Genome-wide sequence identification and expression analysis of *N*^{6}-methyladenosine demethylase in sugar beet (*Beta vulgaris* L.) under salt stress

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N^6-methyladenosine (m^{6}A) is the most abundant and highly conserved RNA modification in eukaryotes. m^{6}A demethylase can remove the m^{6}A marker and dynamically regulate the m^{6}A level in vivo, which plays an important role in plant growth, development and response to abiotic stress. The confirmed m^{6}A demethylases in *Arabidopsis thaliana* include ALKBH9B and ALKBH10B, both belonging to the ALKB family. In this study, BvALKB family members were identified in sugar beet genome-wide database, and their conserved domains, gene structures, chromosomal locations, phylogeny, conserved motifs and expression of *BvALKB* genes were analyzed. Almost all BvALKB proteins contained the conserved domain of 2OG-Fe II-Oxy. Phylogenetic analysis suggested that the 10 proteins were clustered into five groups, each of which had similar motifs and gene structures.

Three *Arabidopsis* m^{6}A demethylase homologous proteins(BvALKBH6B, BvALKBH8B and BvALKBH10B) were of particular concern. Expression profile analysis showed that almost all genes were up-regulated or down-regulated to varying degrees under salt stress, especially *BvALKBH10B* homologous to *AtALKBH10B* was significantly up-regulated, suggesting that the genes were in response to salt stress. This study provides a theoretical basis for further screening of m^{6}A demethylase in sugar beet, and also lays a foundation for studying the role of ALKB family proteins in growth, development and response to salinity stress.

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Abstract

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Keywords: Sugar beet, *N*^{6}-methyladenosine, demethylase, ALKB, salt stress, bioinformation

Introduction

N^6-methyladenosine(m^{6}A) is the most abundant modification in mRNA among all higher eukaryotes , manifested as methylation at the sixth *N* of adenosine , which has been a hot spot of epigenomic studies in recent years(*Huang & Yin., 2018*). Previous studies have shown that m^{6}A, including methytransferase complex (METTL3, METTL14, WTAP, etc.), demethylases(FTO, ALKBH5, etc.) and RNA binding proteins (YTHDF1/2/3, YTHDC1/2, etc.) *(Desrosiers, Friderici K & Rottmanl, 1974*; *Ortega et al., 2003*; *Jia et al., 2011)*, is a reversible and dynamic co-regulation process(*Miao et al., 2020*). In animals, genes encoding m^{6}A-related proteins have been identified and characterized(*Wei, Gershowitz & Moss, 1976*; *Levis & Penman, 1978*), and their important role in animal development has been demonstrated, but the function of these proteins in plants is only now being revealed. m^{6}A is generally enriched near the stop codon and the 3 'UTR, as well as at the long introns and transcription start sites*(Meyer et al., 2012*), which are common in mammals. m^{6}A is found to be enriched near the start codon in *Arabidopsis thaliana*, which may play a role in the plant-specific pathway *(Luo et al., 2013*; *Wan et al., 2015*). A recent explosion of molecular studies centered on m^{6}A methylation has revealed its role in eukaryotic transcriptome regulation, RNA stability, and translation efficiency*(Niu et al., 2013*; *Pan, 2013*; *Yue et al., 2019*). Some proteins are involved in regulating the formation of plant cells and tissues(*Zhong et al., 2008*; *Shen et al., 2016*; *Bhat et al., 2020*; *Scutenaire et al., 2018*), while others regulate the expression of drought and high temperature signal related genes in plants(*Zhao X, 2014*; *Lu et al., 2020*), which play a significant role in plant stress resistance.

The reversibility of RNA methylation is achieved by demethylases, which was confirmed in the paper by He et al(*Jia et al., 2011*). Proteins identified as m^{6}A demethylases belong to the ALKB family and contain highly conserved synthase-like domains. m^{6}A demethylases found in mammals mainly include obesity-related genes FTO and ALKBH5 (*Jia, Fu & He, 2013*; *Liu & Jia, 2014*). The unique C-terminal long loop structure of FTO may determine its function of promoting protein-protein or protein-RNA interactions. Compared with FTO catalyzed m^{6}A to A through intermediates, ALKBH5 could directly catalyze m^{6}A to A(*Mauer et al., 2017*; *Wei et al., 2018*). Due to differences in tissue specificity and substrate, FTO and ALKBH5 play different roles in mRNA processing and metabolism. Studies have shown that FTO can regulate the binding of precursor RNA with splicing factor SRSF2 to affect its splicing maturation, and ALKBH5 is related to the nuclear transport mRNA (*Zhao et al., 2014*).

Bioinformatics analysis revealed that there were 14 ALKB homologous proteins in *Arabidopsis*, among which ALKBH9A, ALKBH9B, ALKBH9C, ALKBH10A and ALKBH10B had the most similar amino acid sequence to ALKBH5. Proteins that have been confirmed as m^{6}A demethylases include ALKBH9B and ALKBH10B. ALKBH10B is highly abundant in all tissues, especially in flowers. It has a specific catalytic function on m^{6}A modified mRNA, and experiments have shown that it can mediate the early flowering transition by regulating the demethylation of *FT*, *SPL3* and *SPL9*(*Duan et al., 2017*). As the only ALKBH5 homologous protein in the cytoplasm, ALKBH9B was responsible for removing *N*^{6}-methyladenosine from ssRNA in vitro and participating in mRNA silencing or degradation. In addition, it also plays a role in plant protection against specific viral pathogens, and through interaction with viral cap protein, modulates the m^{6}A demethylation modification of the AMV genome to affect its life cycle and infection capacity (*Martínez-Pérez et al., 2017*), but has no effect on the activity of cucumber mosaic virus. m^{6}A demethylase has not been found in other plants.

Previous studies have demonstrated the role of some ALKBH members in plant growth and development. The stress response of plant demethylase was mainly studied in model plant *Arabidopsis*. ALKBH9A was highly expressed in roots under salt stress, and ALKBH10A was significantly down-regulated under heat stress(*Růžička et al., 2015*). Under drought, cold or ABA treatment, ALKBH1 levels were signicantly up-regulated, while ALKBH6, ALKBH8B and ALKBH10A expressions were decreased(*Hu, Manduzio & Kang, 2019*), indicating that ALKBH members may play an important role in abiotic stress. In recent studies, it was found that ALKBH6 could bind to m^{6}A marked mRNA and remove the mark in *Arabidopsis*, which may be a potential m^{6}A demethylase. Under drought or heat stress, the survival rate of the *alkbh6* mutant was lower than that of the wild type, but not under salt stress. In addition, ALKBH6 affected ABA response by regulating the expression of genes related to ABA signaling(*Huong, Ngoc & Kang, 2020*). These results suggest that RNA demethylation plays a crucial role in plant responses to abiotic stress.

Sugar beet is one of the most abundant sugar-producing crops, and its yield and quality are of great significance to agricultural production. In China, the saline-alkali land highly coincides with the sugar beet production area. Although the sugar beet has a certain salt tolerance, it is limited in extent. The high salinity of the land not only affects the seed germination and growth, but also causes great damage to the sugar industry. Therefore, the analysis of sugar beet m^{6}A will be helpful to understand its transcriptional modification and expression regulation, and reveal its salt-tolerant mechanism to cultivate new stress resistant strains. m^{6}A demethylase is involved in the response to abiotic stress(*Hu et al., 2021*), so far there has been no specific analysis of sugar beet under salt stress. In this study, bioinformatics analysis of m^{6}A demethylase was carried out based on the sugar beet genome database, and demethylase genes related to salt treatment was identified, which provides a theoretical basis for breeding beet varieties.

Materials & Methods

Materials

The salt-tolerant strain "O"68 of beet was used as the experimental material in this experiment(*Shi et al., 2008*). The seeds were soaked under running water for 12 h, then disinfected with 75% ethanol and washed aseptic for 3 times. The seeds were sown into the wet sponge and cultured in the dark at 24 h for 2 days. After germination, it was transferred to a culture pot containing nutrient solution (light for 16 h, dark for 8 h). After the growth of three pairs of true leaves, 300 mM NaCl solution was used to replace the nutrient solution for 24 h, and the other conditions remained unchanged. The control group was set without salt treatment. After the salt stress, leaves and roots were sampled. Sugar beet samples in control group and experimental group were premixed in advance respectively, and divided into several parts, each containing 0.2 g samples. Then they were immediately precooled in liquid nitrogen and stored in a refrigerator at -80 ^{o}C until analysis.

Screening and identification of sugar beet m^{6}A demethylase

The whole genome database of sugar beet was published (http://bvseq.molgen.mpg.de/index.shtml). The seed sequence of the demethylase conserved domain 2OG-Fe II-oxy(PF13532) was downloaded from Pfam. The *e*-value < 1e^{-5} was set on HMMER(http://www.hmmer.org/), and the beet genome-wide database was searched. Pfam online tool was used to analyze the domain of candidate proteins, and the proteins with the conserved domain were considered to be BvALKB proteins. DNAMAN7.0 was used to multiple sequence alignment of BvALKB proteins, and Weblogo was used for conserved domain identification (http://weblogo.berkeley.edu/).

Bioinformatics analysis of BvALKB family

ExPASY (https://web.expasy.org/protparam/) was used to analyze physical and chemical properties of proteins, including the average molecular weight, isoelectric point, the average number of amino acids, etc(*Gasteiger et al., 2003*). Protein subcellular localization was predicted by CELLO (http://cello.life.nctu.edu.tw/). MapGene2Chrom(http://mg2c.iask.in/mg2c_v2.0/) was used to map the position of genes on chromosomes. MEME (http://meme-suite.org/tools/meme) was used to predict protein motifs(*Bailey et al., 2006*), and the number of searching motifs was set to 20, with other parameters for tacit recognition. Gene intron and exon structures were analyzed in Splign(https://www.ncbi.nlm.nih.gov/sutils/splign/splign.cgi?textpage=online&level=form). A phylogenetic tree (1000 replicates) was constructed by neighbor-joining method using MEGA7 for protein sequence progression and multi-sequence alignment between *Arabidopsis* and sugar beet(*Kumar, Stecher & Tamura, 2016*).

Expression analysis of *BvALKB* genes and gene cloning

Sugar beet samples collected after salt stress treatment were quickly frozen in liquid nitrogen with a mortar and pestle, and ground into a fine powder. Total RNA was extracted using Trizol reagent and the concentration of RNA was determined using the MicroDrop spectrophotometer. Total RNA was reverse transcribed into cDNA by using PrimeScript ™ II 1st Strand cDNA Synthesis Kit(TaKaRa, Japan). In order to detect the gene expression level, qRT-PCR was performed using the CFX96 real-time system and the iTaq^{TM} Universal SYBR Green Supermix Kit(BIO-RAD, USA). The primers were designed using Primer 5 and the sequences were listed(Table 1). In order to avoid experimental error, *UBQ5* and *PP2A* were used as internal controls of leaves, and *25S rRNA* and *PP2A* were used as internal controls of roots. All experiments were repeated at least three times. Data analysis was calculated by $2^{-\Delta\Delta Ct}$ method. The relative expression of each gene was expressed by mean±standard deviation.

PCR primers were designed for genes with significantly different expressions. The PCR product was purified and cloned into pMD19-T vector to construct the recombinant plasmid. The recombinant plasmid was transformed into competent cells of *E. coli* DH5α and the positive clones were screened for the further analysis.

Results

Identification of sugar beet m^{6}A demethylase

The seed sequence of the conserved domain (PF13532) was downloaded from Pfam and searched in the beet genome database by HMMER. A total of 10 homologous proteins were identified, and they were named BvALKBH1B-10B(Table 2). The *e*-value of all the other proteins was less than 1e^{-5} except BvALKBH10B, which was 0.016. Among the 10 candidate proteins, 6 proteins were confirmed to belong to ALKB family by BLAST comparison with NCBI, while BvALKBH2B、BvALKBH3B、BvALKBH8B、BvALKBH10B were not described before and belonged to new ALKB family members.

The sequences of 10 candidate proteins were analyzed by Pfam for conserved domain(Fig. 1). Except BvALKBH10B, all the 9 candidate proteins have complete or partial 2OG-Fe II-Oxy domain, indicating that these proteins are highly conserved. In terms of domain distribution, the domains of BvALKBH6B and BvALKBH8B were at the internal, the domains of BvALKBH7B were at the N-terminus, and the domains of other proteins were all at the C-terminus. The RRM domain of BvALKBH5B was related to mRNA and rRNA processing, RNA output and RNA stability by query. However, due to low sequence similarity, the *e*-value of BvALKBH10B in Pfam database comparison is 0.023, and it has a high possibility of possessing the 2OG-Fe II-Oxy domain, so it will be regarded as a member of this family for subsequent analysis.

The alignment results of DNAMAN7.0 showed certain homology but low conservatism in the domain sequences(Fig. 1). The homology was very high at sites 162, 212, 215, 222, 255, 259, etc, which might be related to the function of the domain and amino acids at these specific locations.

Analysis of physicochemical properties of BvALKB proteins

Physical and chemical properties analysis showed that the average length of the coding region of 10 genes was 1260 bp (783-1755 bp), the average number of amino acids encoding proteins was 416 (260-584), the average molecular weight was 46.41 kDa (28.91-64.97 kDa), and the average isoelectric point was 7.12 (5.11-9.02)(Table 3).

Chromosomal localization of genes

The sugar beet has nine pairs of chromosomes. Chromosome localization analysis showed that each gene tended to be dispersed, and members of this family were found on chromosomes 3 to 8, while *BvALKBH2B*, *BvALKBH3B* and *BvALKBH9B* were concentrated on chromosome 7(Fig. 1). *BvALKBH10B* has no specific location information and is only shown on chromosome 7, probably located in the gap region of fragments splicing from whole gene sequencing.

Phylogenetic relationships and gene structures analysis of BvALKB

Multiple sequence alignment was performed on 14 ALKB family proteins of *Arabidopsis* and 10 proteins of sugar beet using MEGA7, and the alignment diagram of protein local domain was analyzed(Fig. 2). For the convenience of observation, proteins with high sequence similarity were compared together, and it could be seen that the reason for the low homology of each domain might be that the domain similarity of different subclasses was not high, and they were the same only at some special sites.

Then a phylogenetic tree (1000 replicates) was constructed using neighbor-joining method to observe the evolutionary relationship between *Arabidopsis* and sugar beet(Fig. 3). It could be seen that most of the bootstrap values are greater than 70, indicating high reliability. All the proteins were divided into five categories: Class I(AtALKBH9-like) includes BvALKBH6B and BvALKBH8B, which are similar to AtALKBH9; Class II(AtALKBH10-like) only contains

BvALKBH10B, which is similar to AtALKBH10; Only one BvALKB protein belongs to Class III(AtALKBH2-like); Class IV(AtALKBH6/8-like) consists of BvALKBH5B, BvALKBH7B and BvALKBH9B; Three members are assigned to Class V(AtALKBH1-like), including BvALKBH1B, BvALKBH2B and BvALKBH3B(Fig. 3). AtALKBH9B and AtALKBH10B in the first two classes have been confirmed to be m^{6}A demethylases, so BvALKBH6B, BvALKBH8B and BvALKBH10B are likely to also have demethylation functions, which should be focused on.

The gene structure analysis revealed that all genes contain introns and are broken genes(Fig. 3). Generally speaking, genes within the same group showed similar intron and exon organization. *BvALKBH6B* and *BvALKBH8B* of Class I have 6 exons, while *BvALKBH2B* and *BvALKBH3B* of Class V have 4 exons, which are due to their sequence similarity. *BvALKBH5B* and *BvALKBH7B* in Class IV are similar in structure, although the number of exons is different. Other genes, such as *BvALKBH1B* and *BvALKBH9B*, are more or less different in structure from similar genes(Fig. 3).

Motifs analysis and subcellular localization prediction of BvALKB proteins

Set the expected number of searching motifs as 20 on MEME, and the search results are sorted from small to large by *e*-value(Fig. 4). In general, almost all of the 10 proteins except BvALKBH10B have motifs 1, 2, 4, and 8, which are probably important components of the 2OG-Fe II-Oxy domain. Proteins belonging to the same group had similar motif composition. BvALKBH6B, BvALKBH8B and BvALKBH10B homologous to AtALKBH9B/10B differ from other proteins in motif composition because they have closely connected motif 3 and motif 6, which may be related to demethylation function.

The scores of different locations of CELLO predicted proteins showed that most of the proteins were located in the nucleus and mainly performed function of demethylation in the nucleus(Table 3). Individual proteins such as BvALKBH10B was located in the cytoplasm, and BvALKBH7B was located in the cytoplasm and extracellular, indicating that they may perform other extranuclear functions.

Quantitative analysis of *BvALKB* genes in sugar beet under salt stress

m^{6}A plays an important role in response to abiotic stresses. In order to understand the changes of potential m^{6}A demethylation genes in sugar beet under salt stress, we compared the expression level of the genes under normal condition and salt stress. The phenotypic changes of sugar beet cultured to three pairs of true leaves were observed by 300 mM salt stress, and the expression of each gene was analyzed by qRT-PCR.

In leaves, all the other genes were up-regulated or down-regulated to varying degrees except *BvALKBH1B*. *BvALKBH2B*, *BvALKBH4B* and *BvALKBH10B* were up-regulated, especially *BvALKBH10B* was highly up-regulated(Fig. 5). *BvALKBH3B*, *BvALKBH5B*, *BvALKBH6B*, *BvALKBH7B*, *BvALKBH8B* and *BvALKBH9B* were down-regulated, and *BvALKBH9B* was significantly down-regulated. In root, *BvALKBH1B*, *BvALKBH3B*, *BvALKBH6B*, *BvALKBH8B* and *BvALKBH9B* were up-regulated, while the other five genes were down-regulated. *BvALKBH2B*, *BvALKBH4B* and *BvALKBH5B* were down-regulated significantly(Fig. 5). Different expression levels in leaves and roots suggest that the expression of these genes is tissue-specific.

Cloning of *BvALKBH10B* gene

Considering that BvALKBH10B is a homologous protein of AtALKBH10B with high expression and significant difference, we designed PCR primers (F:5'-GGAATTCATGTCGCCGGCGGCGGGACCATTGT-3', R:5'-GGGATCCTCACATTATCCTTCCTTCCACACCTGGGTCAGACATGGT-3') and cloned *BvALKBH10B* gene from Beet "O" 68 and sequenced it. The sequencing results were submitted to the Genbank database, and the accession number was MZ358117, which was consistent with the whole genome database of sugar beet.

Discussion

Soil salinization has become a global problem. In China, saline-alkali land is mainly distributed in northwest, northeast and north China, and highly coincides with sugar beet production area, which puts forward higher requirements for sugar beet salt tolerance. Previous studies have shown that ALKB family proteins are involved in plant growth and development and abiotic stress processes, especially the proteins confirmed as m^{6}A demethylases. However, the ALKB family members in sugar beet have not been studied. Therefore, bioinformatics and quantitative methods were used to study the response of ALKB proteins in sugar beet under salt stress and the theoretical basis for screening m^{6}A demethylase in sugar beet was put forward.

Through the beet genome-wide analysis, we found 10 BvALKB family proteins. The number was similar to *Arabidopsis* (14) and rice (12), but far less than which of wheat (29) and quinoa (27) (*Yue et al., 2019*), which might be caused by different copy number during plant evolution.

Phylogenetic analysis can quickly identify the homology and evolutionary relationships of proteins. The phylogenetic tree of BvALKB proteins and AtALKB proteins was constructed using neighbor-joining method. Proteins with high homology to AtALKBH9B/10B could be considered as potential m^{6}A demethylases. All the proteins were divided into five categories. The Class I contains AtALKBH9A/9B/9C proteins, and two BvALKB proteins (BvALKBH6B and BvALKBH8B) belong to this group. The Class II contains AtALKBH10A/10B proteins, with only BvALKBH10B belonging to it. Therefore, BvALKBH6B, BvALKBH8B and BvALKBH10B are likely to be potential m^{6}A demethylases. Only one BvALKB protein belongs to Class III and may be involved in protecting plants from DNA methylation damage(*Meza et al.*, *2012*). Three BvALKB proteins belong to Class IV, which may participate in tRNA modification and DNA repair(*Leihne et al., 2011*; *Zdżalik et al., 2014*). Three BvALKB proteins belong to Class V, associated with redox and tRNA modifications in cytoplasm and mitochondria (*Kawarada et al., 2017*). Most of the BvALKB proteins within a group possessed a similar exon/intron structure, which suggesting their homology.

Subcellular localization analysis indicated that most proteins were located in the nucleus, while some proteins were located in the cytoplasm and extracellular, which might play different roles in transcriptional regulation. Almost all the proteins have the 2OG-Fe II-Oxy domain, suggesting that m^{6}A is evolutionarily conservative.

The structure of a protein determines its primary function. Motif analysis of BvALKB proteins showed that motif 1, 2, 4 and 8 constituted the conserved domain, and the location of these motifs was consistent with that of the previously identified domain. Due to the conservatism of evolution, the composition of the motifs of BvALKB proteins in a group is basically similar. Notably, the three homologous proteins to AtALKBH9B /10B contained unique motif 3 and motif 6, suggesting that they may be involved in demethylation function.

The expression profiles of sugar beet leaves and roots under normal and salt stress conditions were analyzed. In leaves, all other genes except *BvALKBH1B* were induced or inhibited by salt stress. In roots, five genes were up-regulated while five genes were down-regulated, and three genes were highly down-regulated. Except for *BvALKBH5B* and *BvALKBH7B*, the other eight genes showed opposite expression trends in leaves and roots, suggesting tissue specificity of gene regulation. We paid the most attention to the gene expression levels of three homologous proteins. *BvALKBH6B* and *BvALKBH8B* were down-regulated in leaves, while *BvALKBH10B* was significantly up-regulated, and the opposite trend was observed in roots. *BvALKBH10B* is homologous to *AtALKBH10B*, although the *e*-value was minimal in the initial HMMER search. *BvALKBH10B* with high expression and obvious expression differences was selected for cloning and submitted to GenBank, and the accession number was MZ358117. The significant changes in *BvALKBH10B* expression level indicate our strong concern in subsequent functional verification experiments and provide a basis for the study of salt tolerance of sugar beet.

Conclusions

This study identified 10 sugar beet ALKB family proteins. We used bioinformatics method to analyze its gene structures, chromosome location, physical and chemical properties of protein, motifs, subcellular localization and the phylogenetic tree construction etc, and quantitatively comparing the expression of BvALKB under normal conditions and salt stress. In addition, homologous *Arabidopsis* m⁶A demethylase proteins were screened and identified as potential sugar beet m⁶A demethylases, which laid a foundation for further research on its function and provided ideas for the cultivation of new salt-tolerant strains.

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Figure 1

Conserved domain analysis and chromosome localization of BvALKBs.

(A)Conserved domain analysis of BvALKB proteins. (B)Chromosome localization of BvALKB genes. (C)Sequence analysis of the conserved domain in BvALKB proteins.

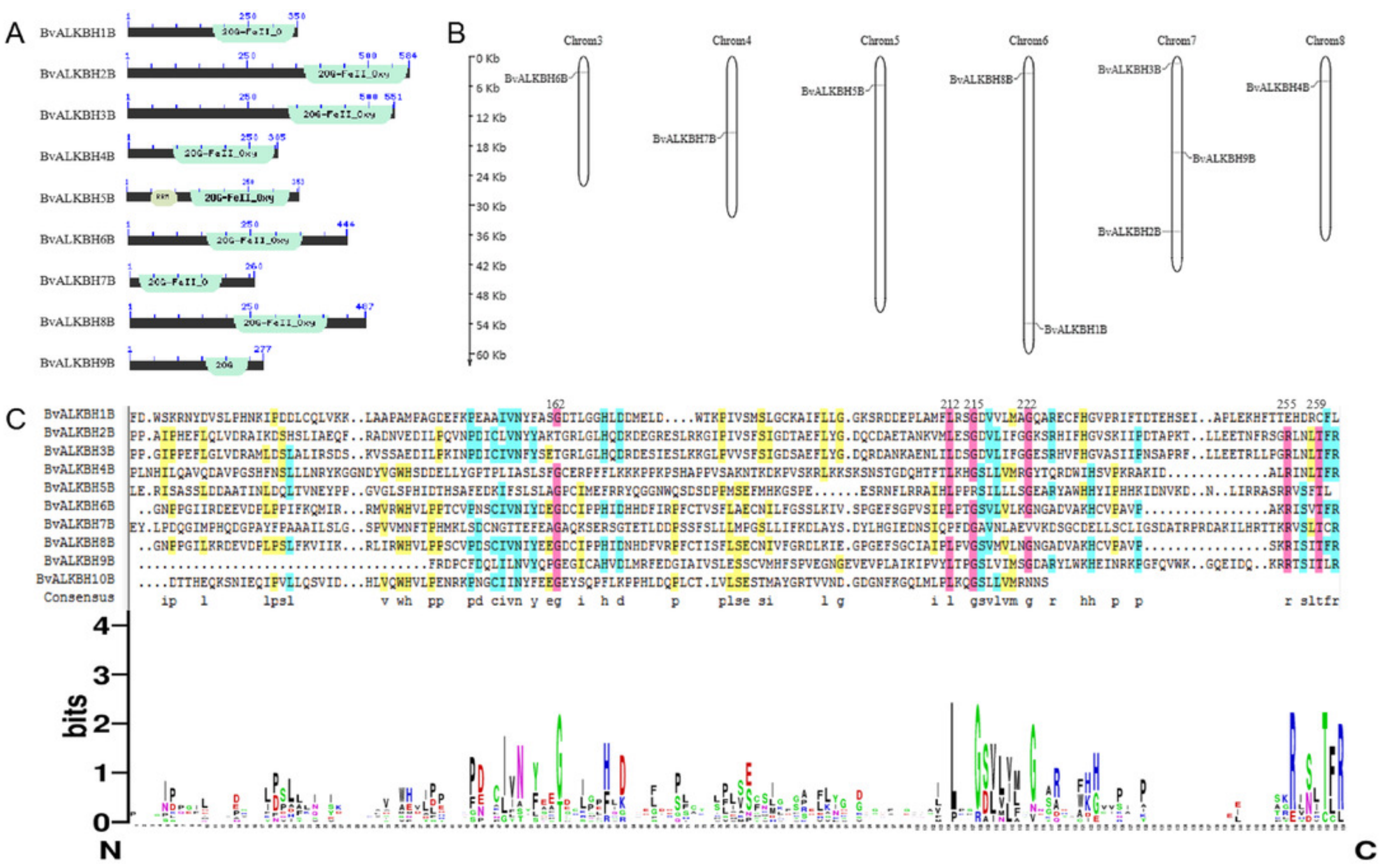

Figure 2

Multiple sequence alignment between BvALKB and AtALKB proteins.

Different colors represent residues with different characteristics.

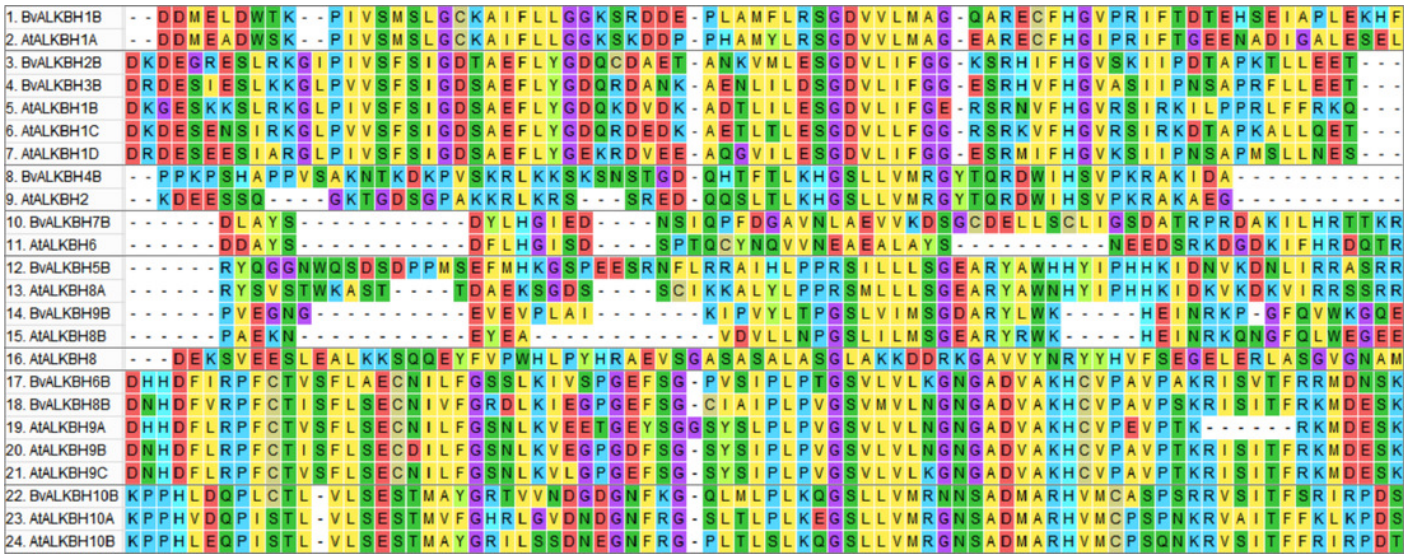

Figure 3

Phylogenetic relationships and gene structures of BvALKBs.

(A)Phylogenetic relationships of BvALKB and AtALKB proteins. The gene class is represented in a different color on the right side of the rootless tree. (B)Gene structures of BvALKB genes.Exon/intron structures of the BvALKB genes are represented in diffenrent ways. Exons and introns are represented by yellow box and lines, respectively.

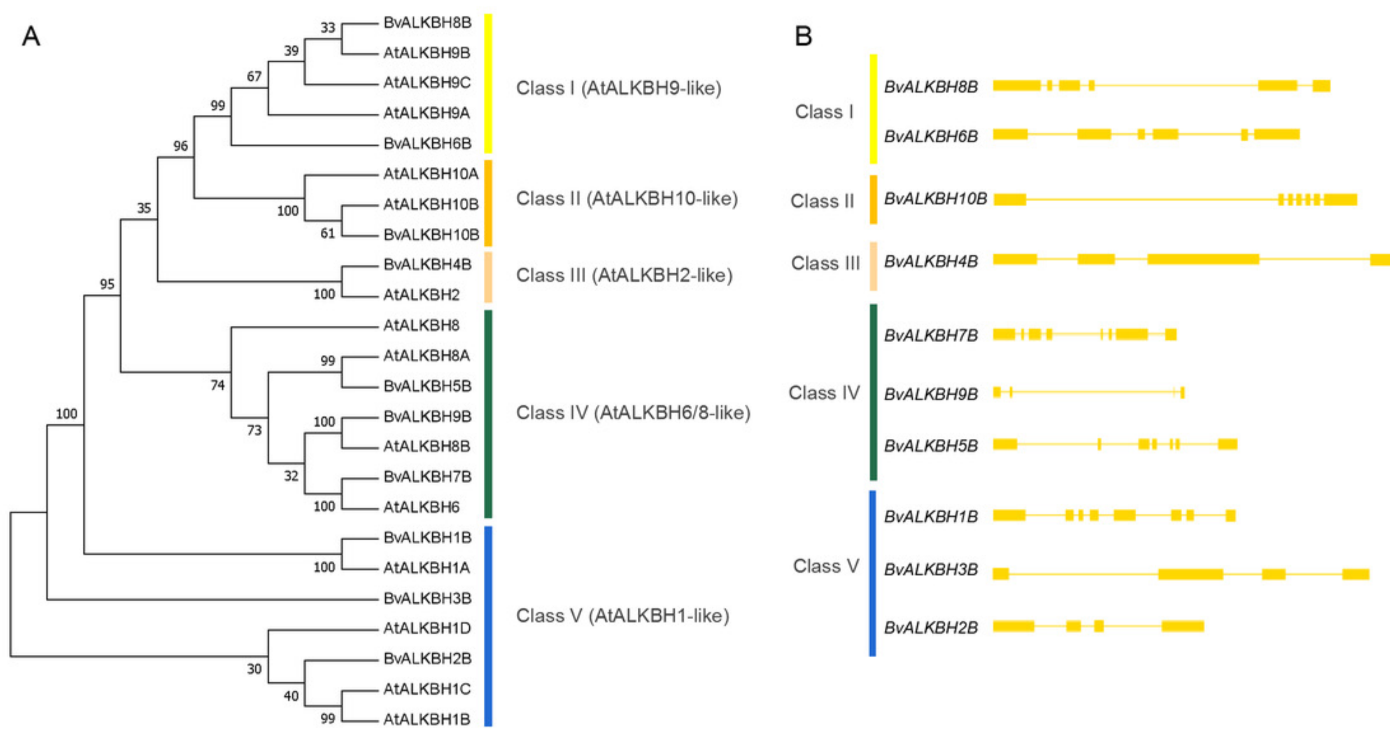

Figure 4

Motif Analysis of BvALKB proteins.

(A)Motifs in BvALKB proteins.The motifs were arranged according to the e-value from small to large, the letters in each motif were amino abbreviation. The size of the letter represented the saliency of the amino acid in the motif. The larger the letter, the higher the saliency, which is, the higher the frequency at which the amino acid appears in the same position in the same motif in different sequences. (B)Analysis of BvALKB proteins motifs. The different color blocks correspond to different motifs. The width of the color block is the length of the motif. The height of the color block represents the saliency of the motifs in the sequence. The higher the saliency, the more able to match the predicted motifs.

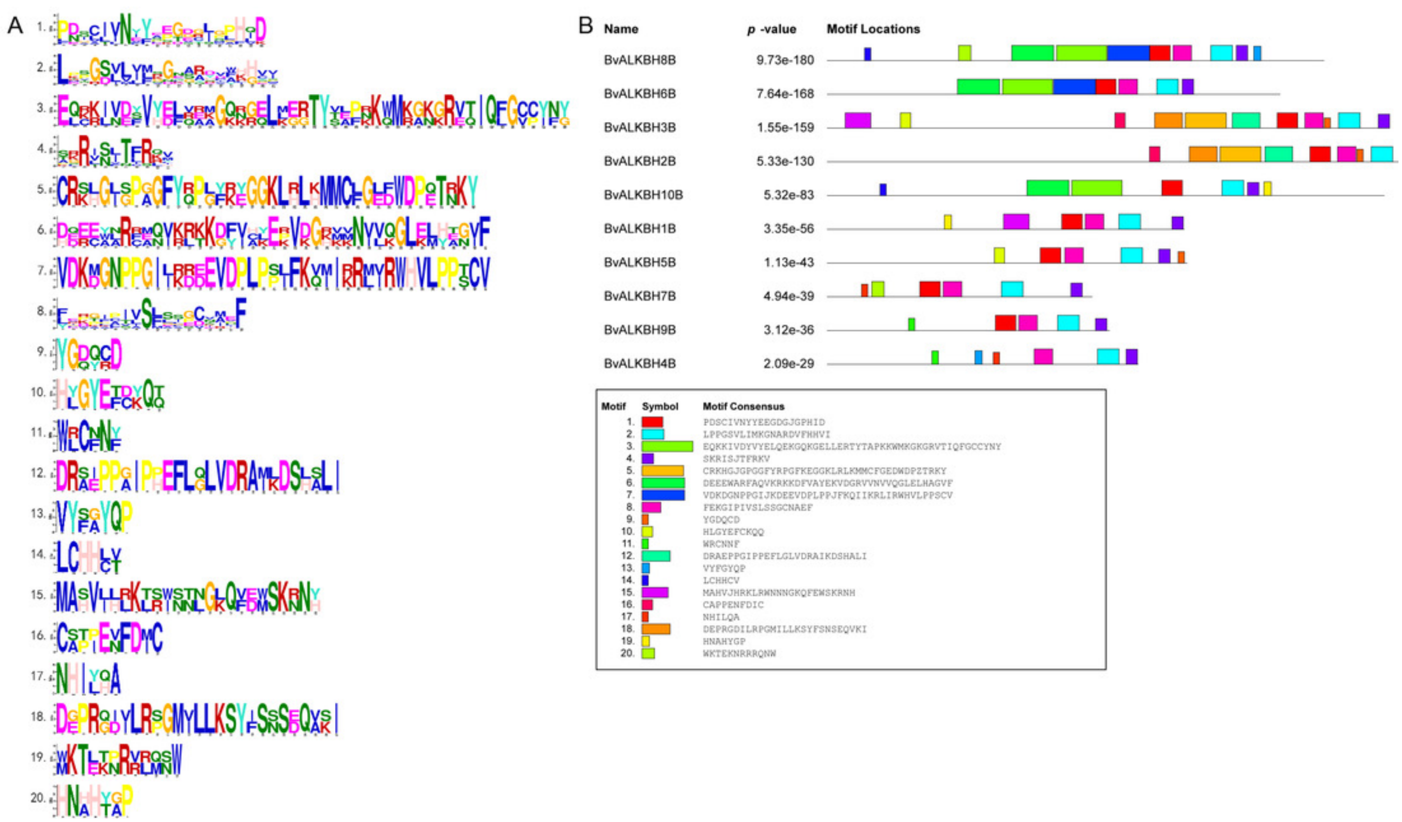

Figure 5

Expression analysis of BvALKB genes under salt stress.

(A)Expression analysis of BvALKB genes in leaves in response to salinity stress. (B)Expression analysis of BvALKB genes in roots in response to salinity stress. Error bars indicate standard deviation. *and**indicate statistically significant differences, as determined by Student's t tests, at p<0.05 and p<0.01, respectively.

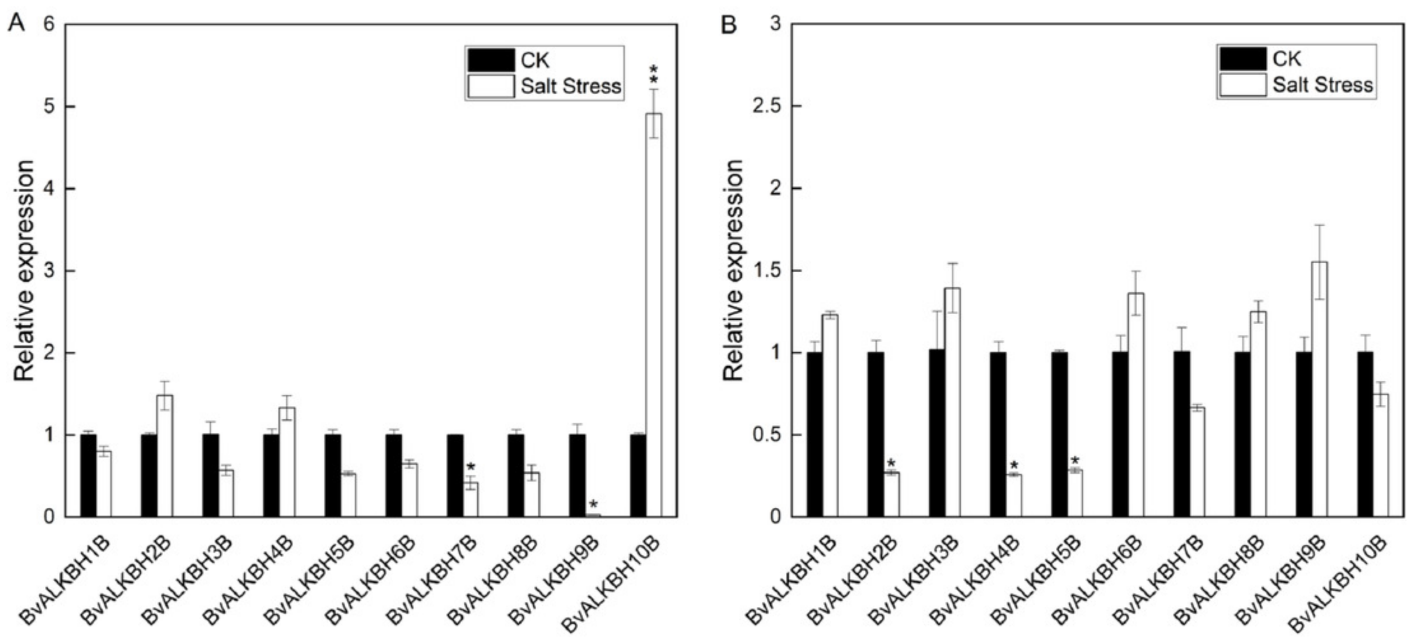

Table 1(on next page)

Primer sequences of BvALKB genes

Table 1 **Primer sequences of BvALKB genes**

Gene	Forward primer(5'-3')	Reverse primer(5'-3')
UBQ5	TCTGCTGGAAGAGCCTTTGG	TTGTCGCCGCTCTTTACACT
25S rRNA	AGACAAGAAGGGGCAACGAG	CACATTGGACGGGGCTTTTC
BvPP2A	TCGTGTCCAAGAAGTGCCTC	CACAACGGTCATCAGGGTCA
BvALKBH1B	AGGGAATGCTTTCATGGGGT	CTCGAACCAAGCTATCCGGG
BvALKBH2B	GTACTTCCAATAAAACGTCACCGT	GTTTTCAGATGAATCACATGTGCCA
BvALKBH3B	TAGCTCGGAACAGGCGAAAA	TGTGGAATTGCCGGTGGTAT
BvALKBH4B	CATATTCTCCAGGCGGTCCA	GGCGTTCACAACCAAAGGAA
BvALKBH5B	AGTCCGGAGGAGTCCAGAAA	AGGTCCTGTTCTGACCTTGC
BvALKBH6B	AAACGGCAGCTTATGGAACG	ATGGGAGGCAAGGGATCAAC
BvALKBH7B	GGCTTTACAGTCGGCTCTGT	GTCAGCCAAGGAGGCAAGTC
BvALKBH8B	TTCCCTTGCCTGTTGGATCG	GCAAAATACACAGGCCGCTT
BvALKBH9B	TACCAGCCAGGTGAGGGTAT	CGAGCATCGCCTGACATGAT
BvALKBH10B	GGTGGGAAACAAGGGAGGAG	CCTCATGTGAGCCTGTGTCA

Table 2 (on next page)

Basic information of BvALKB.

1 Table 2 **Basic information of BvALKB.**

gene name	BvALKB name	NCBI Reference Sequence	Gene ID	Description
Bv6_150770_huzh	BvALKBH1B	XM_010684461.2	104897561	PREDICTED: Beta vulgaris subsp. vulgaris alpha-ketoglutarate-dependent dioxygenase alkB (LOC104897561)
Bv7_169620_pkhc	BvALKBH2B	XM_010686965.2	104899719	PREDICTED: Beta vulgaris subsp. vulgaris hypothetical protein
Bv7_157650_ryeg	BvALKBH3B	XM_010685256.2	104898211	PREDICTED: Beta vulgaris subsp. vulgaris uncharacterized LOC104898211
Bv8_184320_kacr	BvALKBH4B	XM_010688312.2	104900793	PREDICTED: Beta vulgaris subsp. vulgaris DNA oxidative demethylase ALKBH2
Bv5_102160_pgse	BvALKBH5B	XM_010678383.2	104892444	PREDICTED: Beta vulgaris subsp. vulgaris alkylated DNA repair protein alkB homolog 8
Bv3_051230_eskg	BvALKBH6B	XM_010673069.2	104888178	PREDICTED: Beta vulgaris subsp. vulgaris RNA dementhylase ALKBH5
Bv4_083160_sqec	BvALKBH7B	XM_010676670.2	104891030	PREDICTED: Beta vulgaris subsp. vulgaris alpha-ketoglutarate-dependent dioxygenase alkB homolog 6

Bv6_130050_njrf	BvALKBH8B	XM_010681565.2	104895138	PREDICTED: Beta vulgaris subsp. vulgaris uncharacterized LOC104895138
Bv7_164580_swwm	BvALKBH9B	XM_010686203.2	104899068	PREDICTED: Beta vulgaris subsp. vulgaris alkylated DNA repair protein alkB homolog 8
Bv7_179400_uxaj	BvALKBH10B	XM_010698038.2	104908870	PREDICTED: Beta vulgaris subsp. vulgaris hypothetical protein

2

Table 3(on next page)

Physical and chemical properties analysis of BvALKB proteins

Table 3 **Physical and chemical properties analysis of BvALKB proteins**

BvALKB name	ORF(bp)	Amino acid	Molecular weight(Da)	PI
BvALKBH1B	1053	350	39477.03	7.13
BvALKBH2B	1755	584	64923.52	7.15
BvALKBH3B	1656	551	60969.22	8.74
BvALKBH4B	1018	305	34594.96	9.02
BvALKBH5B	1062	353	39620.72	6.53
BvALKBH6B	1335	444	49776.81	8.86
BvALKBH7B	783	260	28912.06	5.70
BvALKBH8B	1464	487	54949.39	6.62
BvALKBH9B	834	277	30792.26	5.11
BvALKBH10B	1641	546	60084.61	6.30