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Functional Analysis of the Castor Bean RcMsc2 Gene Postprint

Authors: Geng Liuting, Li Yanxiao, Zhang Chunlan, Yu Zhongyong, Wang Guiling, Xiang Dianjun, Liu Peng

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Abstract

G2/mitotic-specific cyclin-2 (Msc2) serves as a key regulatory protein in higher plants' response to stress, participating in multiple stress resistance responses. To investigate the function of the RcMsc2 gene, this study successfully cloned RcMsc2 from castor bean leaf tissue, analyzed the structure and potential function of the RcMsc2 protein using bioinformatics, and examined the tissue expression characteristics and abiotic stress expression characteristics of the RcMsc2 gene by qRT-PCR. The results showed that: (1) The RcMsc2 gene is located on the long arm of chromosome 5 in castor bean, with a CDS (coding sequence) region of 1,299 bp, encoding 432 amino acids. (2) The RcMsc2 protein possesses a cyclin family characteristic domain, is an unstable acidic hydrophilic protein, lacks transmembrane domains and signal peptides, and has a relative molecular weight of 49.38 kD. (3) The secondary and tertiary structures of the RcMsc2 protein are primarily composed of α-helices and random coils. (4) The RcMsc2 protein exhibits the highest sequence homology with CYCB2 proteins from Jatropha curcas and Hevea brasiliensis, and all are clustered into Group II. (5) The RcMsc2-GFP fusion protein is localized in the nucleus. (6) The RcMsc2 gene is expressed in all castor bean tissues, primarily functioning in roots and stems; abiotic stress analysis revealed that the RcMsc2 gene can be induced by abscisic acid (ABA), salt, drought, and low temperature treatments, with the RcMsc2 gene showing the most sensitive response to low temperature stress. In summary, this study comprehensively analyzed the structural characteristics, phylogeny, and expression patterns of the RcMsc2 gene, providing a theoretical reference for elucidating the function of the RcMsc2 gene in castor bean growth and development and in response to cold stress.

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Full Text

Functional Analysis of the RcMsc2 Gene in Castor Bean (Ricinus communis)

Geng Liuting[1], Li Yanxiao[1], Zhang Chunlan[2], Yu Zhongyong[3], Wang Guiling[4], Xiang Dianjun[1], Liu Peng[1]*

[1]College of Agriculture, Inner Mongolia Minzu University, Tongliao 028000, Inner Mongolia, China
[2]College of Life Science, Inner Mongolia Minzu University, Tongliao 028000, Inner Mongolia, China
[3]Inner Mongolia Hongbo Seed Industry Technology Co., Ltd., Tongliao 028000, Inner Mongolia, China
[4]Zhalantun Green Industry Development Center, Zhalantun 162650, Inner Mongolia, China

Abstract

G2/mitotic-specific cyclin-2 (Msc2) serves as a key regulatory protein in higher plants' responses to environmental stress, participating in multiple stress-response pathways. To investigate the function of the RcMsc2 gene, we successfully cloned it from castor leaf tissue and employed bioinformatics to analyze its protein structure and potential function. Quantitative real-time PCR (qRT-PCR) was used to characterize its tissue-specific expression patterns and responses to abiotic stress. The results revealed: (1) The RcMsc2 gene is located on the long arm of castor chromosome 5, with a coding sequence (CDS) of 1,299 bp encoding 432 amino acids. (2) The RcMsc2 protein contains characteristic cyclin family domains, is an unstable acidic hydrophilic protein lacking transmembrane domains and signal peptides, and has a relative molecular mass of 49.38 kD. (3) The secondary and tertiary structures of RcMsc2 are dominated by α-helices and random coils. (4) RcMsc2 shows the highest sequence homology with CYCB2 proteins from Jatropha curcas and Hevea brasiliensis, clustering together in Group II. (5) The RcMsc2-GFP fusion protein localizes to the nucleus. (6) RcMsc2 is expressed in all castor tissues, predominantly in roots and stems. Abiotic stress analysis demonstrated that RcMsc2 expression is induced by abscisic acid (ABA), salt, drought, and low temperature treatments, with the most sensitive response observed under low temperature stress. In summary, this study provides a comprehensive analysis of RcMsc2' s structural characteristics, phylogenetic evolution, and expression patterns, offering a theoretical foundation for elucidating its function in castor growth, development, and cold stress responses.

Keywords: castor, RcMsc2, gene cloning, expression characteristics, cold stress

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Castor bean (*Ricinus communis*) is a perennial herbaceous plant in the Eu-

phorbiaceae family native to Africa, cultivable in both tropical and temperate regions (Maghuly et al., 2015). Rich in ricinoleic acid, castor oil has been designated as an important raw material for second-generation biomass green energy (Trabelsi et al., 2018). While castor exhibits strong drought and saline-alkali tolerance, enabling growth in relatively barren lands, it is susceptible to bacterial infection and cold stress during the seedling stage, ultimately reducing plant survival rates and seed quality (Severino et al., 2012; Wang et al., 2022). Tongliao in Inner Mongolia, located in the mid-latitude region, experiences average minimum temperatures ranging from 12.7 °C to 16.1 °C during the crop growing season. Such low-temperature environments severely affect castor seed germination, growth, and biomass accumulation (Tao et al., 2020). Therefore, mitigating the impact of low temperature on castor growth and breeding new varieties with enhanced resistance to abiotic stress are crucial for the future castor cultivation industry and for meeting industrial demand for castor oil.

Cell division represents the most fundamental process in biological growth and development (Van et al., 2010). The eukaryotic cell cycle is primarily controlled by the cyclin-dependent kinase (CDK) family of protein kinases (Suryadinata et al., 2010). Cyclins are categorized based on their functional phases in the cell cycle into cyclin M and cyclin G1 (Canaud et al., 2019). G1 cyclins include types C, D, E, and G, regulating the G1-S transition, while M cyclins include types A and B, functioning during S-M transition, G2-M transition, and M phase (Kõivomägi et al., 2011). G2/mitotic-specific cyclin-2 (Msc2) belongs to the B-type cyclins and is expressed transiently during the G2-M transition, within G2 phase, and during M phase to respond to environmental changes (Hégarat et al., 2020). Recent studies have revealed that CYCB2 genes may participate in plant responses to salt stress, heavy metal stress, ABA, and cold stress (Xu et al., 2010; Huang et al., 2013; Fan et al., 2022). In tobacco (*Nicotiana tabacum*), NtCycB2 expression decreases with prolonged NaCl treatment, while knockout of NtCycB2 enhances plant resistance to NaCl stress (Yan et al., 2021). Transcriptomic studies in sorghum (*Sorghum bicolor*) showed that CYCB2 expression increased under both 100 $\text{mol} \cdot \text{L}^{-1}$ and 150 $\text{mol} \cdot \text{L}^{-1}$ cadmium (Cd) stress, suggesting its involvement in heavy metal stress resistance mechanisms (Roy et al., 2016). In Arabidopsis, the atl17 mutant exhibited significantly higher CYCB2;1 expression than wild-type under various ABA concentrations, indicating that ABA-mediated mechanisms may inhibit primary root growth to resist stress (Xu et al., 2010). Cold stress experiments in cabbage (*Brassica oleracea*) revealed significant differences in CYCB2 expression patterns between 2 days and 7 days of treatment, with CYCB2;1, CYCB2;2, CYCB2;3, and CYCB2;4 showing Log2 values higher than the control, though expression at 7 days was significantly lower than at 2 days, suggesting that CYCB2 genes can enhance cellular differentiation capacity in the short term to mitigate cold damage (Ćosić et al., 2019). However, the potential functions and regulatory mechanisms of CYCB2 genes in castor remain understudied, despite castor' s importance as a biological raw material.

Transcriptome data from castor revealed 848 upregulated differentially

expressed genes (DEGs) under low temperature, among which RcMsc2 (XP_{002521704}.1) was significantly upregulated compared to optimal temperature (25 °C). As a homolog of Arabidopsis CYCB2;3, RcMsc2 likely plays a role in castor' s cold adaptation process (Bai et al., 2019). Therefore, we cloned the RcMsc2 gene, determined its subcellular localization using tobacco leaf cells, and analyzed its expression patterns under various stresses via qRT-PCR. This study aims to: (1) characterize the physicochemical properties, structure, and evolutionary relationships of RcMsc2 protein; (2) investigate the tissue-specific and abiotic stress-responsive expression patterns of castor RcMsc2; and (3) explore the potential function of castor RcMsc2 during cold stress. This research provides potential genetic resources for cold resistance breeding in castor and lays the foundation for elucidating the regulatory mechanisms of RcMsc2 in response to cold stress.

1.1 Experimental Materials

The castor variety 'Tongbi 5' was provided by Tongliao Institute of Agriculture and Animal Husbandry Sciences. The plant total RNA extraction kit (Monzol™ Reagent) and reverse transcription kit (MonScript™ RTIII All-in-One Mix with dsDNase) were purchased from Monad Biotech. T-vector (pMD™ 18-T vector), restriction enzyme (Bsa I), and DNA Ligation Kit were obtained from Takara Bio. High-fidelity PCR enzyme (KOD Master Mix) and PCR product purification kits were purchased from Tiangen Biotech. Maxima Reverse Transcriptase and 2X SG Fast qPCR Master Mix were from Thermo Fisher Scientific (Shanghai). *E. coli* competent cells (DH5α), high-fidelity enzyme (2×Taq Master Mix), primer synthesis, and sequencing services were provided by Sangon Biotech.

1.2 Material Treatment

Healthy castor seeds were sterilized and placed in sterile water for germination at 30 °C for 3 days. After germination, seeds were evenly divided into four portions and arranged in hydroponic trays. When the two cotyledons had fully expanded, seedlings were irrigated with 1/4 Hoagland solution (200 mL daily). At the four-leaf stage, seedling tissues (roots, stems, cotyledons, and true leaves) were collected to examine RcMsc2 tissue expression. Seedlings were then subjected to various stresses: 4 °C, 150 mmol $\cdot$ L^{-1} NaCl, 10% PEG 6000, and 100 mol $\cdot$ L^{-1} ABA. True leaves were harvested at time intervals (0, 2, 4, 8, 12, 24, 30, 48 h), immediately frozen in liquid nitrogen for 24 h, and stored at -70 °C.

1.3.1 Total RNA Extraction and First-Strand cDNA Synthesis

Total RNA was extracted from castor tissues treated at 4 °C for 12 h using the Monzol™ Reagent kit according to the manufacturer' s protocol, and its purity was assessed. The extracted RNA was used as a template for first-strand cDNA

synthesis using the MonScript™ RTIII All-in-One Mix with dsDNase kit, which served as the template for RcMsc2 gene cloning.

1.3.2 RcMsc2 Gene Cloning and Sequencing

Primers RcMsc2-F and RcMsc2-R (Table 1) were designed based on the RcMsc2 gene CDS region (XP_{002521704}.1) from the NCBI database. Using castor cDNA as template, the RcMsc2 CDS sequence was amplified with high-fidelity KOD Master Mix under the following conditions: initial denaturation at 94 °C for 4 min; 35 cycles of 94 °C for 30 s, 58 °C for 45 s, and 72 °C for 90 s; final extension at 72 °C for 10 min. The correctly sized fragment was recovered after electrophoresis, an A-tail was added at the 3 end, and the product was purified and ligated into the T-vector. The pMD™ 18-T-RcMsc2 construct was heat-shock transformed into DH5α competent cells, and positive clones were selected on Kan^+ medium and sent to Sangon for sequencing.

1.4 Bioinformatics Analysis of RcMsc2 Protein

The RcMsc2 coding sequence was translated into protein sequence using the ExPASy tool (https://web.expasy.org/translate/). Castor genome, proteome, and annotation files were downloaded from NCBI (https://www.ncbi.nlm.nih.gov) for local blastp analysis to determine the precise location of RcMsc2. ExPASy-PROSITE (https://prosite.expasy.org) was used to analyze physicochemical properties and hydrophilicity/hydrophobicity. NCBI-CD-SEARCH (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi), PSORT (http://psort.hgc.jp/), and Motif Scan (https://myhits.sib.swiss/cgi-bin/motif_{scan}/) were employed to predict protein domains, subcellular localization, and active sites. SignalP 5.0 (https://services.healthtech.dtu.dk/service.php?SignalP-5.0), DeepTMHMM (https://dtu.biolib.com/DeepTMHMM), and Motif Scan were used to predict signal peptides, transmembrane domains, and active sites. Homologous sequences were downloaded from UniProtKB (https://www.uniprot.org/help/uniprotkb) via online blastp. ClustalW and MEGA 11.0 software were used for sequence alignment and visualization. The alignment results were submitted to ENDscript/ESPr ipt (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi) for refinement, and the phylogenetic tree was beautified using iTOL (https://itol.embl.de). Secondary structure was predicted and visualized using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/secpred_{sopma}.pl). The tertiary structure was predicted using SWISS-MODEL (https://swissmodel.expasy.org/) and evaluated with SAVES v6.0 (https://saves.mbi.ucla.edu/) before analysis.

1.5 RcMsc2 Gene Expression Analysis

qRT-PCR primers RcMsc2-Fx and RcMsc2-Rx (Table 1) were designed based on the RcMsc2 CDS sequence, with RcActin (NC_{063262}.1) as the internal reference. Total RNA was extracted from castor tissues using the Trizol method

and reverse-transcribed into single-stranded cDNA. qPCR was performed on a LightCycler480 II system using 2X SG Fast qPCR Master Mix according to the manufacturer' s instructions: pre-denaturation at 95 °C for 3 min; 45 cycles of 95 °C for 5 s and 60 °C for 30 s. Relative expression levels were calculated using the 2^(-ΔΔCt) method.

1.6 Subcellular Localization of RcMsc2 Protein

The pCAMBIA2300-CaMV 35S-GFP vector was digested with Bsa I, and the target fragment was recovered. Using pMD™ 18-T-RcMsc2 as template, amplification was performed with primers RcMsc2-GFP-Fx and RcMsc2-GFP-Rx. The product was ligated into the pKY-35S-GFP vector using KOD Master Mix, transformed into DH5α competent cells, and positive clones were sequenced. After sequence verification, the pKY-35S-RcMsc2-GFP construct was transferred into *Agrobacterium* (GV3103) for transient expression in tobacco leaf epidermal cells to determine subcellular localization.

Table 1 Primers used in this study

Primer name	Primer sequence (5 -3)	Application
RcMsc2-F	TTGACTGGCTTATTGAGGTG	Gene cloning
RcMsc2-R	CAACCAACCCCACCAACTGA	Gene cloning
RcMsc2-Fx	CGAGCACTAATGGGTTTGGA	qRT-PCR
RcMsc2-Rx	CCATCAATATCCACAATAGGCTC	qRT-PCR
Actin-F	TTCCCAGGCATTGCTGATAG	qRT-PCR
Actin-R	ACATCTGCTGGAAGGTGCTG	qRT-PCR
RcMsc2-GFP-Fx	CAGTGGTCTCACAACATGAATGT[illegible]TGAGAA	Subcellular localization
RcMsc2-GFP-Rx	CAGTGGTCTCATACATGACTGAG[illegible]ATAGAA	Subcellular localization

Note: Underlined sequences indicate Bsa I restriction sites, left side shows protective bases, and right side shows terminal homologous sequences of the fluorescent expression vector.

2.1 RcMsc2 Gene Cloning

Based on preliminary transcriptome data, total RNA was extracted from castor leaves, with quality suitable for reverse transcription (Figure 1 [Figure 1: see original paper]A). Using cDNA as template, target gene amplification yielded a band of approximately 1,300 bp (Figure 1B). Through T-A cloning, transformation, positive colony screening, and sequencing, a 1,299 bp open reading frame (ORF) was obtained, encoding 432 amino acids (Figure 2 [Figure 2: see original paper]). The gene was designated RcMsc2, and local blastp analysis revealed its location on the long arm of chromosome 5.

2.2.1 Basic Physicochemical Properties of RcMsc2 Protein

Protparam analysis revealed that RcMsc2 protein has a molecular formula of $C_{2186}H_{3426}N_{584}O_{662}S_{28}$, totaling 6,886 atoms, with a relative molecular mass of 49.38 kD and a theoretical isoelectric point of 5.26, indicating acidic properties. The protein comprises 20 amino acids, with glutamic acid (Glu) being most abundant (9%) and tryptophan (Trp) least abundant (0.7%). It contains 60 negatively charged residues (Asp + Glu) and 46 positively charged residues (Arg + Lys). The instability coefficient of 45.24 (>40 threshold) classifies it as an unstable protein, while the grand average of hydropathicity of -0.353 (<0) predicts a hydrophilic nature. The aliphatic index is 81.69. Additionally, RcMsc2 contains a Cyclin_C (pfam ID: PF02984) domain and a Cyclin_N (pfam ID: PF00134) domain, confirming its membership in the B-type cyclin family (Figure 3 [Figure 3: see original paper]).

2.2.2 Secondary and Tertiary Structure Analysis of RcMsc2 Protein

Analyzing RcMsc2 protein' s secondary and tertiary structures provides fundamental support for functional studies. Secondary structure prediction showed that RcMsc2 consists of 52.55% α-helices, 40.51% random coils, 5.79% extended strands, and 1.16% β-turns (Figure 4 [Figure 4: see original paper]). The tertiary structure prediction (Figure 5 [Figure 5: see original paper]A) confirmed that α-helices dominate the protein architecture. SAVES v6.0 evaluation (Figure 5B) revealed that 91.1% of the 432 amino acid residues reside in the core region (red zone >90%, A, B, and L regions), with only 8.9% in allowed regions (a, b, l, and p), validating the reliability of the SWISS-MODEL prediction.

2.2.3 Prediction of Signal Peptide, Transmembrane Domain, and Active Sites in RcMsc2 Protein

SignalP 5.0 predicted a signal peptide probability of 0.0012, indicating no signal peptide structure. DeepTMHMM analysis showed no transmembrane domains across the 432 amino acid residues, suggesting RcMsc2 lacks transmembrane capability. Motif Scan identified six potential N-glycosylation sites (positions 2-5, 189-192, 303-306, 370-373, 378-381, and 411-414), four potential tyrosine kinase II phosphorylation sites (positions 63-66, 142-145, 337-340, and 419-422), three potential N-myristoylation sites (positions 20-25, 48-53, and 61-66), and additional active sites at positions 418-420, totaling 20 predicted functional sites. These predictions provide a theoretical basis for understanding RcMsc2 protein' s function under stress conditions.

2.2.4 Multiple Sequence Alignment and Homology Analysis of RcMsc2 Protein

Online Blast analysis revealed sequence identities of 87.4%, 85.5%, 81.1%, 79.7%, 79.7%, 77.5%, 72.7%, and 72.7% with cyclins from *Jatropha curcas* (XP_{012065375}.1), *Hevea brasiliensis* (XP_{021645034}.1), *Salix suchowensis* (XP_{024456907}.1), *Theobroma cacao* (EOX90682.1), *Durio zibethinus* (XP_{022740327}.1), *Gossypium mustelinum* (TYI88728.1), *Glycine max* (NP_{001352035}.1), and *Vigna angularis* (XP_{014521177}.1), respectively. This high conservation across species, particularly in the N-terminal region, suggests similar functional roles. Notably, the substrate-specificity site at position 283 is glutamic acid (E) in RcMsc2 but lysine (K) in other plants, potentially conferring distinct substrate specificity. The neighbor-joining phylogenetic tree constructed with MEGA 11.0 (Figure 7 [Figure 7: see original paper]) clustered the eight plant cyclins into three groups: Group I (soybean and cowpea, 100% bootstrap), Group II (castor with *Jatropha* and rubber tree, 99.9% bootstrap), and Group III (cotton with durian and cacao, 100% bootstrap). These groups correspond to Fabaceae, Euphorbiaceae, and Malvaceae families, respectively, confirming the accuracy of the phylogenetic analysis and revealing the highly conserved evolution of CYCB2 proteins across species. Thus, RcMsc2 is most closely related to *Jatropha* and rubber tree cyclins.

2.3 Subcellular Localization Analysis of RcMsc2 Protein

PSORT predicted nuclear localization with the highest probability for RcMsc2. To verify this, we constructed the pCAMBIA2300-CaMV 35S-RcMsc2-GFP expression vector and expressed it transiently in tobacco epidermal cells via *Agrobacterium* (GV3103)-mediated transformation. Results (Figure 8 [Figure 8: see original paper]) showed that 35S-GFP alone distributed throughout the nucleus, cytoplasm, and cell membrane (Figure 8A-D), whereas 35S-RcMsc2-GFP fluorescence concentrated primarily in the nucleus and cell membrane (Figure 8E). In merged images, 35S-RcMsc2-GFP produced green fluorescence mainly in the nucleus and cell membrane of tobacco leaf cells, though nuclear localization appears most likely, consistent with PSORT predictions. This nuclear localization suggests RcMsc2 may play important roles in cold stress responses.

2.4.1 Tissue Expression Pattern Analysis of RcMsc2 Gene

qRT-PCR analysis revealed that RcMsc2 is expressed in all castor tissues during both seed and seedling stages, with significant differences ($P<0.05$). Expression in roots was significantly higher than in other tissues–2.13-fold, 14.11-fold, and 14.94-fold higher than in cotyledons, stems, and true leaves, respectively (Figure 9 [Figure 9: see original paper]). This indicates distinct tissue-specific expression, suggesting RcMsc2 functions primarily in roots and stems to counteract adverse environmental conditions.

2.4.2 Abiotic Stress Expression Pattern Analysis of RcMsc2 Gene

The expression characteristics of RcMsc2 in castor seedling leaves under low temperature (4 °C), high salt (150 mmol $\cdot$ L^{-1} NaCl), ABA (100 mol $\cdot$ L^{-1}), and drought (10% PEG 6000) stresses were investigated by qRT-PCR. Results showed time-dependent differential expression under various stresses, displaying distinct patterns (Figure 10 [Figure 10: see original paper]). RcMsc2 responded actively to salt and drought stresses, initiating expression at 2 h and 4 h, respectively, and peaking at 4 h with expression levels 3.09-fold and 4.82-fold higher than controls (0 h). Under low temperature and ABA treatments, RcMsc2 exhibited delayed expression patterns, both peaking at 12 h. However, ABA-induced expression dropped sharply after 12 h, whereas low temperature-induced expression persisted until 48 h, indicating that RcMsc2 is likely a cold-inducible gene activated by ABA with sustained expression, suggesting it responds to multiple environmental pressures with particularly prolonged responsiveness to cold.

3 Discussion and Conclusion

Cyclins complex with CDKs to control CDK activity, substrate specificity, and subcellular localization, playing crucial roles in plant cell division processes (Loyer & Trembley, 2020). CYCB2 proteins primarily function during G2 phase (Aydinoglu, 2020). The CYCB2 gene family is multigenic, with 13, 12, and 11 members in soybean (Fonseca-García et al., 2021), alfalfa (Meng et al., 2020), and Arabidopsis (Sterken et al., 2012) genomes, respectively, all containing Cyclin_C and Cyclin_N domains with documented functional studies. In contrast, Msc2 protein function in castor remains poorly characterized. Based on castor cold transcriptome data, we cloned RcMsc2, which encodes 432 amino acids—shorter than Arabidopsis CYCB2;3 (Van et al., 2010) but longer than alfalfa MedtrCycB1;2, MedtrCycB2;1, and MedtrCycB2;2 proteins (Meng et al., 2020). RcMsc2’ s physicochemical properties differ from cyclins in soybean (Fonseca-García et al., 2021), alfalfa (Meng et al., 2020), tomato (Anwar et al., 2019), and sorghum (Roy et al., 2016), which may underlie species-specific involvement in different stress responses.

Sequence analysis revealed that RcMsc2 is primarily composed of α-helices, is hydrophilic, and lacks signal peptides, consistent with previous findings (Lara-Núñez et al., 2015; Sui et al., 2016). Protein N-myristoylation in higher plants helps cope with various adverse environments (Shitani et al., 2000; Podell & Gribskov, 2004). Environmentally induced proteins function cooperatively rather than individually to enhance plant vitality under stress (Dou et al., 2014). The presence of three N-myristoylation sites in RcMsc2 suggests it may collaborate with cold-related proteins to improve castor’ s survival under low temperature. While CYCB2 proteins have been localized to nuclei (Sabelli et al., 2014), spindles (Bulankova et al., 2013), endoplasmic reticulum, cytoplasm, and

cell membranes (Boruc et al., 2010), CYCB2-type proteins primarily localize to nuclei (Lara-Núñez et al., 2021; Chun et al., 2021) and may relocate according to environmental cues (Boruc et al., 2010). Our subcellular localization results confirm RcMsc2' s nuclear localization, suggesting its crucial role as a nuclear protein during cold stress.

Plant CYCB2 family members activate various defense mechanisms to enhance survival under multiple stresses (Huang et al., 2020; Zhang et al., 2021). These members exhibit distinct spatiotemporal expression specificity under stress. In sorghum, CYCB2 expression correlates positively with Cd concentration, being significantly lower at 100 mol $\cdot$ L^{-1} than at 150 mol $\cdot$ L^{-1} Cd (Roy et al., 2016). In cabbage, four CYCB2 genes (CYCB2;1–CYCB2;4) showed similar expression patterns during 2–7 days of cold treatment, all peaking at day 2 (Ćosić et al., 2019). RcMsc2 was expressed in leaves under ABA, PEG, 4 °C, and NaCl stresses, with similar expression patterns under high salt and drought, peaking within 4 h. Delayed expression occurred under ABA and low temperature, with sustained expression until 48 h under cold conditions, indicating that RcMsc2 is a cold-regulated gene likely activated by ABA. Additionally, cyclin family members show tissue-specific expression; Arabidopsis mutants (gig1 and uvi4) exhibited distinct tissue expression patterns for cycb2;2 and CYCB1;1 in cotyledons and hypocotyls, potentially functioning in hypocotyls under dexamethasone induction (Iwata et al., 2012). Our study demonstrates that castor RcMsc2 is expressed during both seed and seedling stages with clear tissue specificity, likely functioning in stems and leaves under low temperature, though the regulatory mechanisms in different tissues require further investigation. This study provides a reference for understanding the molecular mechanisms of castor RcMsc2 in cold stress responses.

References

AYDINOGLU F, 2020. Elucidating the regulatory roles of microRNAs in maize (Zea mays L.) leaf growth response to chilling stress [J]. *Planta*, 251(2): 1-15.

BAI X, CAO S, XIANG DJ, et al., 2019. Transcriptome analysis of castor seeds at germination stage under low temperature [J]. *Mol Plant Breed*, 17(12): 3834-3844.

BORUC J, MYLLE E, DUDAM, et al., 2010. Systematic localization of the Arabidopsis core cell cycle proteins reveals novel cell division complexes [J]. *Plant Physiol*, 152(2): 553-565.

BORUC J, VAN DEN DAELE H, HOLLUNDER J, et al., 2010. Functional modules in the Arabidopsis core cell cycle binary protein–protein interaction network [J]. *Plant Cell*, 22(4): BULANKOVA P, AKIMCHEVA S, FELLNER N, et al., 2013. Identification of Arabidopsis meiotic cyclins reveals functional diversification among plant cyclin genes [J]. *PLoS Genet*, 9(5): e1003508.

CANAUD G, BROOKS CR, KISHI S, et al., 2019. Cyclin G1 and TASCC

regulate kidney epithelial cell G2-M arrest and fibrotic maladaptive repair [J]. *Sci Transl Med*, 11(476): eaav4754.

CHUN JI, KIM SM, KIM H, et al., 2021. SlHair2 regulates the initiation and elongation of type I trichomes on tomato leaves and stems [J]. *Plant Cell Physiol*, 62(9): 1446-1459.

ĆOSIĆ T, RASPOR M, SAVIĆ J, et al., 2019. Expression profiles of organogenesis-related genes over the time course of one-step de novo shoot organogenesis from intact seedlings of kohlrabi [J]. *J Plant Physiol*, 232: 257-269.

DOU YJ, CAO F, MA Y, et al., 2014. Cloning of bHLH78 gene expressed specifically in fruits of cultivated strawberry and construction of overexpression vector [J]. *Mol Plant Breed*, 12(3): 456-465.

FAN W, XIA ZQ, LIU CY, et al., 2022. Ionomics, transcriptomics and untargeted metabolomics analyses provide new insights into the Cd response and accumulation mechanisms of mulberry [J]. *Environ Exp Bot*, 196: 104821.

FONSECA-GARCÍA C, NAVA N, LARA M, et al., 2021. An NADPH oxidase regulates carbon metabolism and the cell cycle during root nodule symbiosis in common bean (*Phaseolus vulgaris*) [J]. *BMC Plant Biol*, 21(1): 1-16.

HÉGARAT N, CRNCEC A, SUAREZ PEREDO RODRIGUEZ MF, et al. 2020. Cyclin A triggers Mitosis either via the Greatwall kinase pathway or Cyclin B [J]. *EMBO J*, 39(11): e104419.

HU X, CHENG X, JIANG H, et al., 2010. Genome-wide analysis of cyclins in maize (*Zea mays*) [J]. *Genet Mol Res*, 9(3): 1490-1503.

HUANG CZ, LEI XU, SUN JJ, et al., 2020. Allelochemical p-hydroxybenzoic acid inhibits root growth via regulating ROS accumulation in cucumber (*Cucumis sativus* L.) [J]. *J Integ Agric*, 19(2): 518-527.

HUANG Y, SRAMKOSKI RM, JACOBBERGER JW, et al., 2013. The kinetics of G2 and M transitions regulated by B cyclins [J]. *PLoS ONE*, 8(12): e80861.

IWATA E, IKEDA S, ABE N, et al., 2012. Roles of GIG1 and UVI4 in genome duplication in *Arabidopsis thaliana* [J]. *Plant Signal Behav*, 7(9): 1079-1081.

KÕIVOMÄGI M, VALK E, VENTA R, et al., 2011. Dynamics of Cdk1 substrate specificity during the cell cycle [J]. *Mol Cell*, 42(5): 610-623.

LARA-NÚÑEZ A, ROMERO-SÁNCHEZ DI, AXOSCO-MARÍN J, et al., 2021. Two cyclin Bs are differentially modulated by glucose and sucrose during maize germination [J]. *Biochimie*, 182: 108-119.

LARA-NÚÑEZ A, VENTURA-GALLEGOS JL, ANAYA AL, et al., 2015. Phytotoxicity of *Sicyos deppei* during tomato germination and its effects on the role of ABA and cell wall enzymes [J]. *Bot Sci*, 93(4): 771-781.

LOYER P, TREMBLEY JH, 2020. Roles of CDK/Cyclin complexes in transcription and pre-mRNA splicing: Cyclins L and CDK11 at the cross-roads of cell cycle and regulation of gene expression [J]. *Seminars Cell Dev Biol*, 107: 36-45.

MAGHULY F, VOLLMANN J, LAIMER M, 2015. Biotechnology of euphorbiaceae (*Jatropha curcas*, *Manihot esculenta*, *Ricinus communis*) [J]. *Appl Plant Genom Biotechnol*: 87-114.

MENG J, PENG MD, YANG J, et al., 2020. Genome-wide analysis of the cyclin gene family and their expression profile in *medicago truncatula* [J]. *Int J Mol Sci*, 21(24): 9430.

PODELL S, GRIBSKOV M, 2004. Predicting N-terminal myristoylation sites in plant proteins [J]. *BMC Genom*, 5(1):37.

ROY SK, CHO SW, KWON SJ, et al., 2016. Morpho-physiological and proteome level responses to cadmium stress in sorghum [J]. *PLoS ONE*, 11(2): e0150431.

SABELLI PA, DANTE RA, NGUYEN HN, et al., 2014. Expression, regulation and activity of a B2-type cyclin in mitotic and endoreduplicating maize endosperm [J]. *Front Plant Sci*, 5: 561.

SEVERINO LS, AULD DL, BALDANZI M, et al., 2012. A review on the challenges for increased production of castor [J]. *Agron J*, 104(4): 853-880.

SHITANI M, LIU JP, HALFTER U, et al., 2000. SOS3 function in plant salt tolerance requires N-myristoylation and calcium binding [J]. *Plant Cell*, 12(9):1667-78.

STERKEN R, KIEKENS R, BORUC J, et al., 2012. Combined linkage and association mapping reveals CYCD5;1 as a quantitative trait gene for endoreduplication in *Arabidopsis* [J]. *Proc Natl Acad Sci*, 109(12): 4678-4683.

SUI ZP, WANG TY, LI HJ, et al., 2016. Overexpression of peptide-encoding OsCEP6.1 results in pleiotropic effects on growth in rice (*O. sativa*) [J]. *Front Plant Sci*, 7: 228.

SURYADINATA R, SADOWSKI M, SARCEVIC B, 2010. Control of cell cycle progression by phosphorylation of cyclin-dependent kinase (CDK) substrates [J]. *Biosci Rep*, 30(4): 243-255.

TAO ZX, HUANG WJ, WANG HJ, 2020. Soil moisture outweighs temperature for triggering the green-up date in temperate grasslands [J]. *Theor Appl Climatol*, 140(3): 1093-1105.

TRABELSI ABH, ZAAFOURI K, BAGHDADI W, et al., 2018. Second generation biofuels production from waste cooking oil via pyrolysis process [J]. *Renewable Energy*, 126: 888-896.

VAN LEENE J, HOLLUNDER J, EECKHOUT D, et al., 2010. Targeted interactomics reveals a complex core cell cycle machinery in *Arabidopsis thaliana* [J]. *Mol Syst Biol*, 6(1): 397.

WANG XY, WU Y, SUN MD, et al., 2022. Dynamic transcriptome profiling revealed key genes and pathways associated with cold stress in castor (*Ricinus communis* L.) [J]. *Ind Crops Products*, 178: 114610.

XU J, GAO GL, DU JJ, et al., 2010. Cell cycle modulation in response of the primary root of arabidopsis to ABA [J]. *Pak J Bot*, 42(4): 2703-2710.

YAN XX, GUAN YY, LIU XY, et al., 2021. NtCycB2 gene knockout enhances resistance to high salinity stress in *Nicotiana tabacum* [J]. *Ind Crops Products*, 171: 113886.

ZHANG H, LU XY, WANG ZJ, et al., 2021. Excretion from long glandular trichomes contributes to alleviation of cadmium toxicity in *Nicotiana tabacum* [J]. *Environ Pollut*, 285: 117184.

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