# Identification and Analysis of R2R3-MYB Genes in Sweet Potato Genome 

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#### Abstract

The plant MYB is a transcription factor family large in number and with important functions. In this study, the MYB family genes were screened and identified via bioinformatic methods from the raw sequence of sweet potato (Ipomoea batatas) genome and the gene structure and function of R2R3-MYB were analyzed. The results showed that there were 88 R2R3-MYB genes with intact R2 and R3 conservative domains, which contained 8 and 9 highly conserved basic amino acids. The results of MEME analysis showed that there were 10 conserved motifs within the I. batatas R2R3-MYB protein sequences. For the I. batatas R2R3-MYB protein sequences, over $80 \%$ contained motif 1 , motif 2 , motif 3 , motif 4 , motif 5 and motif 7 . The R2R3-MYB genes were distributed unevenly across the 15 chromosomes. The number of R2R3-MYB genes in chromosome No. 5 was 15 , which was the largest; the numbers of R2R3-MYB genes in chromosome No. 4 and 13 were both only 2, which was the smallest. Analysis of the sequence alignment showed that there were 6 pairs of interchromosomal duplication and there were 20 pairs of intrachromosomal duplication, 19 of which existed in clusters. The function prediction and categorization via sequence analysis showed that 44 R2R3-MYB genes of the $I$. batatas could be categorized to the 13 subgroups of the $A$. thaliana R2R3-MYB genes, which were involved in the responses to biotic stress and abiotic stress, anthocyanin biosynthesis, anther development, etc. Further analysis showed that 36 R2R3-MYB genes might play important roles in dealing with biotic stress and abiotic stress, 9 of which showed significant up/down-regulation under Fusarium oxysporum f. sp. batatas stress and 27 of which showed significant up/down-regulation under low temperature stress. The domains of I. batatas R2R3-MYB transcription factors were highly conservative, which contained highly conserved motifs within R2 and R3 domains. The phylogenetic tree and transcriptomics data analysis showed that some R2R3-MYB genes might play roles in growth and development, metabolism regulation, biotic stress and abiotic stress, which lent support to $I$. batatas breeding.


Keywords Ipomoea batatas; R2R3-MYB; Transcription factor; Bioinformatics
MYB transcription factor is an important transcription factor that covers a large number of genes that play important roles in plant growth and development. MYB proteins contain highly conserved DNA binding domains, the repetitive numbers of which are taken for categorization into four types, i.e., 1R-MYB, 2R-MYB (R2R3), 3R-MYB (R1R2R3) and 4R-MYB. In plant, the main MYB transcription factor is R2R3 type (Millard et al., 2019), participating in various kinds physiological activities such as the regulations of primary and secondary metabolisms, the biosynthesis of anthocyanin (Zhu et al., 2016), cell differentiation regulation and leaf morphological genesis, cell cycle control and biotic and abiotic stresses (Cominelli and Tonelli, 2009).

Research on the plant MYB transcription factors has been reported in recent years. Fagopyrum tataricum (Tartary buckwheat) FtMYB21 took part in the response to abiotic stresses of drought and salinity (Huang et al., 2015). Astragalus membranaceus (Astragalus) AmMYB44 might be involved in the regulation during abiotic stresses such as drought and low temperature (Li et al., 2019). Dubos et al. (2010) classified the R2R3-MYB gene family of Arabidopsis thaliana (Arabidopsis) into 23 subgroups and the genes were functional annotated.

Sweet potato is an important food crop and serves as a novel energy crop as well. Studies have been conducted at different levels including breeding, cell culture, gene cloning and functional analysis, et al. However, little has been reported on the number, structure and functional analysis of MYB transcription factors in the sweet potato genome. Based on the genome data of sweet potato cultivar Taizhong 6, which was presented jointly by Shanghai Chenshan Plant Science Research Center, Institute of Plant Physiology and Ecology, Germany Max Planck Institute for Molecular Genetics and Max Planck Institute of Molecular Plant Physiology, we utilized bioinformatic methods to predict the sequences of R2R3-MYB and performed analysis on the gene structure, conservative domain, chromosomal localization and phylogenetic tree. Further, the differential expressions of R2R3-MYB genes under Fusarium oxysporum f. sp. batatas (Fob) stress and under low temperature stress were analyzed. This study lent support to the investigation of response mechanism of sweet potato to biotic and abiotic stresses and provided scientific reference for resistance breeding.

## 1 Results and Analysis

### 1.1 Identification of R2R3-MYB genes in I. batatas genome

The snap program is applied to predict the CDS within the $I$. batatas genome sequence and 178458 CDS sequences were obtained (Huang et al., 2020) (Figure 1). With the MYB_DNA-binding HMM model, the hmmsearch program is used to search the whole protein sequences of $I$. batatas genome and 270 sequences with MYB domains were picked up. The sequences with high specificity ( $E$-value $\leq 1 e-30$ ) were selected to rebuild the HMM model specific to I. batatas for searching the whole protein sequences of I. batatas genome. The found 181 sequences were then verified with Interproscan. The 56 R2R3-MYB protein sequences after Interproscan verification were used to build the database of the R2R3-MYB transcription factors of the I. batatas. The database was then aligned to the whole protein sequences of I. batatas genome and 186 sequences ( $\mathrm{E}-\mathrm{value} \leq 1 \mathrm{e}-5$ ) were returned. The sequences were merged and sorted and 186 sequences containing MYB domains were obtained. According to the number of MYB domains within the sequence, $442 \mathrm{R}-\mathrm{MYB}$ transcription factors with intact R2R3 domains were screened. For the remaining 146 sequences of 1R MYB or with incomplete R2/R3 domains, the location information within the chromosome corresponding to the ZFF file was used to define the interval within the chromosome for a new search of the CDS. The consecutive sequences with an overlapped interval were considered one single gene and thus combined, and 44 2R-MYB transcription factors with intact R2R3 domains were supplemented. After sequence classification and count, the sequences without MYB domain or with severelyincomplete MYB domain were excluded and 88 R2R3-MYB transcription factors were obtained eventually.


Figure 1 Screening the R2R3-MYB genes of I. batatas

### 1.2 Conservative domain analysis of R2R3-MYB family

Multiple sequence alignment was carried out for the 88 R2R3-MYB transcription factor sequences, with reference to the amino acid sequence information of the $A$. thaliana R2 and R3 domains for defining the $I$. batatas R2 and R3 conservative domains (Figure 2). The amino acid sequence of the most likely I. batatas R2 domain was KGPWTPEEDEKLINYI+KHGEGNWRALPKKAGLQRCGKSCRLRW+NYLRPDI (the underlines indicated the highly conserved amino acids), with amino acid sequence length 52 and number of highly conserved tryptophan residues 3. The amino acid sequence of the most likely I. batatas R3 domain is KRGNFSPEEEELIIRLHALLGNRWSAIAARLPGRTDNEIKNYWNTHLKKKI, with amino acid sequence length 51 and number of highly conserved tryptophan residues 2 . In addition, we found that there were highly conserved basic amino acids (arginine R, lysine K and histidine H ) in the R2 and R3 domains, with 8 in R2 domain and 9 in R3 domain (Figure 2).


Figure 2 Conservation analysis of I. batatas R2R3-MYB transcription factors

### 1.3 Domain and motif analysis of the $I$. batatas MYB genes

The conservative motifs of the $I$. batatas R2R3-MYB protein sequences were analyzed with MEME program. Ten conservative motifs were obtained and labelled corresponding to the locations in the phylogenetic tree (Figure 3). The results showed that among the domains of the 88 R2R3-MYB transcription factors, the pattern of the motif arrangement was motif 6 -motif 3-motif 7-motif 1-motif 5-motif 4-motif 2-motif 8-motif 9 -motif 10 . Of these motifs, the motif 10 was the least in number and existed in only 6 sequences that belonged to

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the same branch of the phylogenetic tree. Also, the motif 9 was found only in 9 sequences that fell into the same branch of the phylogenetic tree.


Figure 3 Motif distribution of I. batatas R2R3-MYB transcription factors

The amino acid sequences of the 10 motifs were aligned to the amino acid sequences of the R2 and R3 domains (Table 1). The results showed that motif 1 and motif 3 belonged to R 2 domain and motif 2, motif 4 , motif 5 and motif 7 resided in R3 domain. All of the 6 motifs were distributed among over $80 \%$ MYB protein sequences, suggesting a high domain conservation for the identified I. batatas R2R3-MYB transcription factors. Besides, the motif 9 existed in only 9 sequences that were in the same branch of the phylogenetic tree, similar to the situation of motif 10 , which were only found in 6 sequences that stayed in the same branch of the phylogenetic tree, thus the motif information could serve as the reference for classifying the subgroups of R2R3-MYB transcription factors to a certain extent.

Table 1 The conserved motifs of I. batatas R2R3-MYB transcription factors and their corresponding domains

| Name | Motif | E-value | Width | Predicted domain |
| :--- | :--- | :--- | :--- | :--- | :--- |
| motif 1 | 年 |  |  |  |

### 1.4 Chromosomal localization and duplication analysis of the R2R3-MYB transcription factors

The location of the 88 I . batatas R2R3-MYB sequences within the chromosomes were shown in Figure 4. It could be seen that the R2R3-MYB transcription factors were distributed among the 15 chromosomes in an uneven style. The No. 5 chromosome possessed the largest number of R2R3-MYB transcription factors, which was 15. The number of R2R3-MYB transcription factors in No. 12 chromosome was 10 , while those in No. 4 and No. 13 chromosomes were the least, which were both 2 .

Generally, gene duplication within the genome occurred during the evolution process of plants. The judgement of whether or not two genes had a duplication relationship relied on two conditions (Vatansever et al., 2016), the coverage of the aligned area exceeded $75 \%$ of the sequence length of the longer gene, and the similarity between the two aligned sequences should be no less than $75 \%$. We conducted pair-wise alignment for the DNA sequences of 88 R2R3-MYB genes. The pairs with over $75 \%$ similarity were verified with needle program (Rice et al., 2000). The pairs that satisfied the two conditions were regarded as showing potential duplication relationships, which were linked with lines in Figure 4 (Blue lines represent interchromosomal duplication relationships and orange lines represent intra-chromosomal duplication relations-
hips). The results showed that there were both intra-chromosomal duplication relationships and interchromosomal duplication relationships among the $I$. batatas R2R3-MYB genes. The number of gene pairs with potential inter-chromosomal duplication relationship was 6 and that with potential intra-chromosomal duplication relationship was 20 . Furthermore, the gene pairs of the potential intra-chromosomal duplication relationship were arrange in clusters, such as CM008334.1-snap. 11416 and CM008334.1-snap. 11444 (in No. 4 chromosome), CM008338.1-snap. 2208 and CM008338.1-snap. 2213 (in No. 8 chromosome), and CM00-8342.1-snap. 9594 and CM008342.1-snap. 9602 (No. 12 chromosome). Each two of the four genes that were clustered in No. 8 chromosome, i.e., CM008336.1-snap.4536, CM008336.1-snap.4552, CM008336.1-snap. 4639 and CM008336.1-snap.4645, showed potential duplication relationships.


Figure 4 Chromosomal localization and duplication relationship of the R2R3-MYB genes in I. batatas
Note: Blue lines: Inter-chromosomal duplication relationship; Orange lines: Intra-chromosomal duplication relationship

### 1.5 Phylogenetic analysis of the R2R3-MYB gene families of I. batatas and A. thaliana

The R2R3-MYB protein sequences of the $I$. batatas were used to build the phylogenetic tree together with those of the A. thaliana (Figure 5) and the categorization and functional prediction of the I. batatas R2R3MYB sequences were performed in the light of functionally annotated subgroups of A. thaliana R2R3-MYB genes. It has been demonstrated that the A. thaliana R2R3-MYB family could be classified into 23 subgroups (Dubos et al., 2010). Each subgroup of the A. thaliana R2R3-MYB family was marked with one color and it was found that 44 of the I. batatas R2R3-MYB transcription factor family were listed to the 13 subgroups of the A. thaliana R2R3-MYB family including S1 (response to biotic and abiotic stresses), S4 (control of
metabolic pathway), S6 (control of anthocyanin biosynthesis), S9 (control of early inflorescence development and seed germination), S13 (influencing lignin deposition, mucilage production and stomatal aperture), S14 (partially redundant regulators of axillary meristem formation and root development), S18 (involved in anther/pollen development), S19 (control of anther development), S20 (related to stress responses), S21 (control of axillary meristem formation), S22 (modulating auxin, implicated in stress responses), S24 (lateral root development and fatty acid synthesis) (Gibbs et al., 2014; To et al., 2020) and S25 (play roles in embryogenesis). Moreover, in accordance with the motifs shown in Fig. 3, the S9 subgroup and S22 subgroup both had a unique motif, which were motif 9 for S9 subgroup and motif 10 for subgroup S22.


Figure 5 Phylogenetic tree of R2R3-MYB transcription factors in I. batatas and A. thaliana

### 1.6 Differential expression analysis of $I$. batatas R2R3-MYB genes under Fob stress and under low temperature stress

Fusarium wilt is an important fungal disease of sweet potato during plantation and the main pathogen is the $I$. batatas Fob. Previously, we isolated the strain F04 and strain F07 of the I. batatas Fob, which were used to infect the highly resistant cultivar JS57 and the highly susceptible cultivar XZH. The stems of the seedlings after 24 h of infection were used for transcriptome sequencing. A total of 101988 unigenes were obtained and 62605 of them were functionally annotated (Lin et al., 2017). At that time, the genome sequence of $I$. batatas
has not been reported yet, thus it was not convenient for us to conduct in-depth analysis on the transcription factors including MYB.

In this study, we plotted the clustering chart of the expression pattern for the identified R2R3-MYB genes by taking advantage of the transcriptomics sequencing data of $I$. batatas under Fob infection and analyzed the expression variations of these genes. The results showed that the expression variations of the 88 R2R3-MYB genes were significantly different with regard to the three treatments. Compared to the control, the JS57_F04 group and JS57_F07 group showed no apparent discrepancy, while there were 9 genes displaying significant differential expression for the XZH_F07 group (Figure 6A), with 3 down-regulated (green) and 6 upregulated (red). Among the 9 genes, except the down-regulated CM008335.1-snap. 6249 and the up-regulated CM008342.1-snap. 6002 that were not categorized into the A. thaliana subgroups, all other 7 genes were included in the A. thaliana subgroups. The down-regulated CM008337.1-snap. 1355 and CM008339.1snap. 5339 were in S24 and S21, respectively. CM008333.1-snap. 5577 and CM008339.1-snap. 11594 were in S20. CM008342.1-snap. 10114 was in S4. CM008345.1-snap. 2167 and CM008345.1-snap. 2380 were in S22 (Figure 5 and Figure 6A). The R2R3-MYB transcription factors in these groups were relevant to the growth, development and stress response, etc.

Ji et al. (2020) stored the tuberous roots of cultivars Xushu15-1 and Xushu15-4 at ${ }^{\circ} \mathrm{C}$ for 0,2 and 6 weeks respectively and conducted transcriptomics analysis. A total of 27636 unigenes were obtained, 525 of which were identified to be differentially expressed under low temperature stress. In this study, we exploited the transcriptomics data to analyze the differential expression of the R2R3-MYB genes (Figure 6B) and differential expressions were observed in 27 of the R2R3-MYB genes, 10 of which varied in 4 treatment groups. Under low temperature stress, most of the differentially expressed R2R3-MYB genes were down-regulated, however, the magnitudes of change for the up-regulated genes were greater than those of the down-regulated genes. It could be seen that several R2R3-MYB transcription factors increased expression under low temperature stress, such as CM008333.1-snap. 5577 and CM008335.1-snap.3524. The expression variation of CM008335.1-snap. 3524 in Xushu15-1 was greater than in Xushu15-4, whereas the expression was down-regulated in Xushu15-1 for 6 weeks compared to that for 2 weeks. Additionally, the expression of CM008336.1-snap. 12430 was significantly down-regulated under low temperature stress, which was taken in A. thaliana S 4 subgroup that was involved in the regulation of plant metabolic pathways (Figure 5).


Figure 6 Differential expression of R2R3-MYB genes of $I$. batatas under Fob stress and cold stress Note: A: Differential expression of R2R3-MYB genes of I. batatas under Fob stress; B: Differential expression of R2R3-MYB genes of I. batatas under cold stress

## 2 Discussion

MYB gene family is the largest transcription factor family in plant and is crucial for plant growth and development. In this study, the bioinformatic methods were applied to identify and obtain the 88 R2R3-MYB protein sequences in sweet potato. This number is relative less compared with those in other plants such as $A$. thaliana (Dubos et al., 2010) (126), Solanum tuberosum L. (Li et al., 2020) (111) and Ananascomosus (L.) Merr (Chen et al., 2019) (103). Sequence alignment of the 88 R2R3-MYB proteins of the $I$. batatas indicated that the R2 and R3 domains were highly conserved and the amino acid sequences of the R2 and R3 domains were very similar to those of the R2 and R3 domains in other plants.

In the present study, the classification of R2R3-MYB transcription factors of $A$. thaliana was used as a reference for the 88 R2R3-MYB transcription factors of $I$. batatas to build the phylogenetic tree together and yield the subgroup information for the $I$. batatas R2R3-MYB sequences. As for the R2R3-MYB transcription factors in the S9 subgroup of A. thaliana, AtMYB16 controlled the shape of petal epidermal cells; AtMYB17 regulated the early development of inflorescence and the germination of seeds; AtMYB106 was a negative regulator of trichome branching. There were 9 R2R3-MYB transcription factors of $I$. batatas enlisted to the S9 subgroup. These 9 I. batatas R2R3-MYB transcription factors all contained the motif 9, which was not found in other I. batatas R2R3-MYB sequences. Furthermore, the motif 10 was seen in only 6 I. batatas R2R3-MYB sequences, i.e., CM008345.1-snap.2167, CM008345.1-snap.2380, CM008345.1-snap.2514, CM008331.1-snap.10375, CM008332.1-snap. 5974 and CM008332.1-snap.13111, which belonged to the S22 subgroup that were pertinent to stress responses.

In this study, we made use of the transcriptomics sequencing data to verify and analyze the R2R3-MYB transcription factors of $I$. batatas and found that of the 88 R2R3-MYB genes, 9 were differentially expressed after Fob infection and 27 were differentially expressed under low temperature stress. Lin et al. (2017) reported that the digital gene expression (DGE) of four genes, i.e., c51550-g2, c52297-g1, c54420-g2 and $c 56184-g l$ were up-regulated by $1.62,1.40,1.71$ and 3.10 folds, respectively. The result of the qRT-PCR verification showed that the four genes were up-regulated by $2.30,4.88,3.85$ and 2.12 folds, which was consistent with the DGE data, suggesting the viability of the sequencing data and DGE data. Ji et al. (2020) selected 8 genes of the DGE results randomly and 4 potential candidate genes that might increase cold resistance for qRT-PCR verification and the results were in line with the expression patterns of these genes as revealed by DGE, indicating the reliability of the sequencing data. We reused these verified sequencing data for the verification and analysis of R2R3-MYB transcription factors and acquired useful results (Figure 6).

With respect to the R2R3-MYB transcription factors identified in this study, the number of differentially expressed genes varied considerably between under the Fob stress and under the low temperature stress. It might be that the infection of Fob into I. batatas was intrusive and some of the host pathways were likely to be blocked so that the number of response genes was less. In contrast, the $I$. batatas was capable of responding to low temperature stress at levels of tissue, cell and molecule, etc. The nonintrusive and systematic stress was less than Fob stress in terms of specificity, thus there were more R2R3-MYB genes in response to low temperature stress. Further, most of the response $I$. batatas R2R3-MYB genes were down-regulated, which was likely to be related to the metabolism down-regulation of $I$. batatas at low temperature. Nonetheless, there were a small number of up-regulated genes such as CM008333.1-snap. 5577 and CM008335.1-snap. 3524 (Fig. 6B), which might cope with low temperature stress. The number of up/down regulated R2R3-MYB transcription factors under Fob stress was relatively small, nevertheless, several genes showed comparative large magnitude of up/down regulation, such as CM008339.1-snap. 5339 (with log2FoldChange -2.32), CM008339.1-snap. 11594 (with log2FoldChange 2.45), which might be implicated in the mechanism of $I$. batatas resistance to Fob.

## 3 Materials and Methods

### 3.1 Genome sequence of Ipomoea batatas

The genome sequence of $I$. batatas was downloaded from https://www.ncbi.nlm.nih.gov/genome/?term= Ipomoea+batatas for the DNA sequences of the 15 chromosomes.

### 3.2 Gene annotation with snap

The snap program was utilized to predict the coding domain sequences (CDS) within the I. batatas chromosomes with the HMM models of A. thaliana (At.hmm), Caenorhabditis elegans (Ce.hmm), Oryza sativa (Os.hmm) and 178458 protein sequences were obtained (Huang et al., 2020).

### 3.3 Prediction of genes with MYB domains

The HMM domain model of Myb_DNA-binding (PF00249) was downloaded from the Pfam website. The hmmsearch program was used to search the whole protein sequences of $I$. batatas. For the protein sequences with MYB domain, those with E-value $\leq 1 \mathrm{E}-30$ were selected to rebuild the Myb_DNA-binding HMM domain model specific to $I$. batatas with the hmmbuild program. The new HMM model was used to search the whole protein sequences of $I$. batatas to obtain the protein sequences with E-value $\leq 0.01$. With these protein sequences, the blastp program was applied to build the model database of the R2R3 type MYB domain of $I$. batatas for alignment to the whole protein sequences of the I. batatas. The MYB domain features of the obtained protein sequences were aligned and analyzed with NCBI Conserved Domains Tool, Interproscan and Clustal Omega. The augustus program was utilized for an alternative search of the CDS for the protein sequences without intact R2R3 domains. Finally, the R2R3 type MYB protein sequences were screened in compliance with the number of MYB domains within the protein sequence and the database of R2R3 MYB transcription factors was established.

### 3.4 Conservative analysis of R2R3-MYB transcription factors

The Clustal Omega was used for the multiple sequence alignment of the amino acid sequences of R2R3-MYB. The TBtools was utilized to select conservative sites and the domain feature sites were marked manually.

### 3.5 Conservative motif analysis

The conservative motifs of the $I$. batatas R2R3-MYB were analyzed with the MEME program, with the maximum number of searched motifs set as 10 and the search window set as $10-70$ amino acids.

### 3.6 Chromosomal localization of I. batatas MYB genes

The sequence lengths of the 15 chromosomes were calculated and the information file was produced, which recorded the positions of the R2R3-MYB transcription factors within the chromosomes in line with the output ZFF file by snap program in section 3.2. The blastn program was applied to align each pair of the R2R3-MYB gene sequences to obtain the potential duplication relationship, which was verified by needle program (Rice et al., 2000). The positions of the R2R3-MYB genes in the chromosomes and the duplication relationships were drawn by circos program.

### 3.7 Construction of phylogenetic tree of R2R3-MYB

The phylogenetic tree of the R2R3-MYB protein sequences was constructed with the MEGA X program by referencing the 122 R2R3-MYB transcription factors of $A$. thaliana. The statistical method is neighbor-joining and the model/method is the p-distance. The gaps/missing data was set pairwise deletion and the No. of bootstrap replications was set 1000 .

### 3.8 Expression analysis of the R2R3-MYB transcription factors under disease stress and under abiotic stress

The transcriptomics data of the sweet potato under Fob stress (Lin et al., 2017) and under low temperature stress (Ji et al., 2020) were downloaded from EBI (https://www.ebi.ac.uk/) website. The sequencing samples of the Fob infection were JS57_F07, JS57_F04, JS57_CK, XZH_F07 and ZXH_CK, with three replicates for
each sample and 15 sequencing samples in total. The sequencing samples of low temperature stress were Xushu15_1_Cold_0w, Xushu15_1_Cold_2w, Xushu15_1_Cold_6w, Xushu15_4_Cold_0w, Xushu15_4_Cold_2w and Xushu15_4_Cold_6w, with three replicates for each sample and 18 sequencing samples in total. The Hisat2 (Kim et al., 2015) was used to build the index file of the I. batatas R2R3-MYB transcription factors for alignment to the transcriptomics sequencing samples. The aligned reads for each R2R3-MYB gene were counted and the DESeq2 program was utilized to standardize the read counts. The genes with fold change of experiment group vs control group $\geq 2$ and P value $<0.05$ were regarded as significantly up or down-regulated. The $\log 2$ FoldChange values (padj<0.05) were used to draw the heatmaps of the R2R3-MYB transcription factors of I. batatas under Fob stress and under low temperature stress.

## Authors' contributions

HXF and BCY designed and conducted the experiments. HXF and BCY carried out the data analysis and wrote the manuscript. HBF, XM and YZJ participated in experimental design and data analysis. LSQ and CXY concepted, supervised and guided the project.

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## References

Chen Z., Hu F.C., Ruan C.C., Fan H.Y., Guo L.J., and Zhang Z.L., 2019, Bioinformatics and gene expression analysis of pineapple R2R3- MYB gene family, Redai Zuowu Xuebao (Chinese Journal of Tropical Crops), 40(10): 1958-1971
Cominelli E., and Tonelli C., 2009, A new role for plant R2R3-MYB transcription factors in cell cycle regulation, Cell Res., 19(11): 1231-1232 https://doi.org/10.1038/cr.2009.123 PMid:19881525
Dubos C., Stracke R., Grotewold E., Weisshaar B., Martin C., and Lepiniec L., 2010, MYB transcription factors in Arabidopsis, Trends Plant Sci., 15(10): 573-581
https://doi.org/10.1016/j.tplants.2010.06.005
PMid:20674465
Gibbs D.J., Voß U., Harding S.A., Fannon J., Moody L.A., Yamada E., Swarup K., Nibau C., Bassel G.W., Choudhary A., Lavenus J., Bradshaw S.J., Stekel D.J., Bennett M.J., and Coates J.C., 2014, AtMYB93 is a novel negative regulator of lateral root development in Arabidopsis, New Phytol., 203(4): 1194-1207
https://doi.org/10.1111/nph. 12879
PMid:24902892 PMCid:PMC4286813
Huang X.F., Bi C.Y., Shi Y.Y., Hu Y.Z., Zhou L.X., Liang C.X., Huang B.F., Xu M., Lin S.Q., and Chen X.Y., 2020, Discovery and analysis of NBS-LRR gene family in sweet potato genome, Zuowu Xuebao (Acta Agronomica Sinica), 46(8): 1195-1207
Huang Y.J., Deng R.Y., Gao F., Lao X.P., and Wu Q., 2015, Cloning and expression analysis of transcription factor gene FtMYB21 from tartary buckwheat under abiotic stress, Jiyinzuxue Yu Yingyong Shengwuxue (Genomics Applied Biology), 34(9): 1939-1945
Ji C.Y., Kim H.S., Lee C.J., Kim S.E., Lee H.U., Nam S.S., Li Q., Ma D.F., and Kwak S.S., 2020, Comparative transcriptome profiling of tuberous roots of two sweetpotato lines with contrasting low temperature tolerance during storage, Gene, 727: 144244
https://doi.org/10.1016/j.gene.2019.144244 PMid:31715303
Kim D., Langmead B., and Salzberg S.L., 2015, HISAT: a fast spliced aligner with low memory requirements, Nat. Methods, 12(4): 357-360 https://doi.org/10.1038/nmeth. 3317 PMid:25751142 PMCid:PMC4655817

Li Y.M., Kui L.W., Liu Z., Allan A.C., Qin S.H., Liu Y.H., and Zhang J.L., 2020, Genome-wide analysis and expression profiles of the StR2R3-MYB transcription factor superfamily in potato (Solanum tuberosum L.), Int. J. Biol. Macromol., 148: 817-832
https://doi.org/10.1016/j.ijbiomac.2020.01.167 PMid:31962068
Li Z.Y., Jiao Z.X., Chen Y., Gao F., and Zhou Y.J., 2019, Cloning and expression analysis of the AmMYB44 gene from astragalus membranaceus, Jiyinzuxue Yu Yingyong Shengwuxue (Genomics Applied Biology), 38(8): 3605-3613
Lin Y.L., Zou W.K., Lin S.Q., Onofua D., Yang Z.J., Chen H.Z., Wang S.L., and Chen X.Y., 2017, Transcriptome profiling and digital gene expression analysis of sweet potato for the identification of putative genes involved in the defense response against Fusarium oxysporum f. sp. batatas, PLoS One, 12(11): e0187838
https://doi.org/10.1371/journal.pone. 0187838
PMid:29131830 PMCid:PMC5683638

Millard P.S., Kragelund B.B., and Burow M., 2019, R2R3 MYB transcription factors - functions outside the DNA-binding domain, Trends Plant Sci., 24(10): 934-946
https://doi.org/10.1016/j.tplants.2019.07.003
PMid:31358471
Rice P., Longden I., and Bleasby A., 2000, EMBOSS: the European molecular biology open software suite, Trends Genet., 16(6): 276-277 https://doi.org/10.1016/S0168-9525(00)02024-2
To A., Joubès J., Thueux J., Kazaz S., Lepiniec L., and Baud S., 2020, AtMYB92 enhances fatty acid synthesis and suberin deposition in leaves of Nicotiana benthamiana, Plant J., 103(2): 660-676
https://doi.org/10.1111/tpj. 14759
PMid:32246506
Vatansever R., Koc I., Ozyigit I.I., Sen U., Uras M.E., Anjum N.A., Pereira E., and Filiz E., 2016, Genome-wide identification and expression analysis of sulfate transporter (SULTR) genes in potato (Solanum tuberosum L.), Planta, 244(6): 1167-1183
https://doi.org/10.1007/s00425-016-2575-6
PMid:27473680
Yang J., Moeinzadeh M.H., Kuhl H., Helmuth J., Xiao P., Haas S., Liu G.L., Zheng J.L., Sun Z., Fan W.J., Deng G.F., Wang H.X., Hu F.H., Zhao S.S., Fernie A.R., Boerno S., Timmermann B., Zhang P., and Vingron M., 2017, Haplotype-resolved sweet potato genome traces back its hexaploidization history, Nat. Plants, 3(9): 696-703
https://doi.org/10.1038/s41477-017-0002-z
PMid:28827752
Zhu T.T., Liang D., and Xia H., 2016, Progress in the study of the regulation of anthocyanin synthesis by R2R3-MYB in fruits, Jiyinzuxue Yu Yingyong Shengwuxue (Genomics Applied Biology), 35(4): 985-991

