Tissue Expression Pattern and Bioinformatics Analysis of *OsKMP2* Gene in Rice

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Abstract Kinesins are a superfamily of proteins widely present in eukaryotes, playing crucial roles in plant cell wall assembly, cell elongation regulation, gravity sensing, and fertility control. In this study, bioinformatics analysis of the *OsKMP2* gene (LOC_Os02g28850) was performed using online tools such as ExPASy-ProtParam, ProtScale, CD-search, and DNAMAN software. Additionally, qRT-PCR was employed to analyze the tissue expression pattern of *OsKMP2*. The results showed that the molecular weight of the OsKMP2 is 118.397 28 kDa, and it is a hydrophilic and unstable acidic protein. Secondary structure prediction revealed that it primarily consists of α-helices (69.45%), random coils (25.19%), and extended strands (5.36%). The gene was expressed in various rice tissues, with the highest expression level observed in leaves. These results indicate that the *OsKMP2* gene exhibits high evolutionary conservation and functional diversity in rice.

Key words Rice; *OsKMP*; Tissue expression pattern; Bioinformatics analysis

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Kinesins are a protein superfamily ubiquitous in eukaryotes and function as molecular motors. They utilize energy released from ATP hydrolysis to propel themselves and the cargo carried at their tails, moving along microtubules to deliver the "cargo" to specific cellular domains[1]. They play a critical biological role in intracellular transport, mitosis, and meiosis[2]. One end of each kinesin contains a catalytic core domain called the "head," followed by a stalk. The "head" and stalk are connected by a neck linker, and finally, there is a "tail" domain[3]. The "head" domain is responsible for ATP hydrolysis and enables kinesin to move along microtubules. The stalk mediates dimerization and contains coil-helix interaction domains, while the tail binds specific cargo[4-5]. The kinesin superfamily comprises 14 subfamilies (kinesin1-kinesin14) plus two additional branches, kinesin14A and kinesin14B. Different kinds of kinesins perform distinct functions[6]. *Arabidopsis thaliana* contains 61 kinds of kinesins, while japonica and indica rice possess 41 and 45 kinds of kinesins respectively. Current studies on rice kinesins reveal that OsPSS1, a member of kinesin-1, plays a critical role in regulating meiotic chromosome behavior during male gametogenesis and fertility control[7]. BC12, a member of the rice kinesin-4 family, participates in cell cycle progression and modulates cell wall properties[8-9]. OsKinesin-13A utilizes its microtubule depolymerization activity to promote microtubule turnover, thereby influencing the orientation of rice cellulose microfibers and cell elongation in rice, ultimately influencing glume length[10].

Grain size is a crucial agronomic trait that influences both rice yield and quality. Each rice grain primarily consists of an outer hull and an inner caryopsis. After fertilization, the caryopsis begins to grow following hull maturation. However, since the caryopsis develops within the mature hull, it is reasonable to hypothesize that the size of the outer hull may constrain the size of the caryopsis, thereby serving as a major determinant of the final grain size[11]. In the study of kinesins, the loss of SRS3, a member of the kinesin-13 subfamily, results in smaller seeds, indicating that SRS3 regulates cell elongation during rice seed formation[12-13]. Research on kinesin-like proteins revealed that the SGL protein modulates grain length and plant height by influencing the expression levels of genes related to GA synthesis and response in rice[14].

Rice is one of the most important staple crops globally, and its yield and quality are crucial for China's food security. Currently, research on how kinesin genes influence grain size in rice remains limited. In this study, with kinesin *OsKMP2* as the research object, the physicochemical properties, conserved functional domains, secondary and tertiary structures and subcellular localization of the OsKMP2 were analyzed using tools such as ExPASy-ProtParam, CD-search, SOPMA, Phyre2 and PSORT, aiming to enhance the understanding of the rice kinesin family and provide new theoretical foundations for future genetic improvement of rice.

Materials and Methods

Experimental materials

The indica rice variety T98B, provided by China National Hybrid Rice Research and Development Center, was used for RNA extraction. The amino acid sequences of kinesin gene family members pss1, DBS1, OsSRS3, KLP, K16, and BC12 were retrieved from NCBI and analyzed.

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Bioinformatics prediction of OsKMP2 protein

The physicochemical properties of the OsKMP2 were analyzed using the online tool ExPASy-ProtParam. Hydrophilicity and hydrophobicity were assessed with the Protscale online software. The conserved functional domains of the protein were analyzed via the online tool CDD. The secondary structure was predicted using the online tool SOPMA, while the tertiary structure was modeled with the Phyre2 software. Subcellular localization was predicted using the online website Psort.

Homologous gene sequence alignment

The sequence of *OsKMP2* was aligned with *pss1*, *DBS1*, *OsSRS3*, *KLP*, *K16* and *BC12* using the DNAMAN software. The websites of the mentioned software tools are listed in Table 1.

Table 1 Names and websites of analysis software

Name	Website
ExPASy-Protparam	https://web.expasy.org/protparam/
Protscale	https://web.expasy.org/protscale/
CDD	https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
SOPMA	https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html
Phyre2	http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index
Psort	https://www.genscript.com/psort.html
NCBI-BLAST	https://blast.ncbi.nlm.nih.gov/Blast.cgi

Analysis on tissue expression pattern of *OsKMP2*

Total RNA was extracted from roots, stems, leaves, stem nodes, and panicles of the japonica rice variety T98B using TRIzol Reagent (Invitrogen, USA). Reverse transcription of RNA was performed following the protocol of StarScript Pro ALL-in-one RT Mix with gDNA Remover (GenStar). Specific primers were designed using software Primer Premier 5.0 as follows: *OsKMP2*-F: 5'-GATACATGGGCAGGGGTTCA-3' and *OsKMP2*-R: 5'-ATGCTGGAAGCACACAACTG-3'. With the reverse transcription product as template and the Acting gene in rice as internal reference, the expression pattern of *OsKMP2* in different tissues was analyzed by quantitative real-time PCR (qRT-PCR) with three replicates for each sample. The PCR reaction system contained: DNA template 1 μl, forward primer (10 μM) 0.5 μl, reverse primer (10 μM) 0.5 μl, 2 × RealStar Fast SYBR qPCR MIX 10 μl, High/Low ROX Reference Dye 0.4 μl, and sterile water added to 20 μl. The PCR program was started with denaturation at 95 ℃ for 2 min, followed by 40 cycles of denaturation at 95 ℃ for 15 s and annealing at 60 ℃ for 20 s.

Results and Analysis

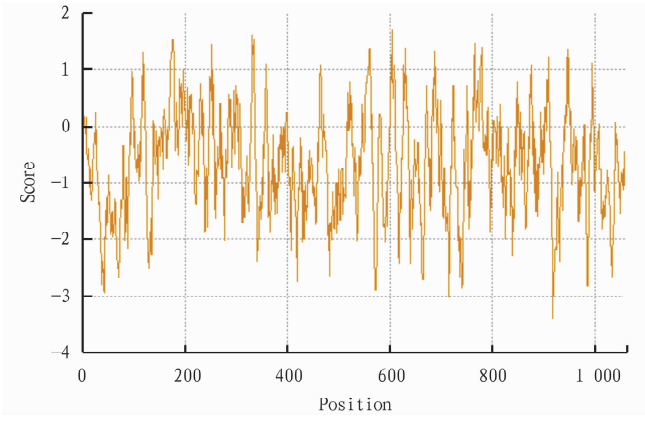
Bioinformatics analysis of OsKMP2

Physicochemical properties of OsKMP2 The *OsKMP2* gene encodes a protein consisting of 1064 amino acids. Analysis of the physicochemical properties using the online tool ExPASy-ProtParam revealed that the molecular formula is $C_{5\,100}H_{8\,191}N_{1\,499}O_{1\,673}S_{35}$, with a relative molecular weight of 118.397 28 kDa. The theoretical isoelectric point (pI) is 5.27. The protein is rich in strongly basic amino acids (including Arg and Lys, 129 in total) and strongly acidic amino acids (including Asp and Glu, 167 in total). The instability index was calculated to be 54.41, classifying it as an unstable protein. It has a fat coefficient of 77.39. Amino acid composition analysis showed that glutamic acid (Glu), serine (Ser), leucine (Leu), alanine (Ala), arginine (Arg), valine (Val) and glutamine (Gln) accounted for relatively high proportions at 10.4%, 9.7%, 9.2%, 7.8%, 6.6%, 5.9%, and 5.6%, respectively, while tyrosine (Tyr) and tryptophan (Trp) showed lower proportions at 1.5% and 0.8%, respectively (Table 2). The hydrophilicity prediction of OsKMP2 revealed that most of its amino acids are hydrophilic, indicating it is a hydrophilic protein (Fig. 1). In conclusion, OsKMP2 is an acidic, unstable and hydrophilic protein.

Table 2 Amino acid contents of OsKMP2 protein

Amino acid	Number	Percentage//%
Ala (A)	83	7.8
Arg (R)	70	6.6
Asn (N)	50	4.7
Asp (D)	56	5.3
Cys (C)	18	1.7
Gln (Q)	60	5.6
Glu (E)	111	10.4
Gly (G)	59	5.5
His (H)	24	2.3
Ile (I)	45	4.2
Leu (L)	98	9.2
Lys (K)	59	5.5
Met (M)	17	1.6
Phe (F)	27	2.5
Pro (P)	52	4.9
Ser (S)	103	9.7
Thr (T)	45	4.2
Trp (W)	8	0.8
Tyr (Y)	16	1.5
Val (V)	63	5.9

Prediction of conserved domains in OsKMP2 The conserved domains of OsKMP2 were predicted using the CD-search tool on NCBI website. OsKMP2 showed specific matches (Superfamilies) to PLN03188 and non-specific matches to KISc_KLP2_like (Fig. 2). Therefore, OsKMP2 belongs to the PLN03188 superfamily (kinesin-12 family protein).



The score value represents the hydrophobicity score of amino acid residues. Higher values indicate stronger hydrophobicity and lower values indicate stronger hydrophilicity.

Fig. 1 Hydrophobicity/hydrophilicity prediction analysis of OsKMP2 protein

Secondary structure prediction of OsKMP2 The secondary structure of OsKMP2 was predicted using the online SOPMA tool. The results showed that the secondary structure primarily consists of α-helices (69.45%), random coils (25.19%), and extended strands (5.36%) (Fig. 3). Therefore, the spatial conformation of this protein crystal is likely based on an α-helix structure.

Tertiary structure prediction of OsKMP2 Prediction analysis of the OsKMP2 tertiary structure model using the online Phyre2 software revealed that the protein is formed by connection of α-helices, random coils, and extended strands (Fig. 4).

Subcellular localization of OsKMP2 Prediction analysis using the online Psort tool indicated that this protein is most likely localized in the nucleus, followed by potential localization in mitochondria and the cytoplasm, and less likely located in the cytoskeleton (Table 3).

Table 3 Subcellular localization of OsKMP2

Subcellular structure	Possibility//%
Nucleus	52.2
Mitochondrion	26.1
Cytoplasm	17.4
Cytoskeleton	4.3

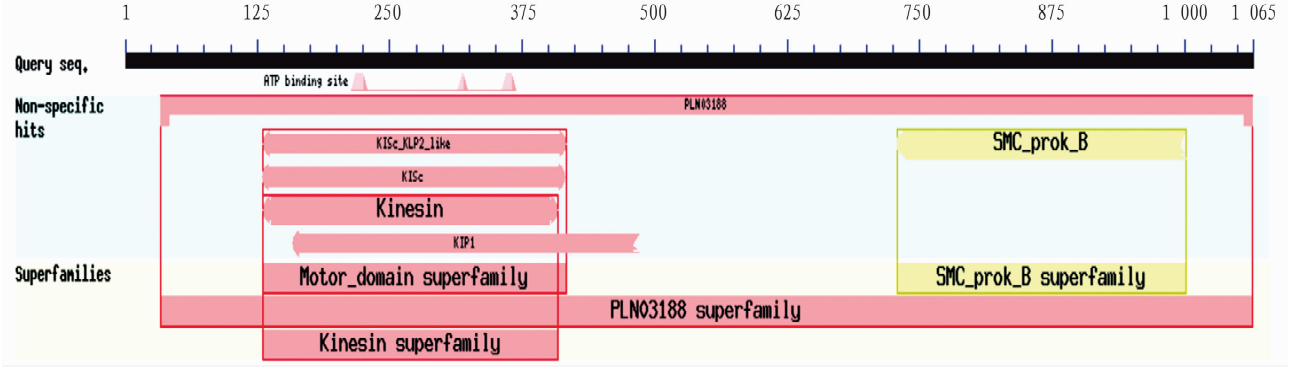
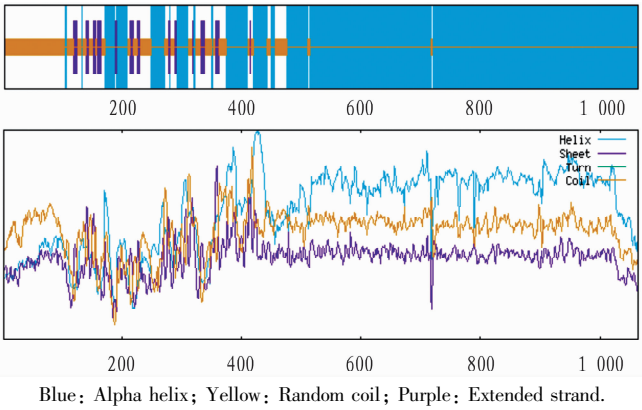


Fig. 2 Conserved domains in OsKMP2 protein



Blue: Alpha helix; Yellow: Random coil; Purple: Extended strand.

Fig. 3 Secondary structure prediction of OsKMP2

Homologous sequence alignment and evolutionary analysis of OsKMP2

Sequence alignment analysis on OsKMP2 and its homologous genes (*pss1*, *DBS1*, *OsSRS3*, *KLP*, *K16*, and *BC12*) revealed that the ORF sequence of *OsKMP2* is 3 195 bp, while those of *pss1*, *DBS1*, *OsSRS3*, *KLP*, *K16* and *BC12* are 1 434, 2 865, 3 299, 3 180, 3 024 and 3 107 bp respectively. Alignment of ORF sequences of *OsKMP2* and homologous genes *pss1*, *DBS1*, *OsSRS3*, *KLP*, *K16* and *BC12* using DNAMAN software showed an overall identity of 46.86%, and individual identity values to homologous *pss1*, *BC12*, *OsSRS3*, *DBS1*, *K16* and *KLP* being 12.28%, 26.67%, 27.56%, 30.27%, 31.25% and 33.72%, respectively (Fig. 5).

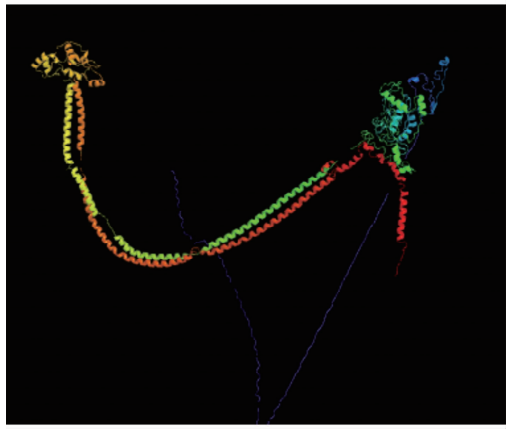


Fig. 4 Tertiary structure prediction of OsKMP2

Fig. 5 ORF sequence alignment of *OsKMP2* with *pss1*, *DBS1*, *OsSRS3*, *K16*, *BC12* and *KLP*

Tissue-specific expression analysis of *OsKMP2* gene

Initially, total RNA was extracted from rice roots, stems, leaves, stem nodes and panicles using a specific method and reverse-transcribed into cDNA. Subsequently, quantitative real-time PCR (qRT-PCR) was employed to examine the expression levels of the *OsKMP2* in different tissues. The results showed that the overall expression level of the *OsKMP2* was relatively high, and the expression was detected in roots, stems, leaves, stem nodes, and panicles. The highest expression was observed in leaves, followed by panicles, while the expression levels in stems and stem nodes were very low, and the level in roots was relatively low (Fig. 7). These findings suggest that the function of OsKMP2 may exhibit potential diversity in different plants.

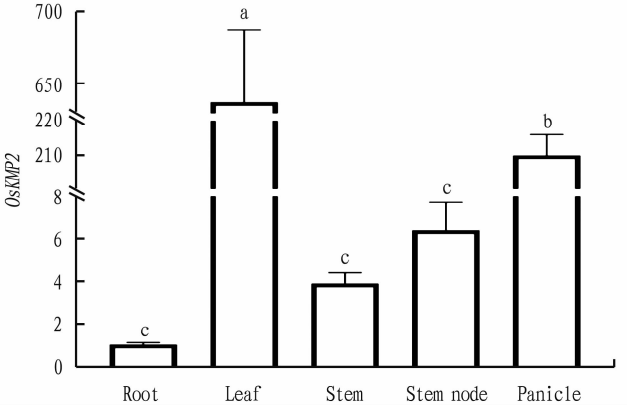


Fig. 6 Expression of *OsKMP2* gene in different tissues of rice

Conclusions and Discussion

This study revealed that *OsKMP2* was expressed at varying levels in rice roots, stems, leaves, stem nodes, and panicles, with the highest expression in leaves, followed by panicles, while the expression was very low in stems and stem nodes and relatively low in roots. Bioinformatics analysis indicated that OsKMP2 is a hydrophilic, conserved and unstable acidic protein belonging to the PLN03188 superfamily (kinesin-12 family protein; Provisional). Secondary structure prediction revealed that the spatial conformation of this protein crystal is likely primarily composed of α-helix structures. The tertiary structure of the OsKMP2 is formed by connection of α-helices, random coils and extended strands. Furthermore, subcellular localization prediction indicated that OsKMP2 is most likely localized in the nucleus, followed by potential localization in mitochondria and the cytoplasm, and less likely localized in the cytoskeleton. These findings suggest that OsKMP2 most likely performs its functions in the nucleus. However, the specific functions of OsKMP2 in the nucleus remain unclear. Studies will be further conducted on OsKMP2 to explore its potential beneficial roles for developing new rice varieties in the future.

Tissue expression pattern analysis revealed that *OsKMP2* was expressed in various tissues, with notably high expression in leaves. Prediction using ExPASy-ProtParam indicated that OsKMP2 is a hydrophilic, conserved and unstable acidic protein. Subcellular localization prediction suggested that this protein likely functions in the nucleus, where it may regulate essential biological processes and mechanisms in rice in a high-level and conserved manner.

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