OsSRK1, a Lectin receptor-like kinase, controls plant height by mediating internode elongation in Oryza sativa L.

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Abstract

LecRLKs (Lectin receptor-like kinases) is a subfamily of RLKs (receptor like kinase) and takes part in mounds of biological processes in plant-environment interaction. However, the roles of LecRLKs in plant development are still elusive. Here, we showed that OsSRK1, belonging to LecRLK family in rice, had a relative higher expression in internode and stem in comparison with that in root and leaf. Importantly, srk1-1 and srk1-2, two genome-edited mutants of OsSRK1using CRISPR/Cas9 system, exhibited obviously a decreased plant height and shorter length of the first internode and second internode compared with those in WT. Subsequently, histochemical sectioning showed that the stem diameter and the cell length in stem are significantly reduced in srk1-1 and srk1-2 compared with WT. Moreover, analyzing the expression of four gibberellin biosynthesis related genes showed that CPS, KAO, KS1 and GA3ox2 expression had similar levels between WT and mutants. Importantly, we further verified that OsSRK1 can directly interact with gibberellin receptor GID1. Together, our results revealed that LecRLKs family member OsSRK1 positively regulated plant height by controlling internode elongation which maybe depended on OsSRK1- GID1 interaction mediated gibberellin signaling transduction.

Introduction

Rice, as the staple food for more than half the population in the world, is the leading food crop and its cultivated areas account for about one third of the total food crop planting areas. Plant height, which caused by stem elongation, is a key factor for lodging resistance and also regulates the grain yield in rice. There are many factors regulating stem elongation, among which the phytohormone gibberellin is one of the most important (wang et al. 2008). Gibberellin precisely controls plant development and growth, including seed germination, hypocotyl elongation, stem elongation and so on (Li et al. 2018). Several reports have indicated that both the synthesis pathway and GA induced signaling pathway can regulate plant height in rice. For instance, the deficiency of *GA20ox2* and *GA20ox3*, two key enzymes for GA synthesis, exhibit dwarf phenotype caused by GA53 accumulation and GA20 and GA1 reduction in rice (Sasaki et al. 2002, Ye et al. 2015, Xue et al. 2013). GA3ox2, another key enzyme for GA synthesis, also regulated plant height by controlling the content of activated GA1 (Itoh et al. 2001, Tong et al. 2014, Hu et al. 2018). Moreover, DOG can regulate cell elongation through balancing the level of activated GA by regulating the transcriptional level of GA3ox2, GA2ox1 and GA2ox3 (Liu et al. 2011). Beside the GA content, GA induced signal transduction also takes part in plant height regulation. For example, the deficiency of GA receptor GID1 caused dwarf phenotype in rice (Ueguchi-Tananja et al. 2005).

RLKs also play crucial roles in plant height. The BR receptor BRI1 and its coreceptor BAK1, two important RLKs, synergistically regulated BR signaling dependent internode elongation (Yamamuro et al. 2011, Zhang et al. 2015, Gui et al. 2016). Moreover, another LRR-RLK XIAO also regulated plant height by mediating BR signaling (Jiang et al. 2012). Besides that, a RLK PSRK2 mediated GA signaling to affect internode elongation (Li et al. 2018).

LecRLKs (Lectin receptor-like kinases) belonged to RLK family and named after its lectin/lectin-like ectodomain (Wang J. et al., 2019). There are 173 LecRLKs in rice and 75 LecRLKs in Arabidopsis implying they had multiple functions in plant. Consistent with speculation, a large number of works had showed that LecRLKs play crucial roles in plant abiotic and biotic stresses, such as salt stress and pathogen stress (Sun et al. 2020). For example, *Pseudomonas syringae* DC3000 induced overexpress of *LecRK-IX.2*, which is depend on FLS2, is required for pathogen defense (Desclos-Theveniau et al. 2012). Aside from the functions of LecRLKs in plant stresses, several LecRLKs have been identified as regulators in seed germination, pollen development and grain yield. However, it is still not be revealed about the specific molecular mechanism of LecRLKs in plant development and growth.

In this work, gene expression assay showed that OsSRK1 had a relative higher expression in internode and stem. Furthermore, the mutants of OsSRK1 exhibited obviously decreased plant height and shorter length of the first internode and second internode compared with those in WT. Moreover, using multiple method evidenced that OsSRK1 can directly interact with gibberellin receptor GID1, which regulated GA signaling pathway. Our results revealed that LecRLKs family member OsSRK1 positively regulated plant height by controlling internode elongation which maybe depended on OsSRK1- GID1 interaction. This work lay a solid foundation for new research direction of rice morphological formation and also provided materials for molecular breeding in rice.

Materials And Methods

Plant materials and growth conditions

Rice (*Oryza sativa ssp. japonica*) variety 9522 was used as the wild-type (WT) and the background for all subsequent mutations. The two lines of srk1 mutant were obtained using CRISPR/Cas9 technology as previously described (Zhang et al., 2014). All plants were grown in a paddy field in Hainan and in a greenhouse with a 30/24 ± 1°C day/night temperature, 50 to 70% relative humidity, and a light/dark period of 13 h/11 h.

Phylogenetic tree construction

Using an amino acid sequence of SRK1 (LOC_Os01g10710), homologous sequences were searched with the BLAST in NCBI (http://www.ncbi.nlm.nih.gov). Sequences of the most similar were downloaded from NCBI and aligned using ClustalX2.1 and Bioedit with default settings. A phylogenetic tree was built with MEGA5 software using the Neighbor-Joining method.

Fluorescence imaging analysis

For detection of the subcellular localization of SRK1, the *pCAMBIA1305-SRK1-GFP* was transfected into protoplasts, which were isolated from one-week-old rice leaves through cellulase and macerozyme digestion. The transfected protoplasts were incubated in the dark at 28 °C for 18 h to allow for the expression of the SRK1 proteins. The transfected protoplasts with vector of *pCAMBIA1305-GFP* was a

negative control. The VENUS fluorescence in root detection with a confocal microscope (Leica TCS SP8 X, excitation 488 nm; emission 500-550 nm).

GUS staining

For construction of the promoter fusion vector pCAMBIA1301- proSRK1::GUS, a 1,536 bp promoter fragment containing the upstream region of ATG and the first exon was amplified using the specific primers proSRK1-F and proSRK1 -R. The proSRK1 fragment was assembled via restriction sites into pCAMBIA1301 (Ohta et al. 1990). Subsequently, the transgenic plants *proSRK2::GUS* were obtained by transformation of (*Oryza sativa ssp. japonica*) variety 9522. Tissues from the homozygous plants were stained with GUS (β-glucuronidase) stain solution as previously described (Jefferson et al. 1986) and images were taken under a Canon camera and Nikon phase-contrast microscope.

Genetic analysis and histological analysis

Plant height and internode length were quantified as previously described (Zhang et al. 2010). Two lines of srk1 transgenic mutant plants (n = 30) and the wild-type control plants (n = 30) were grown in a paddy field, after which the plant main culm was measured at the heading stage. The four elongated internodes (Internode-I, Internode-II, Internode-III, Internode-IV, from top to bottom) were harvested from different individual wild-type and transgenic plants. The statistical tests were performed by t-test, and the variation was expressed as the standard deviation (SD).

The second internodes were collected at the tillering stage and the seedling stage, then fixed in FAA (50% ethanol/0.9 M glacial acetic acid/3.7% form- aldehyde, 18:1:1, v/v/v, by vol) overnight at 4 °C, dehydrated with a graded series of ethanol, infiltrated with xylene, and embedded in paraffin. The 9-μm-thick sections were cut and transferred onto poly-L-lysine- coated glass slides, deparaffinized in xylene, stained with 1% safranin and solid green (formulated with 95% ethanol), and then dehydrated through an ethanol series. Light microscopy was performed using a Nikon microscope.

Total RNA extraction and quantitative PCR assay

Total RNA was isolated from rice tissues (root shoot, leaf, lemma/palea, and anthers) at different stages with the Trizol Reagent kit (Invitrogen) according to the manufacturer's protocol. The stages of rice anthers were classified according to Zhang and Wilson (2009). After treatment with DNase (Promega), the isolated 0.5 mg RNA was reverse transcribed to synthesize first-strand cDNA using the ReverTra Ace-a-First-Strand cDNA synthesis kit (Fermentas). The reverse transcription products were used as templates in the following quantitative PCR. Quantitative PCR analysis of *CPS*, *KS*, *KAO* and *GA3ox2* were performed with SYBR Premix EX Taq (TAKARA) on a Bio-Rad detection system. Each experiment was repeated three times. Data acquisition and analyses were per- formed using the method described by Roter-Gene software (Version 6.0; Build 38). Samples were normalized using *ACTIN1* expression as described previously (Zhou et al. 2014).

Yeast two-hybrid assays (Y2H)

Y2H assays were performed as described previously (Yu et al., 2010). The cytoplasmic domain of RK1 was amplified and constructed into pGADT7 to make an infusion with GAL4-AD as bait. The full CDS of GID1 was cloned into the prey vector pGBKT7. Different plasmid pairs were co-transformed into yeast cells AH109, respectively. The transformants were dilution plated onto synthetic dropout medium lacking tryptophan/leucine agar (-Trp-Leu) and synthetic dropout medium lacking tryptophan/leucine/histidine (-Trp-Leu-His) supplemented with 10 mM 3-AT (3-Amino-1, 2, 4-triazole) for 3-5 days to test the interaction.

GST pull-down assays

The GST-SRK1-CD protein were incubated by GST Sepharose resin for four hours in a tube with the pull-down binding buffer (50 mM Tris-HCl, 150 mM NaCl and 10 mM MgCl2, pH 8.0) at 4°C. GST protein was a negative control. After a brief centrifugation at 100 g for five minutes at 4°C, the buffer was removed, and 1 μg His-GID1 proteins were added to the resin, along with the fresh binding buffer, respectively. Then, the tube was rotated at 4°C for 12 hours for protein binding. Afterwards, the resin was washed for five times with washing buffer (20 mM HEPEs, 40 mM KCl and 1 mM EDTA, pH 8.0) to remove the nonspecifically bound protein, combined with 60 μL washing buffer and 20 μL 4× SDS loading buffer, and boiled for 10 minutes. After centrifugation at 12,000 × g for one minute at 22°C, the supernatant was subjected to immunoblotting analysis. The pull-down was analyzed by western blotting using the GST antibody (CMC, SC-80998) and His antibody (Abmart, M20001).

LUC assays to examine interaction

To investigate the interaction, SP-nLuc-SRK1 with GID1-cLuc were mixed and infiltrated into N. benthamiana leaves. The plants were grown for 36 h before the infiltrated leaves were sprayed with 1 mM D-luciferin, and then the luciferase signal was detected with a chemiluminescence imaging system (Tanon 5200 Multi, Tanon Biomart).

Results

The plasma membrane protein SRK1 belongs to LecRLKs family

To investigate the function of SRK1, we performed phylogenetic analysis by MEGA v7.0 and Clustal X. SRK1 is founded in *Arabidopsis thaliana*, *Oryza sativa*, *Sorghum bicolor*, *Vitis vinifera* and *Brassica napus*, indicating SRK1 is quite conserved in plant. Furthermore, we constructed a phylogenetic tree using SRK1 and its homologs in 13 species (Fig. 1a). The phylogenetic tree contains two subfamilies, one of which was w'a'sestablished in monocotyledons, and the other evolved in dicotyledon. We further aligned the sequences of SRK1 homologs from *Oryza sativa L.*, *BGIOSGA002962*, *ORUFI01G07230*, *SORBI_3003G025300* and *KQL04887*. The results revealed that SRK1 shares high sequence similarity with SRK1 proteins of other four plants.

We analyzed the structure of SRK1 from 13 species mentioned earlier. Except for *TraesPAR_01G000100.1* and *Zm00001d025065*, SRK1 protein containing signal peptide domain, lectin ectodomain, one transmembrane domain and kinase domain belonged to LecRLKs family (Fig. 1b). Whereas, SRK1 in *TraesPAR_01G000100.1* and *Zm00001d025065* lacking of signal peptide domain belonged to S_TKc family. Then SRK1 localization was analyzed using transient expression system in protoplast. And SRK1-GFP was mainly localized at plasma membrane (Fig. 1c), indicating that SRK1 is a typical plasma membrane localized protein as other LecRLKs.

SRK1 gene expression pattern in rice

To investigate the *SRK1* expression pattern in rice, we performed GUS staining assay in *proSRK1-GUS* transgenic plant. Histochemical staining showed that GUS was expressed throughout roots, leaves and stems in seedling stage and transition stage from vegetative growth to reproductive growth (Fig. 2a-f). And GUS staining intensity was stronger in internode and stem (Fig. 2d-e). Moreover, the expression level of GUS is higher in panicle at stage I and stage II, then the level decreased with panicle development, but it will not disappear (Fig. 2g). We further verified these results using RT-PCR to directly detecting the transcriptional level of *SRK1*. As shown in Fig. 2h, SRK1 was expressed in all tissues and stem and internode had a higher expression of SRK1 in comparison with other tissues (Fig. 2h). These results showed that *SRK1* had a stronger expression in stem and internode, indicating the function of SRK1 was potentially associated with stem and internode development.

The deficiency of SRK1 affected internode elongation

To further investigate the function of SRK1, we try to generate genome-edited mutants of Os*SRK1* using CRISPR/Cas9 system. Firstly, we designed a 19-nt target sequence (TS237), which between base 254 to base 274 in SRK1 genome. Then we cloned the target sequence into pYLCRISPR/Cas9 vector and generate the vector of pYLCRISPR/Cas9-MT-SRK1. Using callus transformation system, we respectively generated three kinds of mutants for SRK1 named *N237-7*, *N237-13* and *N237-23* (Fig. 3a). The sequencing results of the three mutants showed that *N237-13* and *N237-23* carry large DNA fragment deletions at transmembrane domain and kinase domain that lead to the premature termination of SRK1 mRNA translation (Fig. 3b).

We compared the phenotype and agricultural traits between SRK1 mutants and WT. As shown in Fig. 3b and 3k , the average height of *srk1-1* and *srk1-2* are 58.62±1.82 cm and 60.32±1.79 cm respectively, which are significantly lower than the 72.78±2.30 cm height of WT (Fig. 3b and 3k). Internode length is closely related with stem length, therefore we detected the internode length in mutants and WT. The first internode length of *srk1-1* and *srk1-2* are 17.00±0.85 cm and 17.16±0.96 cm respectively and the second internode length of *srk1-1* and *srk1-2* are13.10±1.13 cm and 14.64±0.69 cm respectively, which are both shorter than those in WT (the first internode length is 23.36±0.88 and the second internode length is 17.22±0.34) (Fig. 3c, 3d and 31). Aside from those, the average panicle length of *srk1-1* and *srk1-2* are 12.70±0.54 cm and 13.10±0.70cm respectively, which are significantly lower than the 15.44±1.35 cm length of WT (Fig. 3b, 3c and 3k). Interesting, there is no significant difference in number of primary

branches and spikelet per panicle between mutants and WT (Fig. 3m-n). Follow, we analyzed the effective panicle per plant (Fig. S1a), seed length/width (Fig. S1b), and grain weight per plant (Fig. S1c). There is no significant difference in these agronomic traits between WT and mutants. To investigate the reason why the internode lengths of mutants are shorter than those in WT, we analyzed the diameter of the stem and stem cell length using paraffin section. The transverse sectioning of the stem showed that the inner and outer diameter of the stem is obviously smaller than that in WT (Fig. 3e-g and 3o). And the longitudinal sections of stems showed that the cell lengths of *srk1-1* and *srk1-2* are 77.32±18.24 μm and 74.09±15.73μm respectively, which are significantly lower than the length of 104.16±26.07 μm in WT (Fig. 3h-j and 3p).

SRK1 directly interacted with the GA receptor GID1

As GA playing an important role in internode elongation, we further detected the four genes related to GA biosynthesis. The results showed that there was no significant changing of *CPS*, *KAO*, *KS1* and *GA3ox2* expressions between the mutants and WT, indicating that the deficiency of SRK1 may not affect the endogenous GA concentration (Fig. S2a-d).

Therefore, we further screening the interactors of SRK1 using Y2H screening system. We constructed SRK1-CD-BD by cloning the gene segment which expressing cytosolic domain of SRK1 into pGBKT7 vector. The sequencing results shown that one of the potential interactors is GA receptor GID1. Then, we constructed GID1-AD and verified the screening result by Y2H using ONPG as substrate (Fig. 4a-b). Furthermore, we purified GID1-His and SRK1-CD-GST proteins and GST-pulldown assay showed that SRK1-CD-GST directly interacted with GID1-His in vitro (Fig. 4c). Moreover, we can detect the signals when expression GID1-nLUC and SRK1-cLUC in tobacco together, revealing that SRK1 can interact with GID1 in vivo (Fig. 4d). Together, SRK1 may regulate internode elongation by interacting with GA receptor GID1.

Conclusion

As sessile organism, plant developed a complex system to sense and adapt to the changing environment for their survival. RLK play vital roles in plant sensing processes because it can specificity perceive ligand by ectodomain and deliver signals inside cell by kinase domain (Li et al. 2018). LecRLKs belonged to RLK family and are specifically widespread in plants. Although a large number of works showed that LecRLKs play vital roles in plant stresses, there are few works focusing on the function of LecRLKs in plant development. In this work, we identified a plasma membrane localized SRK1 as a member of LecRLK family and SRK1 had a relative higher expression in stem and internode. We showed that SRK1 is a positive regulator for plant height, revealing that the LecRLK SRK1 takes part in rice development. Moreover, plant height is closely associated with lodging resistance and grain yield. Therefore, to study the molecular functions of SRK1 will lay a solid foundation for new research direction of rice morphological formation and molecular breeding in rice.

Rice height are regulated by multiple factors and GA plays a crucial role in this process. The concentration of GA and GA induced responses both can affect plant height. In this work, we found that

there was no significant changing of *CPS*, *KAO*, *KS1* and *GA3ox2* expressions, which are regulator of three steps of GA biosynthesis, between the *SRK1* mutants and WT. This result indicated that SRK1 may not affect the endogenous concentration. Importantly, this work evidenced that SRK1 can directly interact with GA receptor GID1, indicating that SRK1 positively regulated plant height by controlling GID1 dependent GA responses.

RLK always function in a complex to deliver signals at plasma membrane. And the post transcription modification of RLK is vital for RLK mediated signal transduction. Increasing evidences shown that RLK are closely with phytohormone signals, such as auxin, gibberellin and Strigolacton. In this work, we found that SRK1 takes part in GA mediated plant height. Moreover, the localization of RLK is important for its function. For instance, the cytoplastic domain of LRR-RLK TMK4 can be cleaved and transported into nucleus to control auxin signaling. In our work, we found that the plasma localized SRK1 can directly interact with GA receptor GID1, which located in cytoplasm and nucleus. Therefore, to uncover where and how SRK1 interacted with GID1 will provide more information on the mechanism by which RLK functions.

Declarations

Author contribution

Li L. and Chong H. conceived the project; Bin L. and Yi L. performed the experiments with the help of Xiu L., Hao D. and Mu Q.; Y. L. and B. L. analyzed the sequencing data; Bin L. and Xuan L. designed the experiments and interpreted the results; Li L. and Bin L. wrote the manuscript.

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This article is original.

Competing interests

The authors declare no competing interests.

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Figures

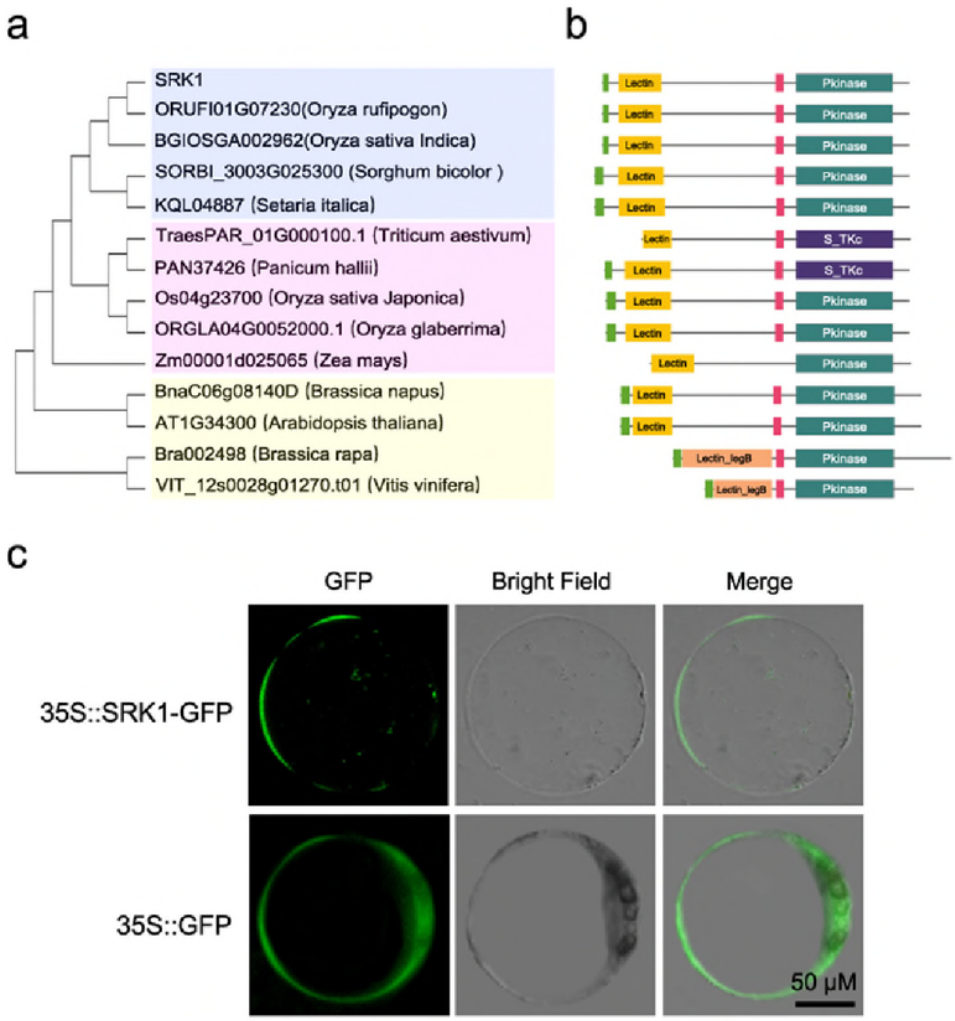

Figure 1

Evolutionary relationship and subcellular localization of SKR1.**a** Phylogram of SRK1. **b** Schematic structure of the SRK1 and homologous gene. Yellow is lectin domain purple is transmembrane domain and blue is kinase domain. **c** SRK1-GFP in protoplasts. Bar= 50 μM.

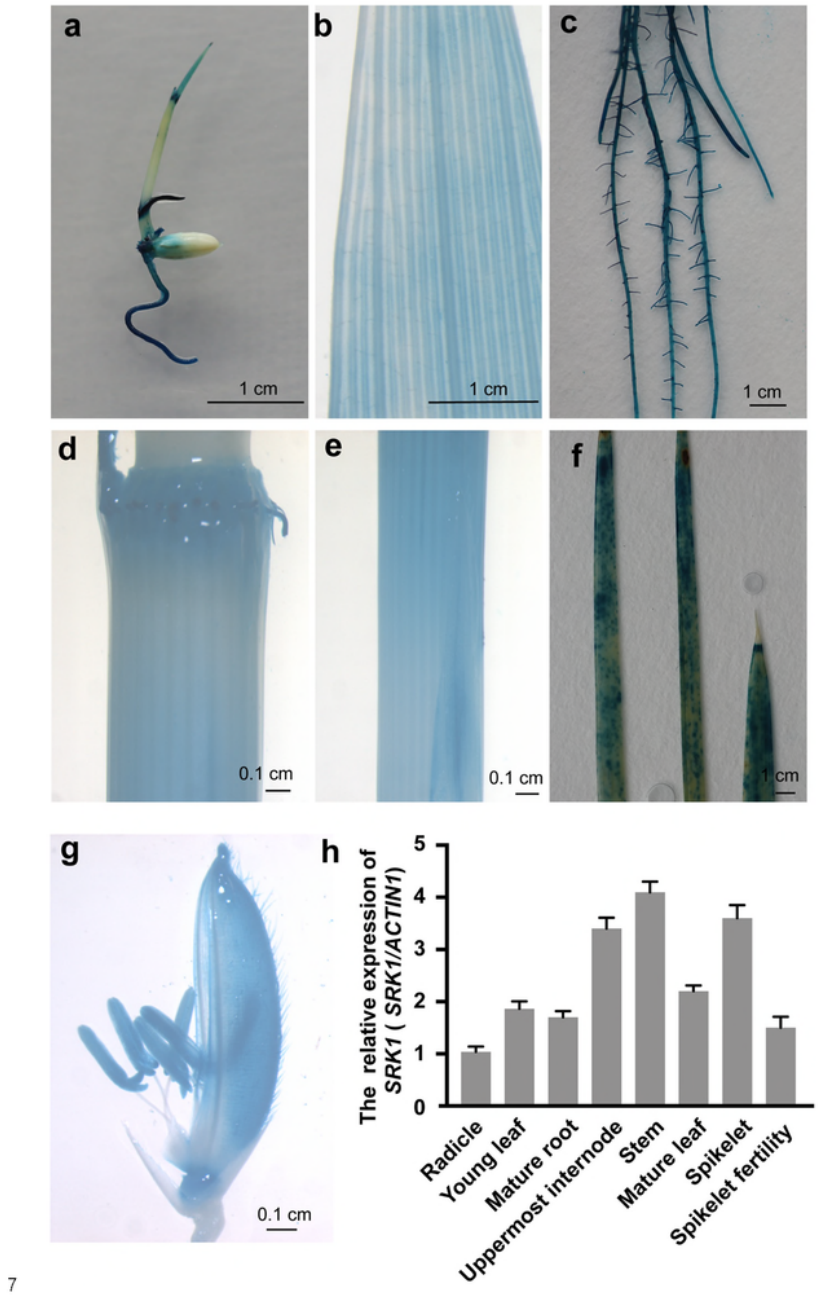

Figure 2

Expression patterns of SRK1 in Arabidopsis plants. **(a–g)** GUS staining of **(a)** 6-d-old plantlets, **(b)** leaf of 45-d-old plants, **(c)** mature root,**(d)**uppermost internode, **(e)** stem of 45-d-old plants, **(f)** mature leaf and **(g)** spikelet. **h** Relative expression of *SRK1* in the plants tissue. Gene expression was determined by quantitative real-time PCR and was normalized against the expression of *Actin1*. Data are means (±SD), *n*=3.

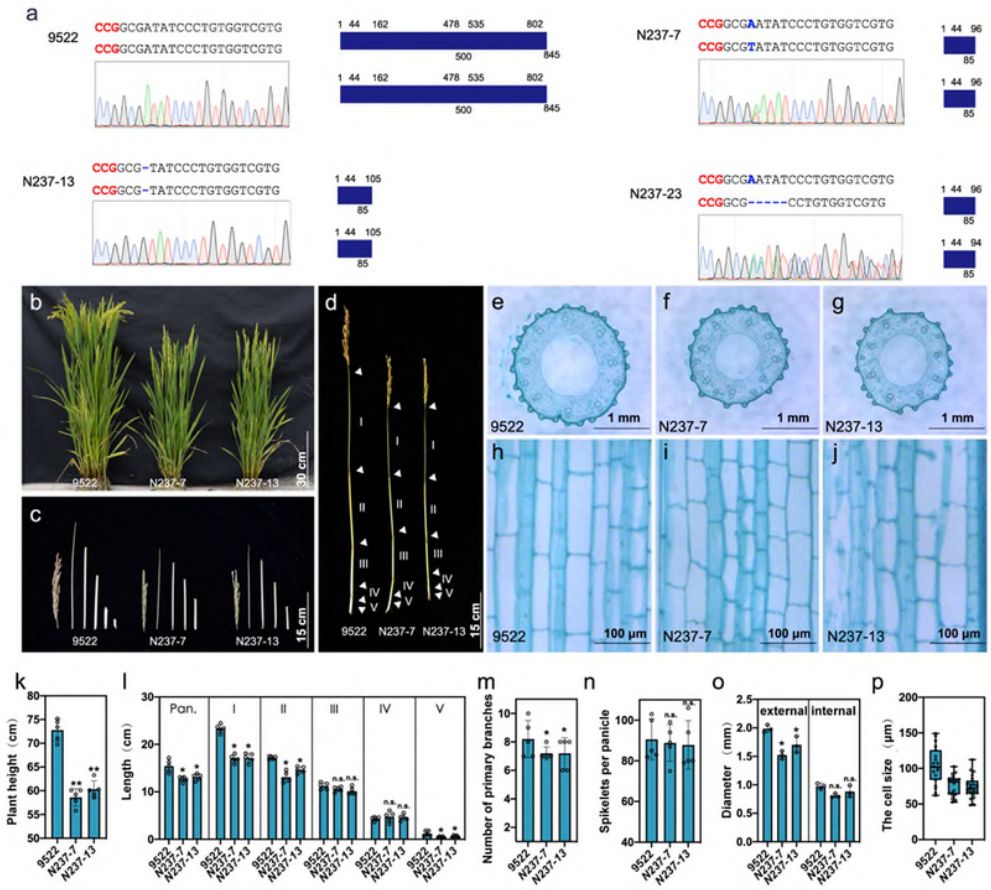

Figure 3

The phenotype analysis of the wild type (WT) and the*SRK1* transgenic plants. **a** Analysis of mutant type of the *SRK1* mutants in *9522.* **b** Morphological character of WT and two *SRK1* transgenic plants lines in heading stage. The plant height statistical analysis in **(k)**, the number of primary branches statistical analysis in **(m)** and the spikelets per panicle statistical analysis in **(n)**. **c-d** Comparison of length in tassel and internodes and the statistical analysis in **(i)**. **e-g** Analyze the transection of stem in WT and two *SRK1*transgenic plants lines, the external and internal statistical analysis in **(n)**. **h-j** The cell size assays, and the length statistical analysis in **(p)**. Data represent the mean ±SD. ** is for p-value <0.01. * is for p-value <0.05. n.s. is for not significant.

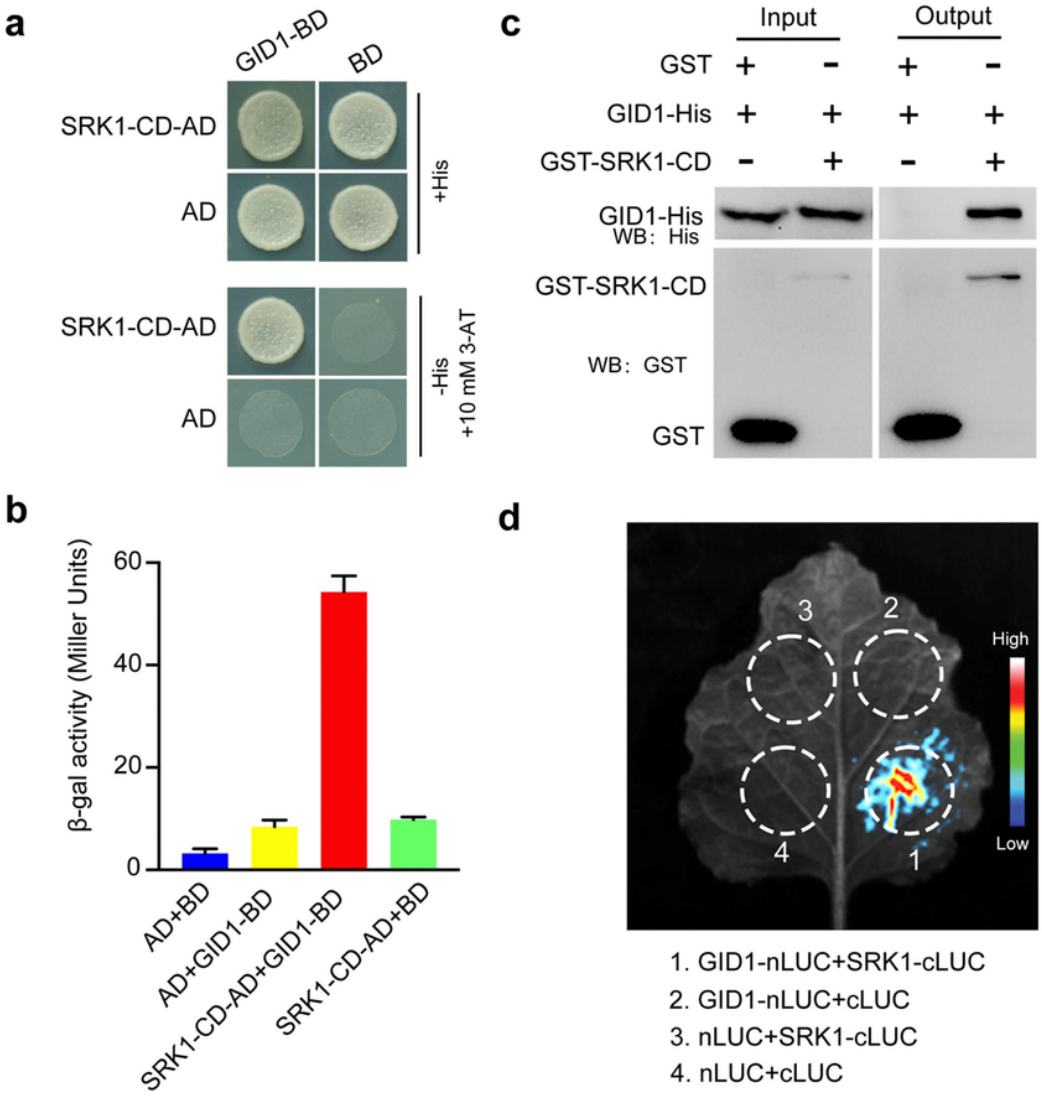

Figure 4

SRK1 interacts with GID1. **a** Y2H assays showed the association between SRK1 and GID1. Yeast cells were grown on SD/-Ade/-Leu medium with or without His. **b** β-galactosidase assay the interaction between SRK1 and GID1using CPRG as substrate. AD+BD as a negative control. **c** Analysis of the interaction between SRK1 and GID1 was carried out by GST pull-down assays. d Luciferase complementation (LUC) assay showing the interaction of SRK1 with GID1.

Supplementary Files

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