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ORIGINAL PAPER

Identification and functional analysis of miR156 family and its target genes in foxtail millet (*Setaria italica*)

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Abstract

miR156s, one of the most conserved miRNA families, are widely involved in multiple growth and development processes in plants. However, the *MIR156* gene family has not yet been identified in foxtail millet. In this study, a total of 11 *MIR156* genes, named as *Sit-MIR156a* to *Sit-MIR156k*, were identified in foxtail millet. A comprehensive bioinformatics analysis of the *Sit-MIR156* gene family was presented, including chromosomal locations, phylogenetic relationships, base conservativeness and secondary structures. Eleven *Sit-MIR156s* were distributed on seven chromosomes. Phylogenetic analysis showed that the *Sit-MIR156* family can be roughly divided into two clusters, and *MIR156* in foxtail millet were more closely related to those in rice compared to Arabidopsis and tomato. All the precursors of *Sit-MIR156* can form into the stable stem-loop secondary structures, and the mature sequences are highly conserved. The expression profile analysis showed that Sit-miR156s were expressed in flowers, leaves and roots with obvious tissue-specific patterns. The target genes of Sit-miR156s are mainly *SPL* transcription factor genes, as well as the genes encoding Acyl-CoA N-acyltransferase, UDP-Glycosyltransferase, DNA-glycosylase, and so on. The diverse expression patterns of these target genes in various tissues suggested that miR156 may play essential roles in plant growth and development and response to environments in foxtail millet. Our results provide an overview and lay the foundation for future functional characterization of the foxtail millet *Sit-MIR156* gene family.

Keywords Foxtail millet · *MIR156* · Target genes · Bioinformatics

Introduction

Non-coding RNAs represent a large group of molecules in a eukaryotic cell (Hombach and Kretz 2016; Si et al. 2020). Among these molecules, microRNAs (miRNAs) have attracted wide interest of the scientific community as vitally important gene expression regulators (Carrington and Ambros 2003; Yu et al. 2017); they are short RNAs, 21–24 nucleotides in length, which play an important role in post-transcriptional gene regulation in animals and plants (Bartel et al. 2004; Kidner and Martienssen 2005; Zhang et al. 2005; Wu et al. 2009; Zheng et al. 2019).

miR156 is one of the most abundant and evolutionarily conserved miRNA families in plants. It plays important roles in the growth development of plants and the regulation of abiotic stress response. In *Arabidopsis*, maize (*Zea mays* L.), and rice (*Oryza sativa*), overexpressing miR156 resulted in dramatic morphologic changes, suggesting that miR156 has global regulatory function in plant development (Schwab et al. 2005; Xie et al. 2006; Chuck et al. 2007). In *Arabidopsis*, miR156/*SPL* modules are involved in the proper timing of the lateral root developmental progression. Plants overexpressing miR156 produce more lateral roots whereas reducing miR156 levels leads to fewer lateral roots (Yu et al. 2015). Overexpression of miR156 leads to prolonged juvenile and delayed flowering of many plant species, such as Arabidopsis (Wu and Poethig 2006), potato (Bhogale et al. 2014), alfalfa (Aung et al. 2015), tomato (Zhang et al. 2011), rice (Xie et al. 2006), and corn (Chuck et al. 2007). Besides, miR156 has been reported to mediate responses to recurring heat stress through SPL transcription factors (Stief et al. 2014).

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miR156s target SQUAMOSA-promoter binding-like (SPL) transcription factor genes in plants. SPLs are characterized by a highly conserved SQUAMOSA promoter-binding protein (SBP) domain which binds to a specific cis-element glycidyltrimethylammonium chloride (GTAC)-binding domain (Birkenbihl et al. 2005; Kropat et al. 2005). SPLs form a small gene family, with 17 *SPL* genes in *Arabidopsis*, 18 in rice and 18 in foxtail millet (Guo et al. 2005; Xie et al. 2006; Yue et al. 2021). SPLs are known to regulate multiple important and divergent biological processes, including leaf development (Wu and Poethig 2006), phase transition (Usami et al. 2009), flower and fruit development (Manning et al. 2006; Wang et al. 2016), plant architecture (Wei et al. 2018), sporogenesis (Unte et al. 2003), GA signaling (Chen et al. 2019), as well as response to abiotic stress (Stief et al. 2014; Wang et al. 2019).

Foxtail millet (*Setaria italica*), a member of the Poaceae family, is one of the oldest cereal crops, domesticated in Northern China (Liu et al. 2016). It is rich in essential amino acids, fatty acids and minerals, which is of important significance to human health (He et al. 2015). The strategic roles of foxtail millet in stabilizing grain production, ensuring the global economy and people's livelihood are attracting more and more attention worldwide (Muthamilarasan and Prasad 2021). Furthermore, Foxtail millet possesses attractive qualities, such as small diploid genome (~430 Mb) (Yang et al. 2020), lower repetitive DNA, short life cycle, and C4 photosynthesis (Brutnell et al. 2010; Pan et al. 2018). The whole genome sequence of foxtail millet has become available (Zhang et al. 2012). Along with the progress in transformation techniques, the development of *xiaomi*, a mini foxtail millet with a life cycle similar to that of Arabidopsis, has increased the utility of foxtail millet as a C4 model plant (Yang et al. 2020). Although studies of foxtail millet have recently advanced, the mechanisms of growth, organ development, and responses to biotic and abiotic stressors remain poorly understood.

To date, the *MIR156* gene family in foxtail millet (*Sit-MIR156*) has not been characterized. Given their essential roles in the growth and development of foxtail millet, we carried out a detailed characterization of *Sit-MIR156* genes in foxtail millet. This work will provide deeper insight and understanding of the *Sit-MIR156* genes in foxtail millet. Also, important clues for their functional analysis and applications in improving quality and resistance to abiotic stresses will be provided.

Materials and methods

Chromosome localization analysis of miR156 family in foxtail millet

The precursor sequences, mature sequences and chromosomal location information of *Sit-miR156* family members of foxtail millet were downloaded from the PmiREN database (Guo et al. 2020) (http://www.pmiren.com/). TBtools (Chen et al. 2020) software was used to draw chromosome mapping of *Sit-MIR156* family genes.

Phylogenetic analysis of the miR156 family in foxtail millet

To construct the phylogenetic tree of miR156s, the precursors sequences of miR156 in tomato, rice, *Arabidopsis* and foxtail millet were downloaded from PmiREN database. Multiple sequence alignments were performed on miR156 precursor sequences. The phylogenetic tree was constructed by MEGA and the neighbor-joining method, and the Bootstrap value was set to 1000.

Base conserved analysis of miR156 sequences in foxtail millet

WebLogo 3 (Crooks et al. 2004) (http://weblogo.Threepluson.com/create.cgi) was used to analyze the base conservation of Sit-MIR156 precursor sequences and mature sequences.

Secondary structure prediction of miR156 precursor in foxtail millet

RNAfold (Denman 1993) (http://rna.Tbi.univ.ac.at.cgi-bin/rnafold.cgi) was used to predict the secondary stem-loop structure of Sit-MIR156 precursors using. Select the minimum free energy (MFE) and partition function using the folding algorithm and basic options.

Tissue specific expression analysis of miR156 in foxtail millet

The expression data of Sit-miR156 family members in roots, leaves and flowers were downloaded from PmiREN database, and the data were mapped into heat maps using TBtools.

Prediction of cis-acting regulatory elements

Promoter sequences (−2000 bps) of *Sit-MIR156* family genes were obtained from the PmiREN database (https://www.pmiren.com/). The sequence of CNGC genes were searched for a variety of cis-acting regulatory elements using PLACE software (http://www.dna.affrc.go.jp/PLACE/signalscan.html) (Higo et al. 1998).

Target gene prediction

The potential targets of miR156 were predicted using the psRNATarget (http://plantgrn.noble.org/psRNATarget/) with default parameters (Dai and Zhao 2011).

In this prediction, the database of foxtail millet, i.e., *Setaria italica*, Transcript, JGI Genomic Project, Phytozome 13, 312 V 2.2, were used for all possible target prediction with the parameters of Expectation ≤ 3, UPE ≤ 25. The predicted target genes were sorted out, and NCBI and MDSI (http://foxtail-millet.biocloud.net/home) were used for functional annotation of candidate target genes.

Tissue-specific expression analysis of miR156 target genes in millet

To study the potential expression patterns of above candidate target genes from foxtail millet at different tissues and developmental stages, the fragments per kilobase of the exon model per million mapped (FPKM) values of these genes were obtained from the GeneAtlas v1 Tissue Sample (Phytozome 13), including the data of etiolated seeding (5 days), germ shoot (6 days), shoot (1 week), different leaf (1, 2, 3, 4, 5, 6) at 2 weeks, panicle stage 1 and 2, and root (10 days). These data were submitted to TBtools (Chen et al. 2020) for expression profile mapping.

Results

Identification and analysis of *MIR156* genes in foxtail millet

Firstly, 11 *MIR156* genes were identified in the foxtail millet genome based on the PmiREN database. The genes were listed as *Sit-MIR156a*–*Sit-MIR156k* (Table1). Among these *MIR156s*, only *Sit-MIR156a* produced mature sequences with 21 bases, while the remaining *Sit-MIR156s* (*Sit-MIR156b*–*Sit-MIR156k*) generated miR156 mature sequences with 20 bases.

To determine the localization of these *Sit-MIR156* genes, we mapped those genes to the 9 foxtail millet chromosomes. The chromosomal distribution of *Sit-MIR156* genes in foxtail millet was uneven: seven of nine chromosomes contained *Sit-MIR156* genes (Fig. 1). Among them, chr 5 had the greatest number of *Sit-MIR156* genes (3 genes), chr 1 and 2 contained two *Sit-MIR156* genes, and chr4, 6, and 8 posed only one *Sit-MIR156* gene.

Phylogenetic analysis of the *MIR156* genes

With the aim of understanding the phylogenetic relationships and evolutionary history, we first investigated the phylogeny of *Sit-MIR156* using stem-loop sequences. The phylogenetic distribution suggested that *sit-MIR156* could be classed into two groups (Fig. 2). Seven *Sit-MIR156* genes (*Sit-MIR156a*–*Sit-MIR156d*, *Sit-MIR156i*–*Sit-MIR156k*) were classed into the first group. The remaining four genes belonged to the second group. Among these four genes, the closely related *Sit-MIR156f*, *Sit-MIR156g* and *Sit-MIR156h* located on the same chromosome, especially *Sit-MIR156f*, *Sit-MIR156*g which were closely linked. The results suggested that these genes may expand through tandem duplication.

To further explore the evolutionary relationship of *MIR156* among different species, the *MIR156* sequences from foxtail millet, Arabidopsis, rice and tomato were used to construct a phylogenetic tree (Fig. 3, Supplemental Table 1). As shown in Fig. 3, the closely related *MIR156s* from different species were clustered into the same branches suggesting that *MIR156s* were evolutionary conserved. The phylogenetic tree also revealed that the majority of *Sit-MIR156* members were distributed with species bias. As shown in Fig. 3, there were six sister gene pairs were

Table 1 Basic information of Sit-miR156 family members

miRNA	miRNA locus	Chromosome location	Mature sequence	Base number
sit-miR156a	*Sit-MIR156a*	Chr4: 31435211–31435338 (+)	UUGACAGAAGAGAGCGAGCAC	21
sit-miR156b	*Sit-MIR156b*	Chr1: 30686003–30686130 (−)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156c	*Sit-MIR156c*	Chr1: 5902073–5902198 (+)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156d	*Sit-MIR156d*	Chr2: 30830639–30830765 (+)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156e	*Sit-MIR156e*	Chr2: 34718478–34718606 (−)	UGACAGAAGAGAGAGAGCAC	20
sit-miR156f	*Sit-MIR156f*	Chr5: 11531652–11531788 (+)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156g	*Sit-MIR156g*	Chr5: 11532049–11532174 (+)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156h	*Sit-MIR156h*	Chr5: 32104707–32104835 (+)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156i	*Sit-MIR156i*	Chr6: 28563946–28564101 (+)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156j	*Sit-MIR156j*	Chr7: 24139196–24139325 (−)	UGACAGAAGAGAGUGAGCAC	20
sit-miR156k	*Sit-MIR156k*	Chr8: 10204668–10204833 (+)	UGACAGAAGAGAGUGAGCAC	20

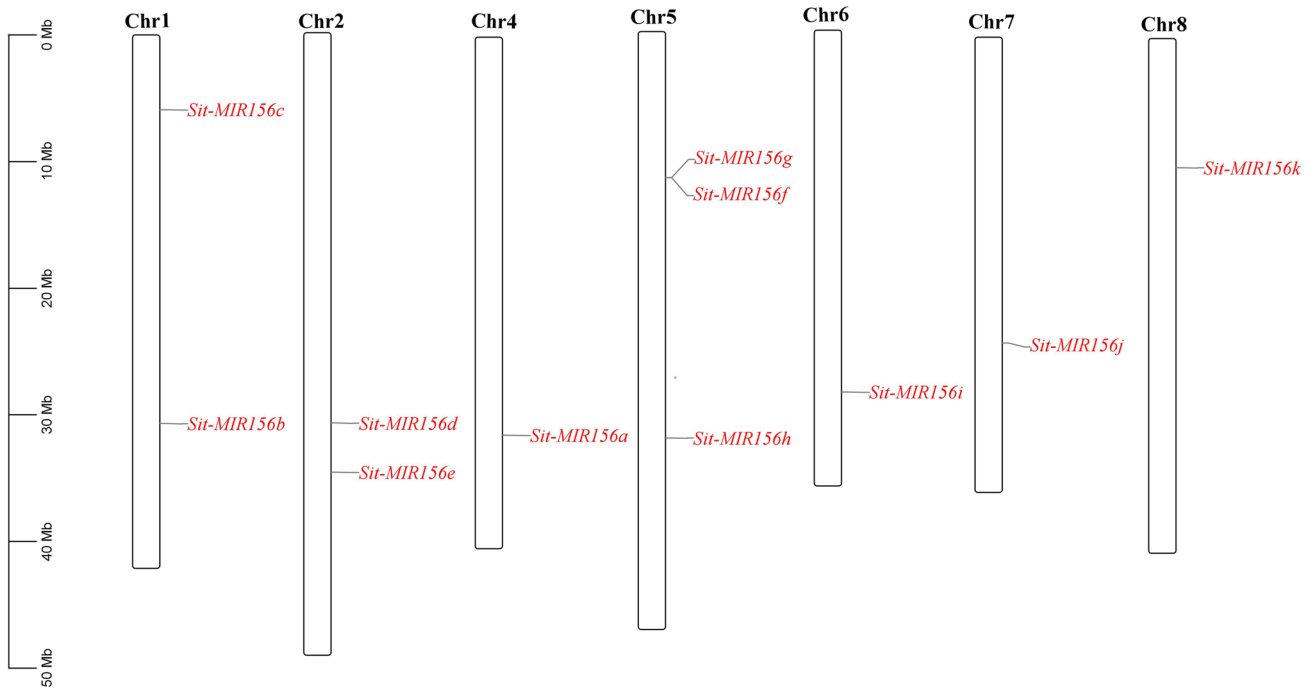

Fig. 1 Chromosome location of *Sit-MIR156* genes in foxtail millet

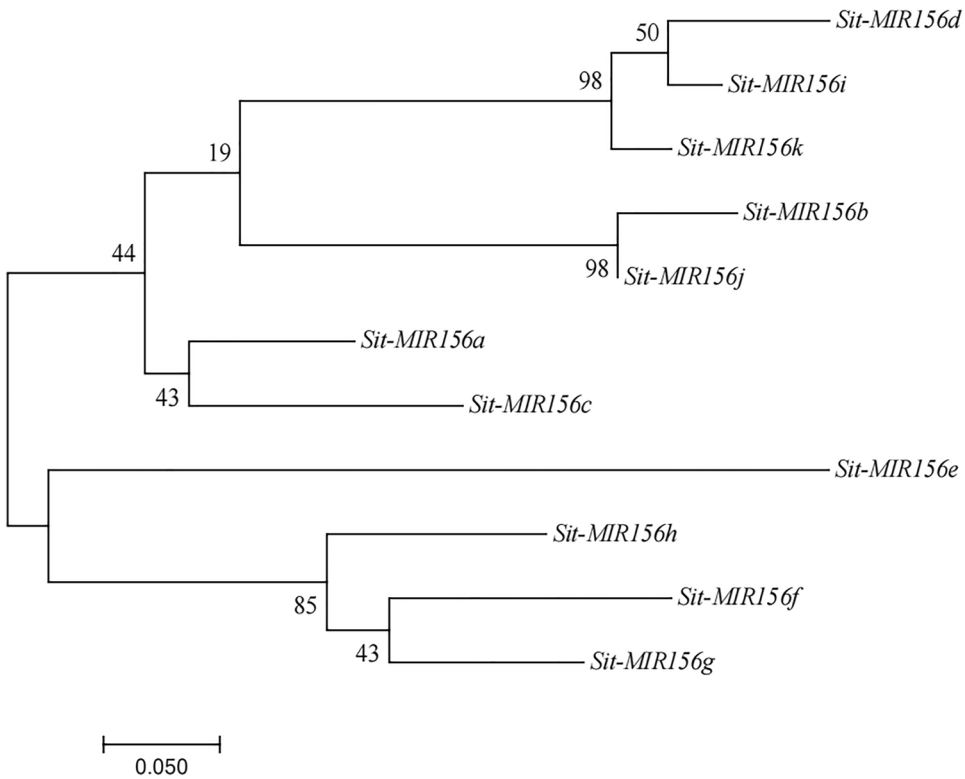

Fig. 2 Phylogenetic analysis of *Sit-MIR156* genes in foxtail millet

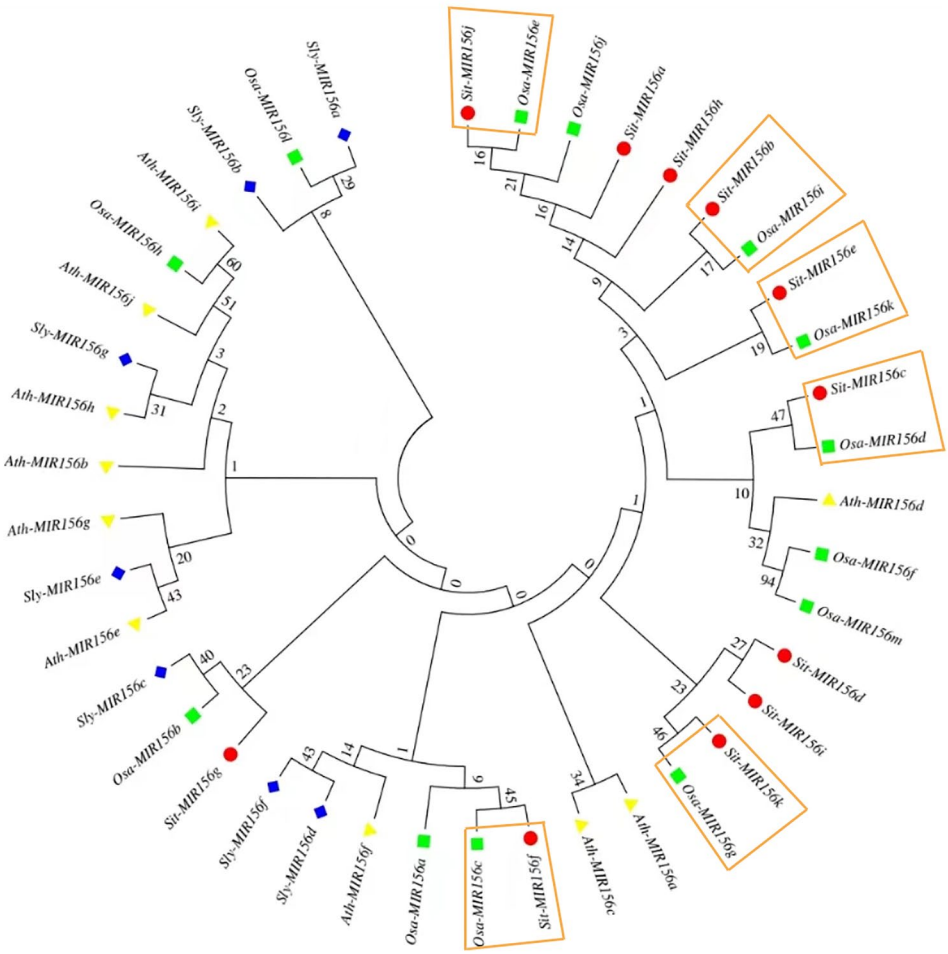

Fig. 3 Phylogenetic analysis of the *MIR156* family in foxtail millet, rice, tomato and Arabidopsis. *Note* The proteins are classified into four groups: I, II, III and IV. Protein from foxtail millet, Arabidopsis, rice and tomato are represented by red solid circle, yellow solid triangle, green solid square, blue solid rhombus. The sister pairs are labeled with orange square

identified between foxtail millet and other species (depicted with orange rectangle in Fig. 3), and all of the six genes were derived from rice. The results suggested that these *MIR156s* in foxtail millet were more closely related to those in rice compared to Arabidopsis and tomato.

Base conserved analysis and secondary structure prediction of *MIR156* genes in foxtail millet

The base conservation of Sit-miR156 stem-loop and mature sequences is shown in Fig. 4. For the stem-loop sequences, the bases of 2–21 and 104–126 were highly conserved, where generated the mature sequences and the star sequences of Sit-miR156 (Fig. 4a). Among the 21 bases in Sit-miR156 mature sequences, 19 bases were completely conserved, and only 2 bases (the first "U" which only existed in Sit-miR156a and the fifth "U/A/G") were slightly less conserved, indicating that foxtail millet miR156 was highly conserved in the evolutionary process (Fig. 4b). In terms of the star sequences, 14 bases base was completely conserved, and the remaining 7 bases were less conserved (Fig. 4c, Supplemental Tab.2).

To further verify these *Sit-MIR156s*, we performed the secondary structure prediction of these miRNA (Fig. 5). The results showed that all 11 *Sit-MIR156s* could form relatively stable secondary stem-loop structures, and the mature miR156 were all generated on the 5' end arm of the precursor, showing strong conservation.

Expression analysis of miR156 in different plant tissues

The expression patterns of Sit-miR156s were analyzed using the sRNA-seq data of different tissues collected from the public online database. As shown in Fig. 6, all of the Sit-miR156s showed the lowest or undetectable expression in the flower, implying that this miRNA might be expressed in the flower at other non-tested developmental stages or under special conditions. According to the expression patterns, Sit-miR156s were clustered into three groups. Sit-miR156a in the first group showed the peak expression in leaf. Seven miR156s in the second group, that is, Sit-miR156b, Sit-miR156d, Sit-miR156f, Sit-miR156h–k, displayed unique expressions in root. Three

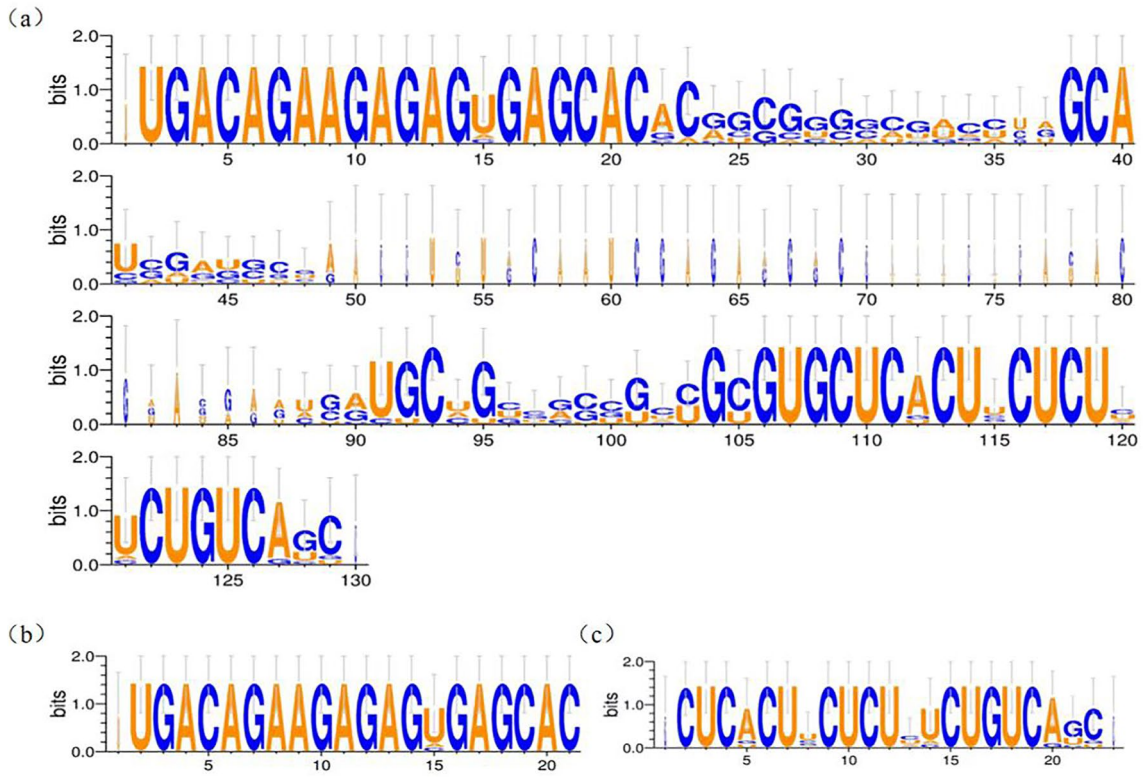

Fig. 4 Base conservativeness of *Sit-MIR*156 sequences. *Note* **a** precursor sequence; **b** mature sequence; **c** star sequence

miR156s in the third group including Sit-miR156c, Sit-miR156e and Sit-miR156g showed predominant expressions in root and lower expressions in leaf. Taken together, the divergent expression patterns of these Sit-miR156s suggested that *MIR156s* play important roles during the growth development of foxtail millet.

To further elucidate the possible regulation mechanisms of *Sit-MIR156s* in the abiotic or biotic stress response, the promoter sequences were analyzed using the PlantCARE database to identify cis-regulatory elements in the promoter region. Thirteen types of stress- and hormone-related cis-acting regulatory elements were detected in the promoters: five hormone-related elements respond to abscisic acid, MeJA, auxin, gibberellin and salicylic acid, respectively; three stress-related elements respond to drought, low-temperature and anaerobic induction; four elements related to circadian control, MYB binding, zein metabolism and meristem expression (Fig. 7). All 11 *MIR156* genes contained 4–9 cis-elements related to stress or hormone response. Among these elements, the ABRE (abscisic acid responsiveness) were detected in all 11 *MIR156* genes. Therefore, these results demonstrated that expression of *Sit-MIR156* genes would be regulated by various environmental factors.

The prediction of miR156 target genes in foxtail millet

To further explore the function of miR156 in foxtail millet, the putative targets of miR156 were predicted by psRNATarget (Dai and Zhao 2011). As shown in Table 2, 19 target genes were pridicted. The results showed that most targets would be *SPLs* (Table 2). In addition, the genes encoding Acyl-CoA N-acyltransferase, UDP-Glycosyltransferase, DNA-glycosylase, potassium channels, F-box protein family, no vein-like and phosphate dehydrogenase were also putative targets. *SPLs*, important transcription factors, play important roles in the multiple important and divergent biological processes. All of the miR156 members can target *SPLs*, indicating *SPLs* are the most important target gene of Sit-miR156s. The *SPL* family members targeted by Sit-miR156s are *Sit-SPL1–4, Sit-SPL11–13* and *Sit-SPL15–18*, which account for a significant portion of the *SPL* genes in foxtail millet, suggesting that miR156-*SPL* module plays important role during the growth of foxtail millet.

Table 2 Prediction of miR156 target genes in foxtail millet

Target gene	miRNA	Alignment area					Function of target gene
Seita.1G069300	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL4
		Target	863	GUGCUCUCUCUCUUCUGUCAG	883		
Seita.1G077000	sit-miR156a	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		Acyl-CoAN-acyltransferase
		Target	760	GUGUUCGAU-UCUUCUGUCAG	779		
Seita.1G091900	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	20	CACGAGUGAGAGAAGACAGU	1		SPL3
		Target	1776	GUGCUCUCUCUCUUCUGUCA	1795		
Seita.2G209700	sit-miR156a	miRNA	21	CACGAGC-GAGAGAAGACAGUU	1		UDP-Glycosyltransferase
		Target	1074	CUGUUCGACCCUCUUUUGUCAA	1095		
Seita.2G254300	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL16
		Target	1256	GUGCUCUCUCUCUUCUGUCAA	1276		
Seita.2G266500	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL17
		Target	1886	GUGCUCUCUCUCUUCUGUCAU	1906		
Seita.2G324900	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL12
		Target	823	AUGCUCUCUCUCUUCUGUCAU	843		
Seita.2G325000	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL1
		Target	655	AUGCUCUCUCUCUUCUGUCAU	675		
Seita.3G324500	sit-miR156e	miRNA	20	CACGAGAGAGAGAAGA-CAGU	1		TOM5
		Target	606	GUGCUCUUUCUCUUUUUGUUA	626		
Seita.4G196500	sit-miR156e	miRNA	20	CACGAGAGAGAGAAGACAGU	1		DNA-glycosylase
		Target	1372	UUGCCCUUUCUCUUUUGUCA	1391		
Seita.4G270400	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	20	CACGAGUGAGAGAAGACAGU	1		SPL11
		Target	2074	GUGCUCUCUCUCUUCUGUCA	2093		
Seita.5G323200	sit-miR156b、c、d、f、g、h、i、g、k	miRNA	20	CACGAGUGAGAGAAGACAGU	1		potassium channels
		Target	347	GUGCUCACUUUCUUC-GUCC	365		
Seita.5G432500	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL2
		Target	2365	GUGCUCUCUCUCUUCUGUCAG	2385		
Seita.6G154000	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		F-box protein family
		Target	3980	GUGCUCUAUUUCUUUUGUCAA	4000		
Seita.6G205500	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL13
		Target	1424	GUGCUCUCUCUCUUCUGUCAA	1444		
Seita.6G223300	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL15
		Target	971	GUGCUCUCUCUCUUCUGUCAG	991		
Seita.8G124900	sit-miR156a、b、c、d、e、f、g、h、i、g、k	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		SPL18
		Target	1285	GUGCUCUCUCUCCUCUGUCAU	1305		
Seita.9G048500	sit-miR156a	miRNA	21	CACGAGCGAGAGAAGACAGUU	1		no vein-like
		Target	7409	UUGCUCGUGUUAUUCUGUCAA	7429		
Seita.9G072000	sit-miR156b、c、d、f、g、h、i、g、k	miRNA	20	CACGAGUGAGAGAAGACAGU	1		phosphate dehydrogenase
		Target	1780	GUGUUGAUUUUCUUUUGUCA	1799		

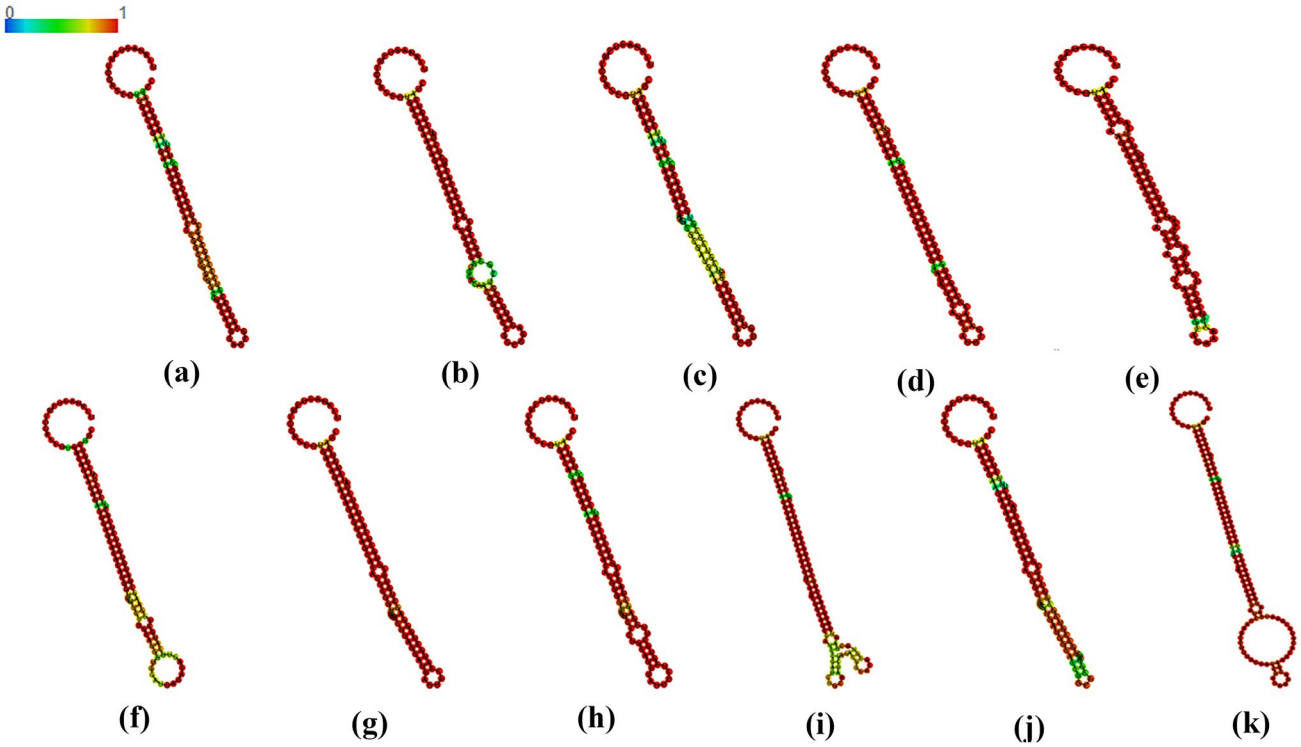

Fig. 5 Secondary stem-loop structure of *Sit-MIR156s*. *Note* **a**–**k** the secondary structures of *Sit-MIR156a*–*Sit-MIR156k*. Colors from blue to red indicate the probability of base pairing from 0 to 1

Expression analysis of miR156 target genes in different plant tissues

MiRNAs affect plant growth and development and respond to the environment by regulating the expression of target genes. As shown in Fig. 8, the expression patterns of *SPLs* targeted by Sit-miR156 were classified into two types. Nine *SPLs* are mainly expressed in panicle, showing obvious tissue-specific expression. It indicated that these genes may be involved in the growth and development of panicle in foxtail millet. The remaining two miR156-targeted *SPLs* (*SPL16* and *SPL18*) were expressed at high levels in etiolated seedling, suggesting both genes may harbor the similar function in the seedling.

Beside *SPLs*, three miR156-targeted genes, encoding NO VEIN-like protein, F-box protein, and phosphate dehydregenase, were more highly expressed in leaves, indicating putative functions of these genes on leaf developments. In *Arabidopsis*, *NO VEIN* gene encodes a plant-specific nuclear factor required for leaf vascular development (Tsugeki et al. 2010). The target gene *Seita.1G077000* encoding an Acyl-CoAN-acyltransferase, displayed peak expression in root, suggesting this gene may play a role in root development.

Discussion

MiR156s play a vital regulatory role in various biological processes during plant development. The regulation of miR156 is mediated by the inhibition of plant-specific SQUAMOSA PROMOTER BIDING-LIKE (SPL) transcription factors (Preston and Hileman 2013). In this study, we identified 11 *MIR156* genes in foxtail millet (Table 1). Our study provided comprehensive information on *MIR156* family in foxtail millet.

In this paper, phylogenetic analysis of *MIR156s* in millet, rice, *Arabidopsis* and tomato showed that the *MIR156* genes of millet and rice were most closely related. So the studies on rice miR156s could serve as good references for research in foxtail millet. Eleven out of 18 *SPLs* in the rice genome are targeted by miR156, which is the same as that in foxtail millet. It was reported that miR156 and their targeting *SPL* genes have pleiotropic effects on a large number of biological pathways in rice (Wang et al. 2009). For example, in rice, overexpression of miR156 can result in prolonged juvenile and delayed flowering (Xie et al. 2006). As we known, branching and internode growth are important traits because they are essential for light reception. In rice, miR156 affects the branching of shoots by cleaving *SPL*

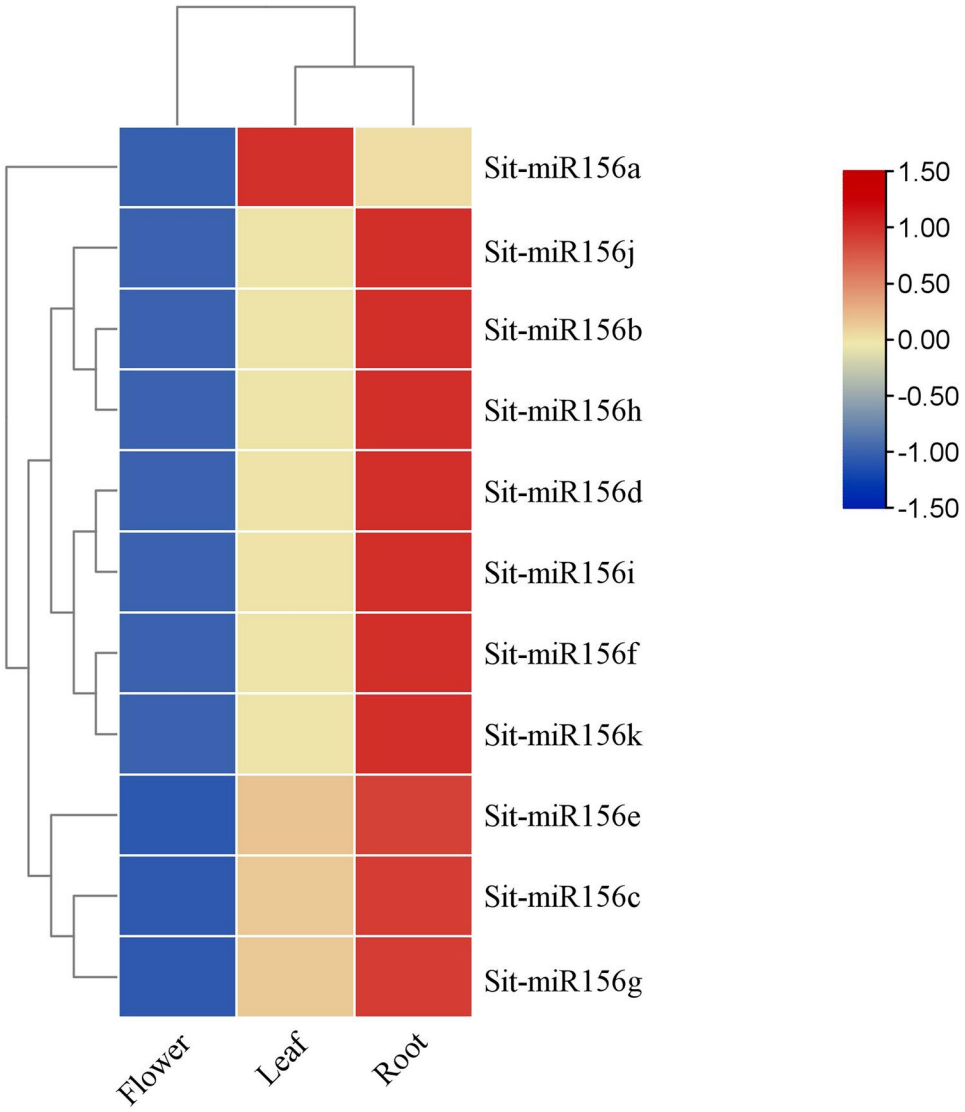

Fig. 6 Expression of Sit-miR156s in different tissues. The color scale represents expression levels from high (red) to low (blue color)

transcripts (Liu et al. 2017). In addition, the overexpression of miR156 in rice can cause dwarfism by inhibiting shoot apical meristems and promoting germination of axillary buds (Wang et al. 2015), and lead to the production of more roots (Xie et al. 2012), which suggests miR156 may play a role in root branching. Besides, in rice, overexpression of miR156 in transgenic seedlings showed a higher salt tolerance. Except for rice, multiple functions of miR156 in *Arabidopsis*, soybeans and maize have been reported previously (Chuck et al. 2007; Wu et al. 2009; Yu et al. 2010; Cao et al. 2015; Sun et al. 2019). In a word, a comparative study may provide useful information for revealing biological functions of miR156s in foxtail millet.

Our study also analyzed the expression profiles of Sit-miR156s and their putative target genes. The results suggest that *MIR156* and the target genes may play important roles in both vegetative and reproductive organs. Comparative analysis showed that miR156a and the target genes *Seita.9G048500*, *Seita.6G154000*, *Seita.9G072000* and *Seita.2G209700* displayed specific expression in leaves, although these target genes showed various expression levels in leaves at different developmental stages. Besides, the target gene *Seita.1G077000* and most of miR156s showed preferential expression in root. The differential expressions of these miR156s and the target genes in various tissues indicates the complex regulatory function of miR156 during the growth of fotail millet. The study will be useful in selecting candidate genes related to tissue development in foxtail millet and pave the way to further functional verification of these *MIR156* genes in foxtail millet.

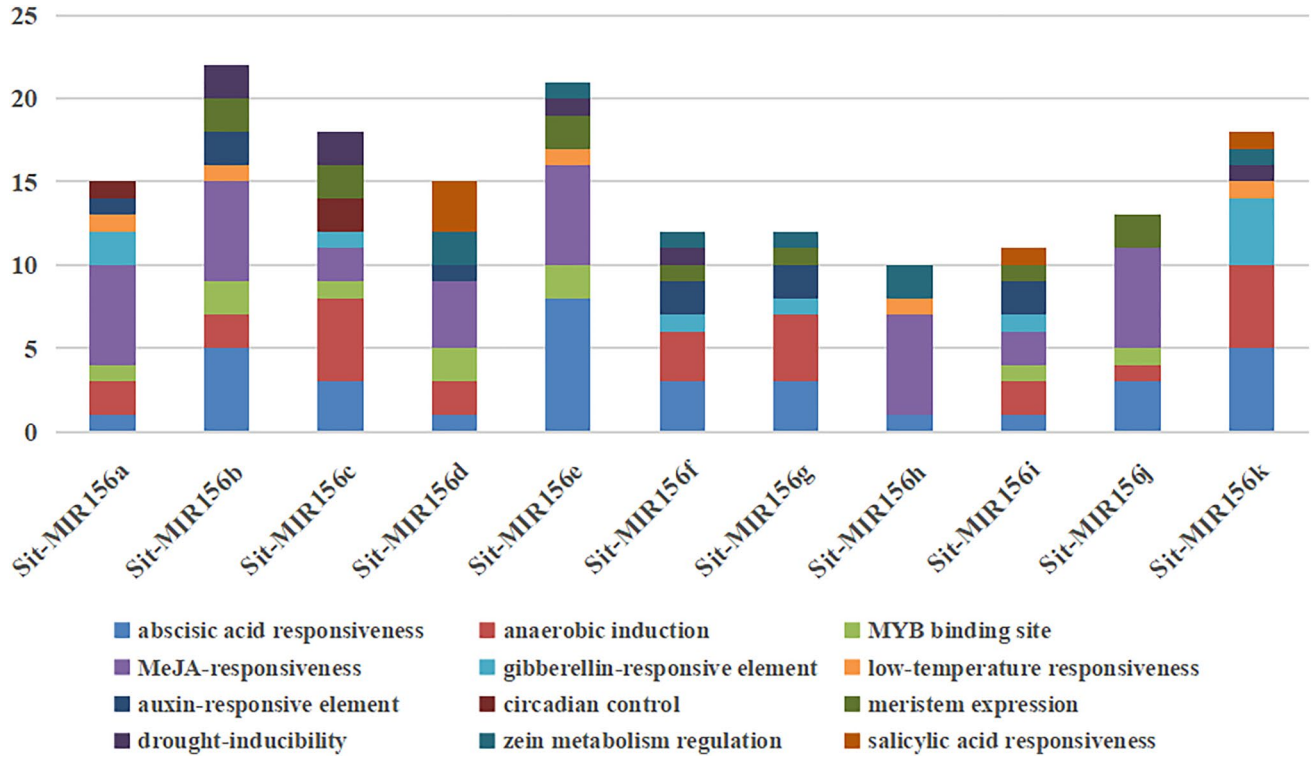

Fig. 7 Prediction of cis-acting regulatory elements of *Sit-MIR156s*

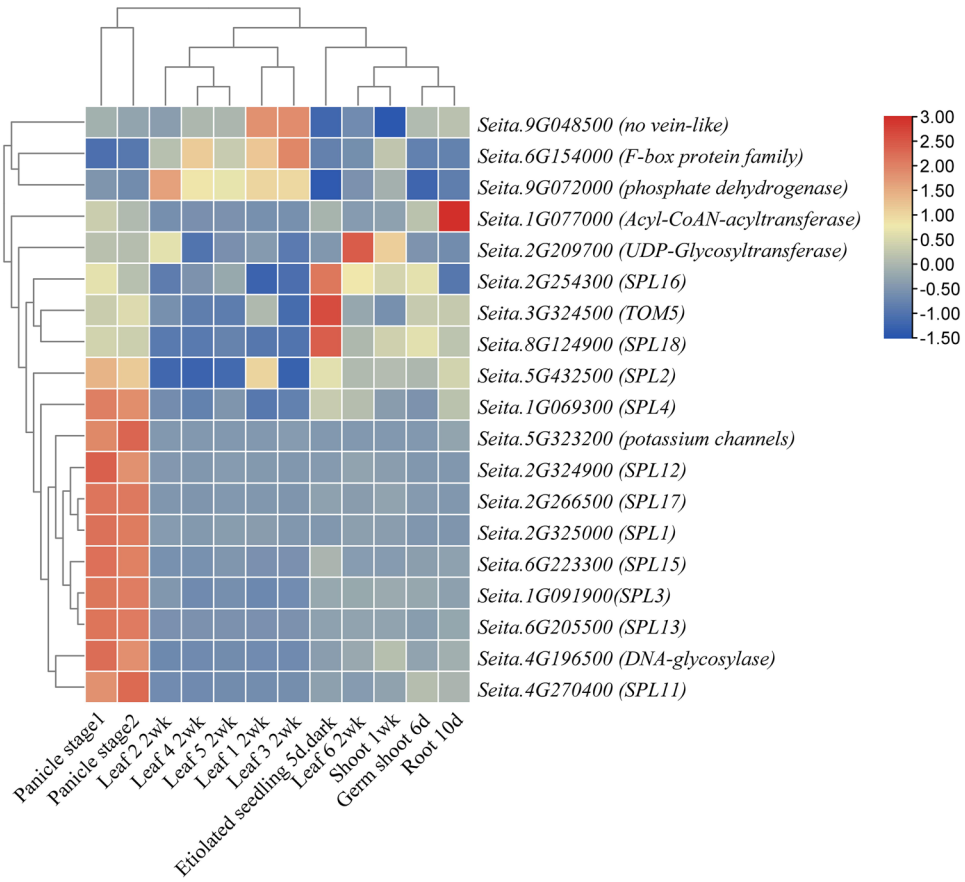

Fig. 8 Expression of Sit-miR156 target genes in different tissues and environment conditions. The color scale represents expression levels from high (red) to low (blue color)

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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