins were detected. According to the RNA helicases conserved motifs, 300 sweetpotato RNA helicase genes were divided into subfamilies DEAD-box (53 DEAD genes), DEAH-box (54 DEAH genes) and DExDH-box (193 DExDH genes) subfamilies (**Supplementary File. 1**). The amount of RNA helicase proteins in *Arabidopsis* and rice was 113 and 115, respectively. It is predicted that there are 300 RNA helicase proteins in sweetpotato, which is much more than the number of *Arabidopsis* and rice (Umate et al., 2010). Then, the 300 RNA helicase genes on 15 sweetpotato chromosomes were named from top to bottom as *IbDEAD1 ~ IbDEAD53*, *IbDEAH1 ~ IbDEAH54* and *IbDExDH1 ~ IbDExDH193* (**Supplementary Figure 1**). Subsequently, the physicochemical properties of 300 sweetpotato RNA helicase proteins were examined. The length and relative molecular mass of the RNA helicase vary greatly. The length of DEAD ranges from 323 aa (IbDEAD34) to 1301 aa (IbDEAD2), the relative molecular mass ranges from 3584.09 to 145261.6 Da, and the isoelectric point ranges from 5.02 (IbDEAD40) to 9.87 (IbDEAD37). The length of DEAH is between 206aa (IbDEAH5) and 2904aa (IbDEAH44), the relative molecular mass is between 23377.03 and 323370.3Da, and the isoelectric point is between 5.16 (IbDEAH22) and 9.25 (IbDEAH36). The length of DExDH ranged from 128aa (IbDExDH7) to 2801aa (IbDExDH30), the relative molecular mass ranged from 13904.77 to 306450.01 Da, and the isoelectric point graded from 4.81 (IbDExDH7) to 9.67 (IbDExDH45). The subcellular location showed that most of the RNA helicase proteins were positioned in the nucleus. Furthermore, the potential phosphorylation sites showed that IbDEAD contains 29 (IbDEAD28) to 141 (IbDEAD2) phosphorylation sites, IbDEAH contains 19 (IbDEAH5) to 280 (IbDEAH44) phosphorylation sites, and IbDExDH contains 18 (IbDExDH141) to 362 (IbDExDH30) phosphorylation sites, all of these sweetpotato RNA helicase proteins restrain more Ser sites than the Tyr and Thr sites (**Supplementary Table. 1**).

3.2 Phylogenetic analysis of the RNA helicase family proteins in sweetpotato

The rootless phylogenetic tree of the identified RNA helicase in sweetpotato and the known RNA helicase in *Arabidopsis thaliana* was constructed to study the evolution and classification of RNA helicase in sweetpotato (**Figure 1**). Appertaining to the classification of the RNA helicase in *Arabidopsis*, they were separated into 3 subfamilies: DEAD, DEAH and DExDH. 53 IbDEAD proteins were separated into 9 subgroups (except IbDEAD5), 54

IbDEAH proteins were separated into 9 subgroups (except *IbDEAH36*), and 193 IbDExDH proteins were separated into 13 subgroups (except *IbDExDH19* and *IbDExDH23*), the V subgroup in IbDEAH and the XII in IbDExDH are unique to sweetpotato. RNA helicase proteins are widely and unevenly distributed in different subfamilies. IbDEAD and IbDEAH have nine subgroups, and VIII is the largest subgroup. The sweetpotato RNA helicase genes contained in subgroup VI, VIII and XI of IbDExDH were the least. Interestingly, *IbDEAD5*, *IbDEAH36*, *IbDExDH19* and *IbDExDH23* do not exist in any of the above three subfamilies, which suggesting that they may have other functions.

3.3 Gene structure and motif composition analyses in sweetpotato

To further understand the structural characteristics of the sweetpotato RNA helicase family, we paralleled the composition of conserved motifs and introns / exons on the basis of the phylogenetic tree of sweetpotato RNA helicase (**Figures 2 and 4**). The structures of the IbDEAD, IbDEAH, and IbDExDH subfamily genes are quite different between different subgroups, but the genes in the same side branch are homologous genes, and their genetic structures is also similar, indicating that the exon-intron structure is highly correlated with phylogenetic relationships (**Figures 2 and 4**). The genetic structure of the RNA helicase family members is relatively complex, which the *IbDEAD* and *IbDEAH* gene members contain multiple exons, the *IbDExDH* gene members have only five genes with only one exon (2.6 %), and other genes contain multiple exons. Next, we analyzed the motifs of each member of these RNA helicases on the MEME website, and found that all sweetpotato RNA helicases contain 15 conserved motifs (**Figures 2 and 4**). Motifs 1, 2, 3, 4, 5, 7, 8, 9 and 15 are current in nearly all proteins, and motif 5 contains extremely conserved sequences DEAD, DEAH, and DExD / H. These conserved motifs are the same in sweetpotato RNA helicase, but the domains and amino acid sequences of these maintained motifs are very different (**Figures 2 and 4**). In general, phylogenetic tree examination of data shows that the system development and features and the divergence of genetic structure and sequence distribution are closely related.

3.4 Chromosome localization of the RNA helicase family in sweetpotato

Physical location detection based on GFF3 genome annotation showed that 53 *IbDEAD* genes, 54 *IbDEAH* genes and 193 *IbDExDH* genes were located on all 15 chromosomes. Among them, Chr6 in *IbDEAD* contains the most abundant *IbDEAD* genes, with 7 members. Chr11 in *IbDEAH* contains the most abundant *IbDEAH* genes, with

9 members. However, the *IbDEAD* and *IbDEAH* genes are not distributed in Chr9. Most chromosomes are rich in *IbDExDH* genes, but Chr10 contains only four *IbDExDH* genes (**Figures 5** and **Supplementary Figure 2**). The distribution of the three subfamilies of RNA helicases on 15 chromosomes is quite different. Description of collinearity analysis manifested that there was a group of tandem duplicated *IbDEAH* and two groups of tandem duplicated *IbDExDHs*, which were *IbDEAH2/24*, *IbDExDH114/168* and *IbDExDH162/185*. However, no tandem duplicated gene was found in *IbDEAD* **(Supplementary Table. 7**).

3.5Cis element analysis of the RNA helicase family gene promoters

To explore the possible regulatory mechanism of sweetpotato RNA helicase on abiotic stress and hormones, we scanned the cis-acting elements in the 2kb promoter upriver of the sweetpotato RNA helicase gene through the PlantCare database (**Figures 6 and 8**, **Supplementary Table. 2**).

The conclusions showed that each promoter region of RNA helicase had multiple cis-acting elements connected with stress- and / or hormones. Among others, nearly 90% of RNA helicase promoters contain multiple stress cis-elements, for instance stress response elements (TC-rich repeats), low temperature response elements (LTR), MAJA response elements (TGACG-motif), drought response elements (MBS), etc. These cis-acting elements can be associated with expression. For example, the expression of several RNA helicase genes providing *IbDEAH-32 / -42* and *IbDExDH-36 / -47 / -96* was increased under different stresses. Correspondingly, stress-related repeats of MBS, TC, or LTR cis elements are more numerous in their promoter regions. However, TC-rich repeats, MAJA response elements and LTR elements were found on the promoters of the *IbDExDH-25 / -48* and *IbDEAH53* genes, their expression was not particularly significant under salt, drought, or cold stress, but there was a certain degree of response under high-temperature stress (**Figure 9**). Furthermore, all sweetpotato RNA helicase promoters restrain many hormone elements, such as abscisic acid response element (ABRE), Me-JA response element (CGTCA motif and TGACG motif) or auxin response element (TGA-box) (**Figures 6 and 8**, **Supplementary Table. 2**). Nevertheless, the expression of the RNA helicase genes in dissimilar tissues of 10 different sweetpotato varieties was also different (**Figure. 14**). Most of the RNA helicase genes are communicated in high amounts in sweetpotato stems and root tissues, in particular, the expression levels of *IbDExDH36* and *IbDExDH48* in the roots were lower than those in other tissues. It shows that RNA helicase is associated to plant development. Among

them, the transcription levels of most RNA helicase genes do not respond significantly to hormonal treatment (**Figure 10**). These data indicate that the cis-acting elements of sweetpotato RNA helicase can be concerned in both hormonal and abiotic stresses.

3.6Identification of RNA helicase family cold-response genes in transcriptomes and their expression profiles under multiple abiotic stress and hormonal treatments

Many studies have shown that the RNA helicase have an influence in various abiotic stresses (such as cold, drought, and heat) and development. To determine the potential biological function of the sweetpotato RNA helicase gene under adversity stress, based on our previous RNA-seq data, we first studied the expression of XuShu 18 under cold stress (Xie et al., 2019). The consequence indicated that eight RNA helicase genes were screened out. Subsequently, we further studied the expression patterns of eight genes (*IbDEAH-32 / -42 / -53*, *IbDExDH-25 / -36 / -47 / -48 / -96*) screened by qRT-PCR under salt, drought, heat and cold stresses, and explore a two-fold cut off value (Zhu et al., 2015). The results showed that all selected genes were up-regulated to varying degrees after salt, drought, cold, and heat treatment. Among them, four stresses could up-regulate *IbDEAH32* and *IbDExDH-36 / -47* transcription, two stresses could up-regulate *IbDEAH42* and *IbDExDH-48 / -96* expression, and one stress could up-regulate *IbDExDH25* and *IbDEAH53* transcription. All RNA helicases could be up-regulated under high temperature stress and salt stress. In particular, *IbDEAH32* and *IbDExDH47* had the highest level of induction after cold treatment, about six times, while the level of induction was relatively low in the transcription of other RNA helicases. Except for *IbDExDH48*, the expression of other genes was significantly enhanced under high temperature conditions, which was 2.3 and 19.3-fold that of the control. Under salt stress conditions, the expression of *IbDEAH42* and *IbDExDH-47 / -48* was enhanced, and the expression of other genes was weakly induced by salt stress. In particular, the response of all genes to drought stress was not very strong, which may be affected by other factors (**Figure 9**). Overall, these data suggest that multiple sweetpotato RNA helicase members can take the lead in reaction to abiotic stress.

Moreover, qRT-PCR was acclimated to further detect the transcription profiles of eight RNA helicase genes under distinct hormone treatments, encircling ABA, IAA, GA and ZT. Unexpectedly, most of the RNA helicase genes were down-regulated when we used double as the cut-off value for differential expression. Just the stress hormone ZT could prompt the expression of *IbDExDH-36 / -47 / -48 / -98* and *IbDEAH42* (**Figure 10**). It is reported that the response of the RNA helicase to hormone treatment is not obvious, mainly

related to development and abiotic stress(Camborde et al., 2022;Li et al., 2022b). Overall, these data propose that multiple members of the sweetpotato RNA helicase can be important players in answer to hormone and / or abiotic stresses.

3.7Collinearity analysis of the RNA helicase genes between sweetpotato and other plants

To furthermore explore the origin and evolutionary mechanism of sweetpotato RNA helicase genes, we compared the homology of 300 RNA helicase genes with 8 representative species-related genes. These species include wild diploid relatives of sweetpotato (*Ipomoea trioba* and *Ipomoea trifida*), two model plants (*Arabidopsis thaliana* and *Oryza sativa*), two cruciferous plants (*Brassica rapa* and *Brassica oleracea*) and two Solanaceae plants (*Solanum lycopersicum* and *Capsicum annuum*). Among them, 42 (79.2%) and 43 (81.1%) *IbDEAD* genes were homologous to genes in *Ipomoea trioba* and *Ipomoea trifida*, respectively, accompanied by *Solanum lycopersicum* (8), *Capsicum annuum* (6), *Arabidopsis thaliana* (6), *Brassica rapa* (2), and *Brassica oleracea* (1), but no homologous genes were found both sweetpotato and rice. 39 (72.2%) and 40 (74.1%) *IbDEAH* genes were also homologous to the genes in *Ipomoea trioba* and *Ipomoea trifida*, respectively, accompanied by *Solanum lycopersicum* (8), *Capsicum annuum* (3) and *Arabidopsis thaliana* (3), nevertheless, no similar homologous genes were noted between sweetpotato and *Brassica rapa*, cabbage and rice. Similarly, 89 (46.1%) and 87 (45.1%) *IbDExDH* genes were homologous to the genes in *Ipomoea trioba* and *Ipomoea trifida*, followed by tomato (24), pepper (10), *Arabidopsis* (7), *Brassica rapa* (3), and cabbage (2), however, no homologous genes were found with rice (**Figures 11 and 13**). It should be mentioned that the collinearity of sweetpotato RNA helicase genes between the *Ipomoea trioba* and *Ipomoea trifida* genes more than the extra six varieties, which can be associated to the wild diploid relationship of sweetpotato.

Moreover, the mass of the RNA helicase genes in our sweetpotato are one-to-one gene pairs with sweetpotato diploids, and a few are one sweetpotato gene corresponding to two or three diploid gene pairs. For example, the sweetpotato genes *IbDEAD* (10), *IbDEAH* (2) and *IbDExDH* (19) have a one-to-many relationship in *Ipomoea trioba*, and *IbDEAD* (10), *IbDEAH* (2) and *IbDExDH* (17) sweetpotato genes have a one-to-many relationship in *Ipomoea trifida* (**Supplementary Table. 3**). Interestingly, we found that *IbDEAH* did not find some collinear gene pairs between sweetpotato and *Oryza sativa* / *Brassica rapa* / *Brassica oleracea*, and there was no collinear gene pair between sweetpotato RNA helicase and rice.

3.8Tissue expression patterns of cold-response genes of the

RNA helicase family of different sweet potato cultivars

Objective to preliminarily understand the role of the RNA helicase gene in the development stage of sweetpotato, the expression contours of the RNA helicase genes in diverse tissues of 10 sweetpotato varieties were analyzed by qRT-PCR, such as young leaves, leaves, stems and roots. The expression contours of eight RNA helicase genes were grouped together on their respective heat maps (**Figure 14**). The expression of the RNA helicase in various tissues is different, and the expression in different varieties of sweetpotato is also quite different. This also shows that the RNA helicase is related to growth and development (Tyagi et al., 2020;Liu et al., 2023). Some the RNA helicase genes were highly communicated in stems and roots of sweetpotato, such as *IbDExDH25*, *IbDEAH42*, *IbDExDH47*, and *IbDExDH96*. Similarly, some RNA helicase genes were also highly communicated in sweetpotato leaves, including *IbDExDH36*, *IbDExDH48*, and *IbDEAH53*. This shows that the expression of the RNA helicase in unequal growth stages of sweetpotato is diverse and have an influence in the development stage of plants. This is steady with the results of other plants in RNA helicase. HS3 is located in the DEAD-box RNA helicase 22 in *Arabidopsis* plastids, which is necessary for proper accumulation of plastid gene mRNA throughout seed germination and plant growth (Kanai et al., 2013;Iglesias-Fernández et al., 2019). The RNA helicase can also concern the development of plants under chilling stress. AtRH7, one of the RNA helicases in *Arabidopsis*, is an RNA chaperone involved in cold adaptation (Huang et al., 2016b). The mutation of rh7 affects the abnormal development of flowers in *Arabidopsis* thaliana, and makes the leaves of *Arabidopsis thaliana* smaller in chilling stress (Liu et al., 2016).

4. Discussion

The RNA helicases are ubiquitous in miscellaneous organisms. It is involved in almost all processes of RNA metabolism, such as transcription, mRNA splicing and output, mRNA translation, etc. It involves almost all aspects of organisms (Vashisht and Tuteja, 2006;Sloan and Bohnsack, 2018;Donsbach and Klostermeier, 2021). In the past, the study of the RNA helicase family in plants mainly focuses on dicotyledonous model plant Arabidopsis thaliana and monocotyledonous rice (Nawaz and Kang, 2019b;Takagi et al., 2020;Banu et al., 2023b), these play an essential role in plant growth and stress response (Kim et al., 2008;Li et al., 2022a). Sweetpotato is a significant food crop, which is broadly used in food, feed and industrial raw materials. It is the seventh largest food crop in the world, with the strong ability to adapt to the environment, varieties

of high yield, strong stress resistance (Yu et al., 2020;Banu et al., 2023a). However, only 17 RNA helicase genes have been nominated in *Ipomoea trifida* (Wan et al., 2020). There is no comprehensive study on RNA helicase in sweetpotato. This study systematically identified RNA helicase genes in sweetpotato, which laid a solid basis for further study on the engagement of the RNA helicase in plant abiotic stress and development.

In the present study, we conducted a comprehensive reasoning of the sweetpotato RNA helicase gene family, containing phylogenetic tree, expression profiles of different sweetpotato varieties under common growth circumstances, and expression profiles under diverse abiotic stresses and hormone stresses. Firstly, 300 RNA helicase genes were nominated in sweetpotato genome, which is a very large gene family. A larger family of the RNA helicase gene exists in sweetpotato, suggesting that RNA helicase plays a leading role in regulating environmental responses. According to the family classification of *Arabidopsis* and rice RNA helicases (Xu et al., 2013a), they were divided into three subfamilies, including IbDEAD (53), IbDEAH (54) and IbDExDH (193) (**Supplementary File. 1**). The chromosomal mapping of 300 sweetpotato RNA helicase genes was mapped, and the distribution of the RNA helicase on 15 sweetpotato chromosomes was analyzed. Chromosome localization reasoning proved that sweetpotato RNA helicase gene was scattered on all 15 chromosomes. Among them, IbDEAD was the most distributed on LG4 and LG6 chromosomes (7 genes), and the least distributed on LG2, LG8, LG10, LG12, and LG14 chromosomes (2 genes). IbDEAH was the most distributed on the LG11 chromosome (9 genes), and the least distributed on the LG4, LG5, and LG12 chromosomes (2 genes). IbDExDH was equally scattered on the sweetpotato chromosomes (**Supplementary Figure 1**).

The sweetpotato RNA helicase gene contains different numbers of exons and different lengths of introns (**Figures 2 and 4**). Intriguingly, five genes in the IbDExDH subfamily contain only one exon, while the IbDEAD and IbDEAH subfamilies do not contain this case and contain multiple exons. In fact, the length of RNA helicase family proteins varies greatly. IbDEAD is 323 to 1301 amino acids, IbDEAH is 206 to 2904 amino acids, and IbDExDH is 128 to 2801 amino acids. The highly different amino acid sequences of these sweetpotato RNA helicases induce diverse protein structures and functions in dissimilar developmental and stress-resistant environments. At the same time, the sweetpotato RNA helicase motif was analyzed, the most conserved motif in different species is Asp-Glu-Ala-Asp, which is divided into three subfamilies according to its difference (Tanner and Linder, 2001;Linder and Jankowsky, 2011;Xu

et al., 2023). According to the structural characteristics and phylogenetic analysis of the Motif V region, the determined helicases can be moreover divided into these subfamilies, comprising IbDEAD, IbDEAH, and IbDExDH. Phylogenetic analysis showed that IbDEAD, IbDEAH and IbDExDH RNA helicase proteins can be moreover divided into nine, or thirteen great subgroups (**Figure 1**). The gene structure results showed that the main RNA helicase genes in *Arabidopsis* were uniform to the AtRH family genes (Aubourg et al., 1999), but the position and length of introns were not fixed. However, this is the first genome-wide examination of the RNA helicase genes family in sweetpotato. The distinct subfamilies and gene structures of sweetpotato RNA helicase genes indirectly indicate the different functions in RNA metabolism, stress resistance and growth and development.

In fact, we establish that most RNA helicase gene promoters contain some cis-regulatory elements, such as plant development, abiotic stress, plant hormones, and light response elements(Huang et al., 2016a;Nawaz and Kang, 2019a). It is worth noting that IbDEAD and IbDEAH have more cis-regulatory elements associated to low temperature and light response, and IbDExDH has more cis-acting elements associated with abscisic acid and plant hormones (**Figures 6 and 8**). According to the study that temperature, abscisic acid and jasmonic acid are involved in abiotic stress processes in plants (Bari and Jones, 2009;Nidumukkala et al., 2019;Bharath et al., 2021). Under hormone and abiotic stress treatments, qRT-PCR data showed that RNA helicase mainly responded to abiotic stress (**Figures 9 and 10**). A myriad of cis-regulatory elements in the promoter of sweetpotato RNA helicase genes indicate that these genes given a paramount importance plant stress resistance. Therefore, we found new candidate genes that can regulate the ability of sweetpotato to resist abiotic stress through RNA metabolism. Considering that sweetpotato is the seventh largest food crop in the world, their economic importance in the world and their adaptation to the environment are great challenges. The revelation of stress-related genes laid a solid foundation for promoting the research of molecular basis of sweetpotato resistance and accelerating the breeding of sweetpotato resistant varieties.

The RNA helicase given a paramount importance in regulating plant development and responding to environmental stimuli. The expression profiles of the RNA helicase genes were analyzed in young leaves, leaves, stems, and roots of different varieties of sweetpotato, and their response patterns were analyzed under four abiotic stresses and four hormone stresses (**Figure 14**). The expression of sweetpotato RNA helicase genes in dissimilar tissues

was significantly different, indicating that these genes were related to plant development. Moreover, the RNA helicase genes mainly answer to abiotic stresses in sweetpotato, such as cold, heat and salt, indicating that these genes are related to plant stress resistance. This is consistent with the functional studies of other plant RNA helicases, for instance *Arabidopsis* (Huang et al., 2016b), rice (Xiaomei et al., 2020), tomato (Capel et al., 2020), rapeseed (Zhang et al., 2022b), chrysanthemum (Zhang et al., 2022a), Zea mays (Yang et al., 2023), barley (Ru et al., 2021) and soybean (Wang et al., 2022). We hypothesized that RNA helicase protein can be a functional gene related to sweetpotato growth and development, or a regulatory factor under different environmental constraints. Therefore, this study provides new experimental ideas and clues for the above speculation.

In conclusion, exploring the reception of the RNA helicase gene in sweetpotato can help transgenic research improve the yield and resistance to stress of sweetpotato. The identification, classification and phylogenetic tree construction of sweetpotato RNA helicase genes were explored through bioinformatics, which produced meaningful information for in-depth study of the biological function of sweetpotato RNA helicase gene. These studies also contribute to understanding the molecular basis of several significant agricultural traits in sweetpotato cultivation. However, the exact regulatory mechanism of sweetpotato RNA helicase gene development and stress response are still unclear and needs to be additional examined.

5. Conclusion

In this research, we conducted an exhaustive genome-wide reasoning of the sweetpotato RNA helicase family, containing chromosome distribution, promoter elements, and protein motif analysis. All of 300 RNA helicase genes were detected, containing IbDEAD, IbDEAH and IbDExDH subfamilies. The expression patterns of eight RNA helicase genes in different sweetpotato varieties and their responses to abiotic stress and hormonal stress were analyzed by qRT-PCR. The expression of the RNA helicase genes was significantly distinct in individual tissues of 10 sweetpotato varieties and notably raised under divergent abiotic stresses. The results showed that RNA helicase was complicated in the direction of extension and the resistance to stress of sweetpotato. This study supplies new inspirations into the development and exploration of the RNA helicase gene families.

Contributions

Zongyun Li, Mingku Zhu and Tingting Dong conceived and designed the research. Fangfang Mu, Hao Zheng and Qiaorui Zhao performed the research and analyzed the data. Fangfang Mu wrote the

manuscript. Mingku Zhu, Lei Kai and Zongyun Li helped to revise the manuscript. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors have no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the attachment.

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Figures

Figure 1

Figure 2

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