SmallOrgan 1 plays an essential role in cell proliferation, cell expansion and cadmium uptake in rice

Peng Qin [1], **Jiangbo Hu** [1], **Weilan Chen** [1], **Guohua Zhang** [1], **Jian Li** [1], **Shijun Fan** [1], **Bin Tu** [1], **Xuewei Chen** [1], **Yuping Wang** [1], **Shigui Li** [1], **Bingtian Ma** [Corresp. 1]

[1] Rice Research Institute, Sichuan Agricultural University, China

Corresponding Author: Bingtian Ma
Email address: btma02@sicau.edu.cn

Background: Organ size is determined by the number and size of cells, which are controlled by cell proliferation and cell expansion. Organ size in rice plays a crucial role in determination of rice yield. Cadmium (Cd), one of the major environmental pollutants, is harmful to human health. Cd-polluted rice grains have been a major source of human Cd-intake, thus reducing Cd-uptake into rice grains isimportant. Strigolactone (SL), a hormone, has been well-studied in terms of regulating plant architecture and in its signal perception, but its biological roles in cell proliferation, cell expansion and Cd-uptake have not been uncovered.**Methods:** We comprehensively investigated agronomic traits and cell size between WT and *so1* . We treated WT and *so1* with Cd, and analyzed Cd content in rice grain using inductively coupled plasma massspectrometry (ICP-MS). We used bulk segregation analysis for SO1 mapping, and analyzed sequences surrounding SO1 in *rufipogo* , *indica*, *japonica* and *intermedia* varieties of rice. **Results:** We identified a spontaneous rice mutant, *small organs 1* (*so1)* , which exhibited small organs,such as small leaves, panicles and grains. Cytological analysis indicated that the small organs of *so1* were due to both impaired cell proliferation and expansion. We found that *so1* grains highly accumulated Cd after Cd treatment. In the *so1* mutant, an 8bp insertion resulting in a premature stop codon was identified in *LOC_Os04g46470* (*High-Tillering Dwarf1*) which is involved in Strigolactone (SL) synthesis. Because both *htd1* and *so1* mutants exhibited dwarf and excessive tillers, the 8bp insertion was responsible for the *so1* phenotype. *SO1/HTD1* was ubiquitously expressed, most highly in stem and sheath. Very few SNP polymorphisms surrounding *SO1/HTD1* were identified in *japonica* group, whereas SNPs were abundant in the *rufipogon*, *indica* and *intermedia* groups.**Discussion:** Our work showed the novel biological roles of *SO1/HTD1* in controlling rice organ size and Cd-uptake, and indicated that the SL signaling pathway is involved in cell proliferation, cell expansion, and Cd-uptake, a role completely different from the previously reported role of HTD1. Therefore, our study is helpful for

understanding the molecular functions of *SO1/HTD1* and SL during rice development.

Small Organ 1 Plays an Essential Role in Cell Proliferation, Cell Expansion and Cadmium Uptake in Rice

Peng Qin[A,B,1], Jiangbo Hu[A,1], Weilan Chen[A], Guohua, Zhang[A], Jian Li[A], Shijun Fan[A], Bin Tu[A,B], Xuewei Chen[A], Yuping Wang[A], Shigui Li[A,B*], Bingtian Ma[A,B*]

[A]Rice Research Institute of Sichuan Agricultural University, Chengdu Wenjiang, Sichuan, 611130, China.

[B]State Key Laboratory of Hybrid Rice, Sichuan Agricultural University, Chengdu Wenjiang, Sichuan, 611130, China.

[1]These authors contributed equally to this work.

*Corresponding author: Bingtian Ma and Shigui Li

Email: btma02@sicau.edu.cn; lishigui@sicau.edu.cn

Abstract

Background: Organ size is determined by the number and size of cells, which are controlled by cell proliferation and cell expansion. Organ size in rice plays a crucial role in determination of rice yield. Cadmium (Cd), one of the major environmental pollutants, is harmful to human health. Cd-polluted rice grains have been a major source of human Cd-intake, thus reducing Cd-uptake into rice grains is important. Strigolactone (SL), a hormone, has been well-studied in terms of regulating plant architecture and in its signal perception, but its biological roles in cell proliferation, cell expansion and Cd-uptake have not been uncovered.

Methods: We comprehensively investigated agronomic traits and cell size between WT and *so1*. We treated WT and *so1* with Cd, and analyzed Cd content in rice grain using inductively coupled plasma mass spectrometry (ICP-MS). We used bulk segregation analysis for SO1 mapping, and analyzed sequences surrounding SO1 in *rufipogon*, *indica*, *japonica* and *intermedia* varieties of rice.

Results: We identified a spontaneous rice mutant, *small organs 1* (*so1)*, which exhibited small organs, such as small leaves, panicles and grains. Cytological analysis indicated that the small organs of *so1* were due to both impaired cell proliferation and expansion. We found that *so1* grains highly accumulated Cd after Cd treatment. In the *so1* mutant, an 8bp insertion resulting in a premature stop codon was identified in *LOC_Os04g46470* (*High-Tillering Dwarf1*) which is involved in Strigolactone (SL) synthesis. Because both *htd1* and *so1* mutants exhibited dwarf and excessive tillers, the 8bp insertion was responsible for the *so1* phenotype. *SO1/HTD1* was ubiquitously expressed, most highly in stem and sheath. Very few SNP polymorphisms surrounding *SO1/HTD1* were identified in *japonica* group, whereas SNPs were abundant in the *rufipogon*, *indica* and *intermedia* groups.

Discussion: Our work showed the novel biological roles of *SO1/HTD1* in controlling rice organ size and Cd-uptake, and indicated that the SL signaling pathway is involved in cell proliferation, cell expansion, and Cd-uptake, a role completely different from the previously reported role of HTD1. Therefore, our study is helpful for understanding the molecular functions of *SO1/HTD1* and SL during rice development.

Introduction

Organ size is determined by the number and size of cells, which are controlled by cell proliferation and cell expansion (Jiang et al. 2012). Organ size in rice, such as panicle and grain size, plays a crucial role in determination of rice yield. So far, various signaling pathway have been reported to be involved in plant cell proliferation and expansion, including plant hormones, such as gibberellin, which can promote rice internode elongation in deep water (Sauter et al. 1995); *DA1,* involved in ABA signaling, which controls organ size by regulating the cell cycle (Li et al. 2008); *ARGOS* functions in auxin signaling pathway upstream of *ANT* (Hu et al. 2003)； and *Xiao*, which is involved in the control of organ size by participating in brassinosteroid signaling (Jiang et al. 2012). An enzyme responsible for cytokinin biosynthesis controls panicle size (Kurakawa et al. 2007). SL (Strigolactone), another hormone, has been well-studied in regulating plant architecture and its signaling perception (Al-Babili & Bouwmeester 2015). However, its biological roles in cell proliferation or cell expansion have not been uncovered.

Cd (Cadmium), one of the major environmental pollutants, is harmful to human health. Cd-polluted rice grains has been a major source of human Cd intake (Uraguchi & Fujiwara 2012). Controlling Cd uptake in rice grains has been an important issue in agriculture. So far, several approaches to reduce Cd accumulation in rice grains have been reported, but those approaches are costly, ineffective or unstable (Clemens et al. 2013; Ueno et al. 2009). Genetic improvement for reduced Cd in rice grain was thought be effective, but not cost-effective costly in Cd-polluted areas. Several genes, such as *OsLCT1* (Uraguchi et al. 2014), *OsHMA3n*, *OsHMA3a* (Kumagai et al. 2014; Miyadate et al. 2011; Sasaki et al. 2014), *OsNramp1* (Takahashi et al. 2011) and *OsIRT1*

(Lee & An 2009), have been reported to be involved in the Cd uptake pathway in rice. However, the molecular mechanism of Cd uptake in rice was largely unknown.

Here, we identified a spontaneous mutant, *so1*, which exhibited small organs. Cytological analysis showed that SO1 is involved in cell proliferation and expansion. Grains of the *so1* mutant accumulated Cd. Mapping-based cloning showed that an 8bp insertion resulting in a premature stop codon was identified in *LOC_Os04g46470* (*HTD1*) whose mutant exhibits similar phenotype with *so1*, and is involved in Strigolactone (SL) synthesis. *SO1* was ubiquitously expressed, and highly expressed in stem and sheath. Very few SNP polymorphisms surrounding *SO1* were identified in the *japonica* group, whereas SNPs were abundant in the *rufipogon*, *indica* and *intermedia* groups. Together, our work highlights biological roles of *SO1/HDT1* in a way different from those previously reported, and is helpful for understanding the molecular functions of SL signaling pathways during rice development.

Methods and material

Plant materials

The *so1* mutant was a spontaneous mutant, and was noticed in an *indica* rice breeding program. For evaluation of all agronomic traits, the wild-type and *so1* were grown in a paddy at Chengdu, Sichuan in 2015, and mean values from three measurements were used for analysis. The tiller number of WT and *so1* was counted once every 7 days from the beginning of tillering (39 day after sowing) to the end of tillering.

Semi-section and SEM

To observe epidermal cell size in the culm, we performed semi-section of the first internode as described by (Qin et al. 2013); the measurement of cell length and width was performed using Image J software. To document cell size of the vascular bundles and glume cells of the spikelet, we used SEM following the protocol described by (Qin et al. 2013). Briefly, the culm at the mature stage and glume at the filling stage were fixed in 0.1M sodium phosphate buffer (2.5% gultaraldehyde, pH=6.8) at 4℃ for overnight, then washed in 0.1M phosphate buffer (pH 6.8)

twice, then fixed in 0.1M sodium phosphate buffer (2% osmium tetroxide) at 4℃ overnight. We used an acetone series from 30-100% to dehydrate, and used liquid CO_2 to dry samples out, and then coated them with gold powder, then observed with Scanning Electron Microscope JEM-1200 EX (Hitachi).

Cadmium treatment and measurement

For cadmium treatment, WT and *so1* plants were first grown in soil without added Cd (background level of Cd is 0.05mg/Kg) for 30 days, then transferred to soil with 1mg/Kg Cd. Mature grains were collected for cadmium measurement, according to (Miyadate et al. 2011).

Gene Mapping

Bulk segregation analysis was used for gene mapping. Briefly, we analyzed the polymorphisms of 608 SSR markers on 12 chromosomes between *so1* and HuaB. 61 SSR markers with polymorphisms were further used to analyze two DNA pools which contained 10 F2 individual with or without excessive tiller phenotype. The markers RM5749, RM6365 and RM6748 on Chr.4 showed polymorphism between the two pools. More markers with polymorphism adjacent to these three markers were analyzed for 463 F2 individuals with excessive tillers.

Expression analysis

Total RNAs of different tissues in Fig. S4 were isolated using Trizol (Invitrogen). For the qPCR assay, we used 600ng total RNA for reverse transcription, with a Primescript RT reagent kit with gDNA eraser (TakaRa), and 200ng/μL cDNA, gene-specific primer pairs and SsoFast EvaGreen supermix (Bio-Rad), with the Bio-Rad CFX96 real-time system. All primers are listed in supplementary table 1.

SNP polymorphism analysis

The *O.rufipogon* haplotype sequence in Hap3 (Huang et al. 2012) and the *Indica*, *Japonica* and *Intermedia* re-sequencing data of 3K rice accessions (project 2014) were used for analyzing

polymorphisms in the adjacent SO1 region using fastPHASE36 (Scheet & Stephens 2006).

Results

The *so1* mutant exhibited small organs

The *so1* mutant was a spontaneous mutant in the *indica* background. The *so1* mutant was dwarf and had excessive tillers (A). The dwarf phenotype of *so1* was due to equally shortened internodes (Fig. S1A and B). The *so1* mutant increased its tiller number until 112 days after sowing, whereas the tiller number of WT did not increase after 53 days after sowing (Fig. S2A). The number of *so1* tillers at the mature stage was up to 91, while only 8 tillers were seen for WT. In addition to the dwarf and excessive tiller phenotype, the organ size of *so1* was much smaller than in WT, such as leaf, panicle grain size and each internode (Fig. 1B-F). Analysis of agronomic traits showed that except for tiller number per plant, all traits, such as plant height, 1000-grain weight, panicle length, grain length and grain width, were significantly reduced in the *so1* mutant (Fig. 1G-N).

SO1 was involved in cell proliferation and expansion

To investigate the cytological reason for the small organ in *so1*, we performed semi-sections and SEM (scanning electron microscopy) of the first internode and outer parenchyma layer of the spikelet hulls, respectively. The number and size of the vascular bundles of the *so1* stem was obviously decreased (Fig. 2A-D). The number and length of the culm epidermal cells was also significantly decreased in *so1* (Fig. 2E, F, I and J). The cell length and width of the spikelet outer epidermal cells were reduced around 18.4% and 13% in *so1* (Fig. 2E, F and K). The number of longitudinal and horizontal cells was increased 14.2% and 4.4% in the spikelet outer epidermis (Fig. 2G, H and L), respectively. We investigated the expression of genes involved in cell proliferation and expansion using stem apex meristem. Consistent with the decreased cell length and number of *so1* culm, most cell proliferation-related genes were significantly down-regulated, and three of six cell expansion-related genes were down-regulated in *so1* (Fig. 2 M and N).

164 **The grain of *so1* mutant accumulated Cd**

165 During the course of mutant screening for aberrant Cd accumulation in rice grain, we surprisingly
166 found that Cd concentration in *so1* was about 2.8 fold more than in WT when 1mg/Kg Cd was
167 added, whereas no difference was found between WT and *so1* without Cd addition (Fig.3A).

168

169 **Genetic analysis and mapping of *SO1***

170 All F1 plants generated from a cross between *so1* and WT exhibited normal tiller number and plant
171 height as WT, and in the F2 population, 331 and 1324 plants exhibited excessive tillers and normal
172 tillers, respectively ($\chi^2(3:1)=2.95 < \chi^2_{(0.05)}=3.84$), indicating a single recessive mutation was
173 responsible for the *so1* phenotype.
174 A bulk segregation approach was performed to map the target mutation of *so1* using a population
175 generated from the cross between *so1* and HuaB. The target mutation was mapping to a 749kb
176 region between AG234 and RM470 on Chr.4 by preliminary mapping, and then narrowed to a
177 76.8kb region by fine mapping. An 8bp insertion was identified in the second exon of
178 *Loc_Os06g46470*, leading a premature stop codon in *so1* (Fig. 4A). Mutants of *Loc_Os06g46470,*
179 including *hdt1* and *osccd7,* exhibited similar phenotype with *so1* (Kulkarni et al. 2014; Zou et al.
180 2006), we therefore concluded that the *so1* phenotype is due to the 8bp insertion in
181 *Loc_Os04g46470*.

182

183 **Expression analysis of *SO1* and genes involved in strigolactone pathway**

184 We then investigated the expression pattern of *so1* among a number of tissues using RT-qPCR.
185 *SO1/HTD1* was ubiquitously expressed in most tissues, and highly expressed in stem and leaf
186 sheath (Fig. 5A). The expression of *D14*/*D88*/*HDT2* and *D53* involved in SL signal perception
187 pathway were respectively up-regulated and down-regulated, and no change for *D3*. Both *D10* and
188 *D27* involved in SL synthesis was up-regulated, whereas *SO1*/*HDT1* was down-regulated (Fig.
189 5B), respectively.

190

191 **SNP diversity surrounding *SO1/HTD1***

192 Given that the expression difference of *D88*, involved in SL signaling transduction, was proposed
193 to contribute to the difference of panicle branch number between 9311 and PA64s (Peng et al.
194 2014), we analyzed SNP polymorphisms of *SO1/HTD1* among 3K rice accessions, interestingly,
195 SNP polymorphisms of *SO1/HTD1* were similar among *rufipogon*, *indica* and *intermedia* group,
196 whereas very few SNP polymorphisms were identified in the *japonica* group (Fig. 6A), indicating
197 that *SO1/HTD1* might have been domesticated during *japonica* breeding selection.

198

199 **Discussion**

200 *HTD1* encodes a carotenoid cleavage dioxygenase involved in SL synthesis, which negatively
201 regulates the growth of axillary buds (Kulkarni et al. 2014; Zou et al. 2006). However, whether
202 and how *HDT1* functions in other tissues was unknown. In this study, we identified a mutant, *so1*,
203 which is allelic to *hdt1*. Phenotypic characterization indicated that *SO1/HTD1* functions in most
204 organs, such as stem, leaf, panicle and seed, and positively regulates organ size. *SO1/HTD1* is
205 involved in SL synthesis pathway, therefore, we determined that SL signaling pathway is involved
206 in cell proliferation and cell expansion. Additionally, we showed that *SO1/HTD1* negatively
207 regulates Cd accumulation in rice grain, the first indication that SL signaling participates in the
208 pathway of Cd accumulation in rice grain. Our study expands understanding of SL signaling
209 pathway in rice development processes. Also, our results suggest a possibility of increasing organ
210 size and reducing Cd accumulation in rice grain by manipulating *SO1/HTD1*.

211 The mutation of 8bp insertion in *SO1/HTD1,* which caused a premature stop codon in the *so1*
212 mutant, is different from the reported mutation in *HTD1*. Therefore, the allelic mutation in *so1*
213 provided a novel haplotype for studying the protein function of *SO1/HTD1*.

214 As *D88*/*HDT2*/*D14* functions in the SL signaling pathway, its natural variation was proposed to
215 be responsible for panicle branch number differences between 9311 and PA64s (Peng et al. 2014).
216 Natural SNP polymorphisms surrounding *SO1/HTD1* were few in *japonica*, whereas diversified
217 in *rufipogon*, *indica* and *intermedia*, suggesting that the SNP haplotype of *SO1/HTD1* in *japonica*

was selected during *Japonica* rice breeding, and is possibly important for some important agronomic traits.

Conclusion

The *so1* mutant exhibited small organs, such as small leaf, panicle and grain size. *SO1* controls organ size by regulating cell proliferation and expansion. *SO1* also negatively regulates Cd accumulation in rice grain. An 8bp insertion in *Loc_Os06g46470* is responsible for the *so1* phenotype, and *so1* is allelic to *htd1*. The *japonica* haplotype of *SO1/HTD1* was selected during rice breeding.

Contribution

Peng Qin and Jiangbo Hu performed most experiments, wrote the manuscript and contributed equally to this work. Weilan Chen performed the Cd measurements. Guohua Zhang and Jing Li performed the sequence diversity analysis. Shijun Fan, Bin Tu, Xuewei Chen and Yuping Wang generated the population for mapping, and Shigui Li and Bingtian Ma designed and supervised the research.

Acknowledgements

Thanks to Sheila McCormick for editing and comments on the manuscript.

References

Al-Babili S, and Bouwmeester HJ. 2015. Strigolactones, a novel carotenoid-derived plant hormone. *Annu Rev Plant Biol* 66:161-186. 10.1146/annurev-arplant-043014-114759

Clemens S, Aarts MG, Thomine S, and Verbruggen N. 2013. Plant science: the key to preventing slow cadmium poisoning. *Trends Plant Sci* 18:92-99. 10.1016/j.tplants.2012.08.003

Hu Y, Xie Q, and Chua NH. 2003. The Arabidopsis auxin-inducible gene ARGOS controls lateral organ size. *Plant Cell* 15:1951-1961.

Huang X, Zhao Y, Wei X, Li C, Wang A, Zhao Q, Li W, Guo Y, Deng L, Zhu C, Fan D, Lu Y, Weng Q, Liu K, Zhou T, Jing Y, Si L, Dong G, Huang T, Lu T, Feng Q, Qian Q, Li J, and Han B. 2012. Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nat Genet* 44:32-39. 10.1038/ng.1018

Jiang Y, Bao L, Jeong SY, Kim SK, Xu C, Li X, and Zhang Q. 2012. XIAO is involved in the control of organ size by contributing to the regulation of signaling and homeostasis of brassinosteroids and cell cycling in rice. *Plant J* 70:398-408. 10.1111/j.1365-313X.2011.04877.x

Kulkarni KP, Vishwakarma C, Sahoo SP, Lima JM, Nath M, Dokku P, Gacche RN, Mohapatra T, Robin S, Sarla N, Seshashayee M, Singh AK, Singh K, Singh NK, and Sharma RP. 2014. A substitution mutation in OsCCD7 cosegregates with dwarf and increased tillering phenotype in rice. *J Genet* 93:389-401.

Kumagai S, Suzuki T, Tezuka K, Satoh-Nagasawa N, Takahashi H, Sakurai K, Watanabe A, Fujimura T, and Akagi H. 2014. Functional analysis of the C-terminal region of the vacuolar cadmium-transporting rice OsHMA3. *FEBS Lett* 588:789-794. 10.1016/j.febslet.2014.01.037

Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato Y, Sakakibara H, and Kyozuka J. 2007. Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* 445:652-655. 10.1038/nature05504

Lee S, and An G. 2009. Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. *Plant Cell Environ* 32:408-416. 10.1111/j.1365-3040.2009.01935.x

Li Y, Zheng L, Corke F, Smith C, and Bevan MW. 2008. Control of final seed and organ size by the DA1 gene family in Arabidopsis thaliana. *Genes Dev* 22:1331-1336. 10.1101/gad.463608

Miyadate H, Adachi S, Hiraizumi A, Tezuka K, Nakazawa N, Kawamoto T, Katou K, Kodama I, Sakurai K, Takahashi H, Satoh-Nagasawa N, Watanabe A, Fujimura T, and Akagi H. 2011. OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytol* 189:190-199. 10.1111/j.1469-8137.2010.03459.x

Peng Y, Gao Z, Zhang B, Liu C, Xu J, Ruan B, Hu J, Dong G, Guo L, Liang G, and Qian Q. 2014. Fine mapping and candidate gene analysis of a major QTL for panicle structure in rice. *Plant Cell Rep* 33:1843-1850. 10.1007/s00299-014-1661-0

project rg. 2014. The 3,000 rice genomes project. *Gigascience* 3:7. 10.1186/2047-217X-3-7

Qin P, Tu B, Wang Y, Deng L, Quilichini TD, Li T, Wang H, Ma B, and Li S. 2013. ABCG15 encodes an ABC transporter protein, and is essential for post-meiotic anther and pollen exine development in rice. *Plant Cell Physiol* 54:138-154. 10.1093/pcp/pcs162

Sasaki A, Yamaji N, and Ma JF. 2014. Overexpression of OsHMA3 enhances Cd tolerance and expression of Zn transporter genes in rice. *J Exp Bot* 65:6013-6021. 10.1093/jxb/eru340

Sauter M, Mekhedov SL, and Kende H. 1995. Gibberellin promotes histone H1 kinase activity and the expression of cdc2 and cyclin genes during the induction of rapid growth in deepwater rice internodes. *Plant J* 7:623-632.

Scheet P, and Stephens M. 2006. A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. *Am J Hum Genet* 78:629-644. 10.1086/502802

Takahashi R, Ishimaru Y, Nakanishi H, and Nishizawa NK. 2011. Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. *Plant Signal Behav* 6:1813-1816. 10.4161/psb.6.11.17587

Ueno D, Kono I, Yokosho K, Ando T, Yano M, and Ma JF. 2009. A major quantitative trait locus controlling cadmium translocation in rice (Oryza sativa). *New Phytol* 182:644-653. 10.1111/j.1469-8137.2009.02784.x

Uraguchi S, and Fujiwara T. 2012. Cadmium transport and tolerance in rice: perspectives for reducing grain cadmium accumulation. *Rice (N Y)* 5:5. 10.1186/1939-8433-5-5

Uraguchi S, Kamiya T, Clemens S, and Fujiwara T. 2014. Characterization of OsLCT1, a cadmium transporter from

indica rice (Oryza sativa). *Physiol Plant* 151:339-347. 10.1111/ppl.12189

Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, and Zhu L. 2006. The rice HIGH-TILLERING DWARF1 encoding an ortholog of Arabidopsis MAX3 is required for negative regulation of the outgrowth of axillary buds. *Plant J* 48:687-698. 10.1111/j.1365-313X.2006.02916.x

Supplementary Table

319

Primers	Forward primer(5`-3`)	Reverse primer(5`-3`)
AG234	GCTCACCTGCACCTAGGTAT	CTACTCGCGAGGATCCATGG
RM470	CCCTCCCGTAGACCTTGTACCC	CCACAGCTAACCAATCCTTCTCC
A1	CCCTATTTAGACTTGCAAACCCA	CTCTCTCATTGCCACGGTAT
A4	CGACCGTGCATATGATCACC	TCCGTAAGCATACAGGCTCT
A6	AGCAGGGCGAATTGAGATCC	ACGGTAGCGTTTTTTACTTGGA
HTD1-1	TAAAGCACGAGAAACGAGCCAACG	GAGGAGGATGTAGTGGGTGTCGGT
HTD1-2	CACTTCACTTTCTACGGTCAGCTC	CACCTCTAGTTTTGCCACAAGTGC
HTD1-3	AAGATAGGGACAGAGAATGCACTT	GGTTTGATTCAGTTCCTATTTATG
HTD1-4	AGAGGATGGTGGCTATGT	TTTATCAATACACGAGGC
Ubiquitin	AGAAGGAGTCCACCCTCCACC	GCATCCAGCACAGTAAAACACG
D3	AAGCCGGTTTATCCAATTCC	GCACCAAGAATCGTCTGGAT
D10	CGTGGCGATATCGATGGT	CGACCTCCTCGAACGTCTT
D14	AGCGACGAGTCAAAACGAAG	TAAAATCCGACGCGGTAAAA
SO1	AGATGCCATCTAGTGGTGCT	GCTCATTCATCTCCCCAGAA
D27	TCTGGGCTAAAGAATGAAAAGGA	AGAGCTTGGGTCACAATCTCG
D53	ATAGGAAACCTGTGCCGACC	GGCGGGAGCAAAACTCCTAA

320

321

Figure 1(on next page)

Figure1-6

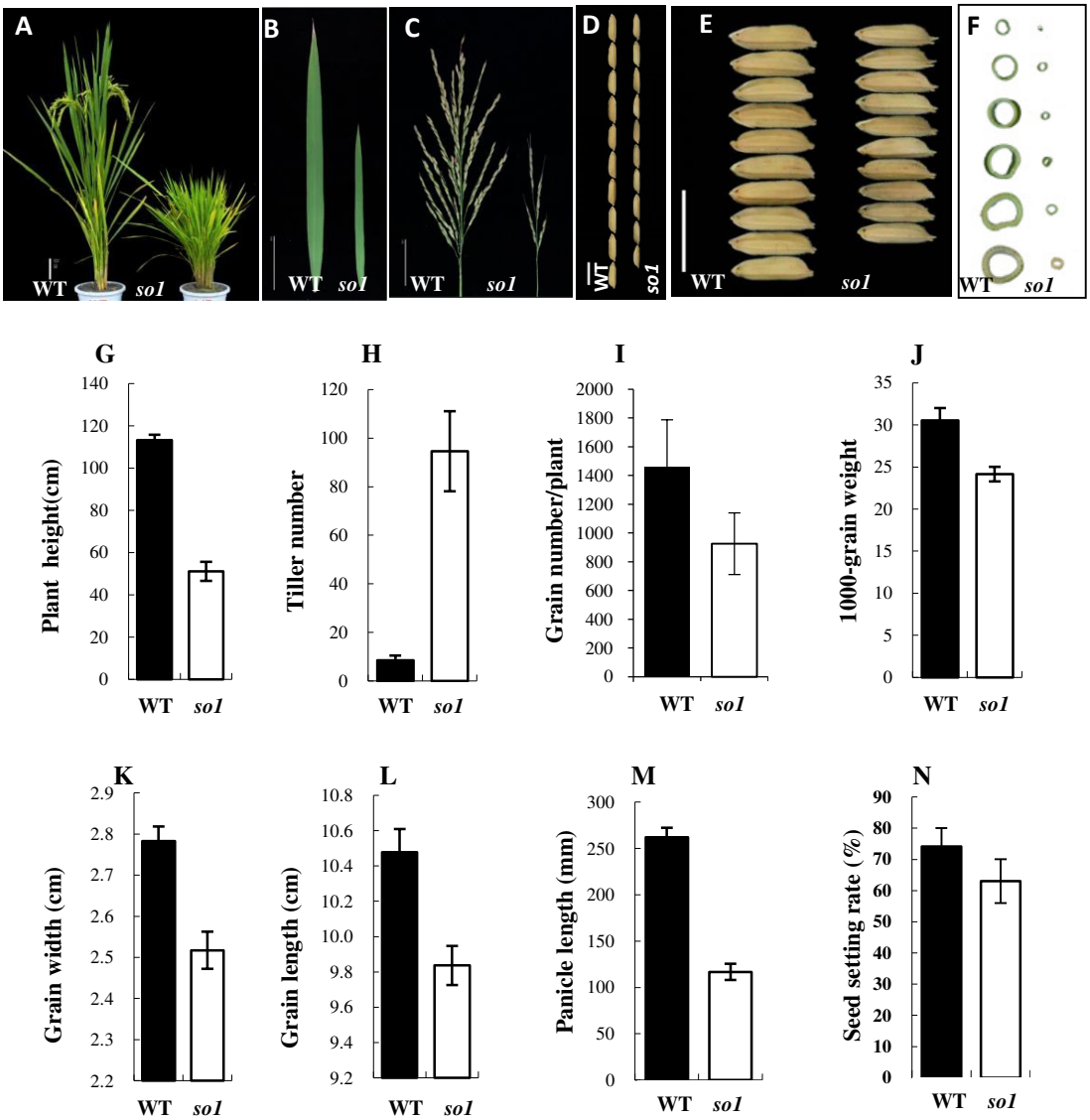

Fig.1 Comparison of plant (A), leaf (B), panicle (C), grain length (D), grain width (E) and each internode (F) between WT and *so1*. The sequence of internodes from top to bottom is the same with that of each plant internode. The agronomic traits between WT and so1, (G) Plant height; (H) Tiller number; (I) Grain number per plant; (G) 1000-grain weight; (K) Grain width; (L) Grain length; (M) Panicle length; (N) Seed setting rate.

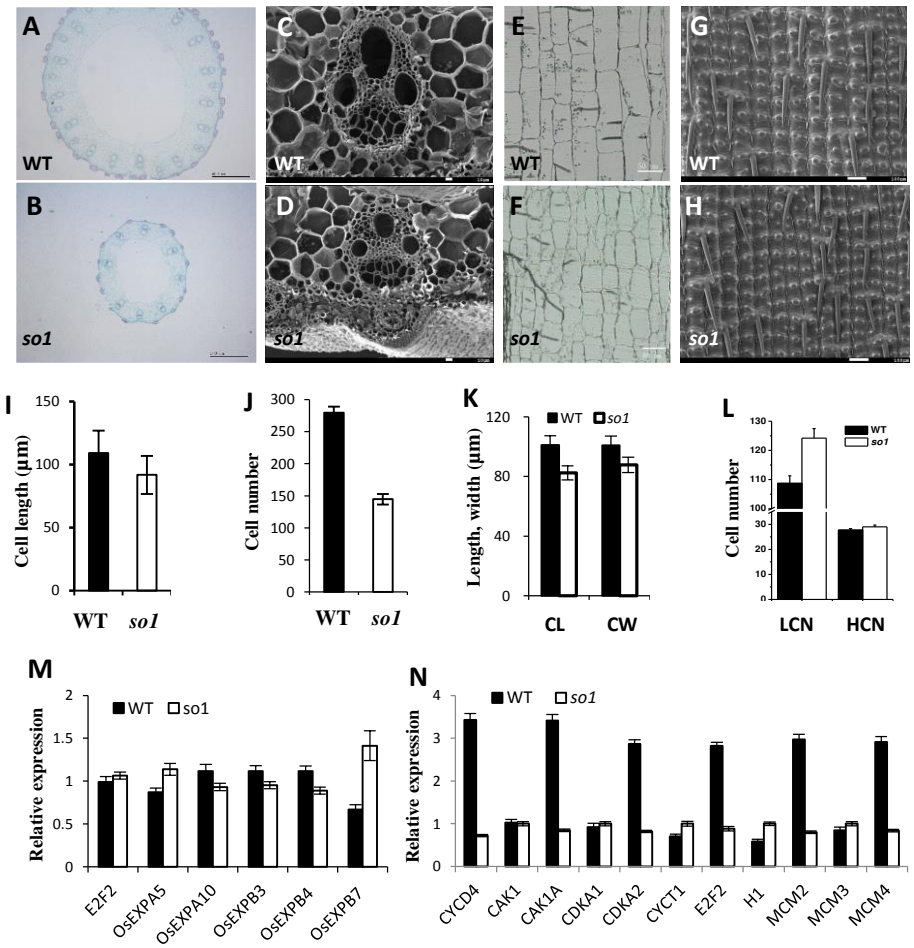

Fig.2 Section comparison of the first internode between WT (A) and *so1* (B). Cell size comparison of vascular bundle by SEM between WT (C) and *so1* (D). Comparison of cell size and number of the first internode epidermal cell by longitudinal section between WT (E) and *so1* (F). Comparison of cell size and number of spikelet hull epidermal cell by SEM between WT (G) and *so1* (H). Quantitative analysis of longitudinal cell length (I) and cell number (J) of the first internode. Quantitative analysis of cell length and width (K), longitudinal and horizontal cell number (L) between WT and *so1* spikelet hull, Expression analysis of genes related cell expansion (M) and cell proliferation (N) between WT and *so1*. CL: cell length, CW: cell width, LCN: longitudinal cell number, HCN: horizontal cell number.

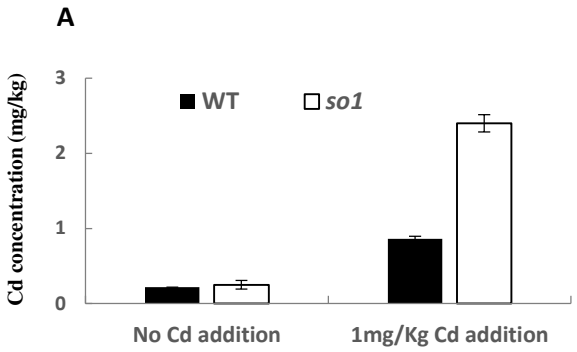

Fig.3 The comparison of Cd concentration in grain between WT and *so1* (A).

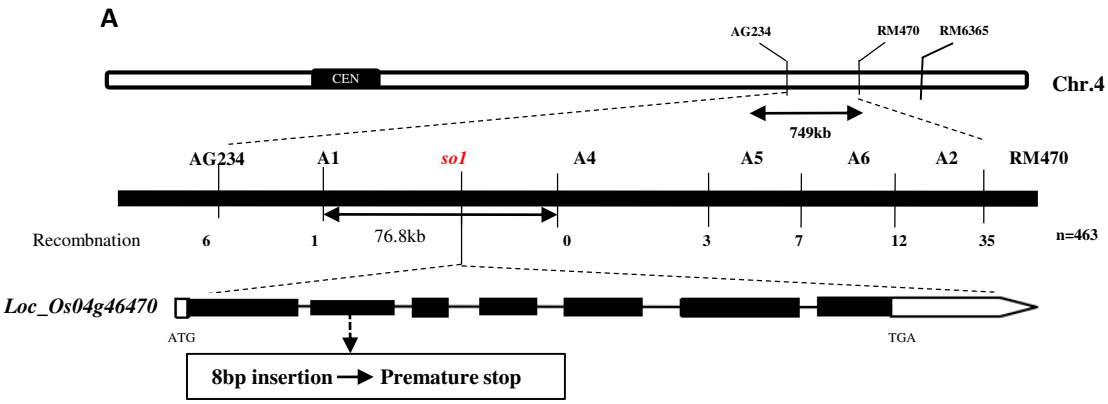

Fig. 4 Gene mapping schematic of *SO1* (A).

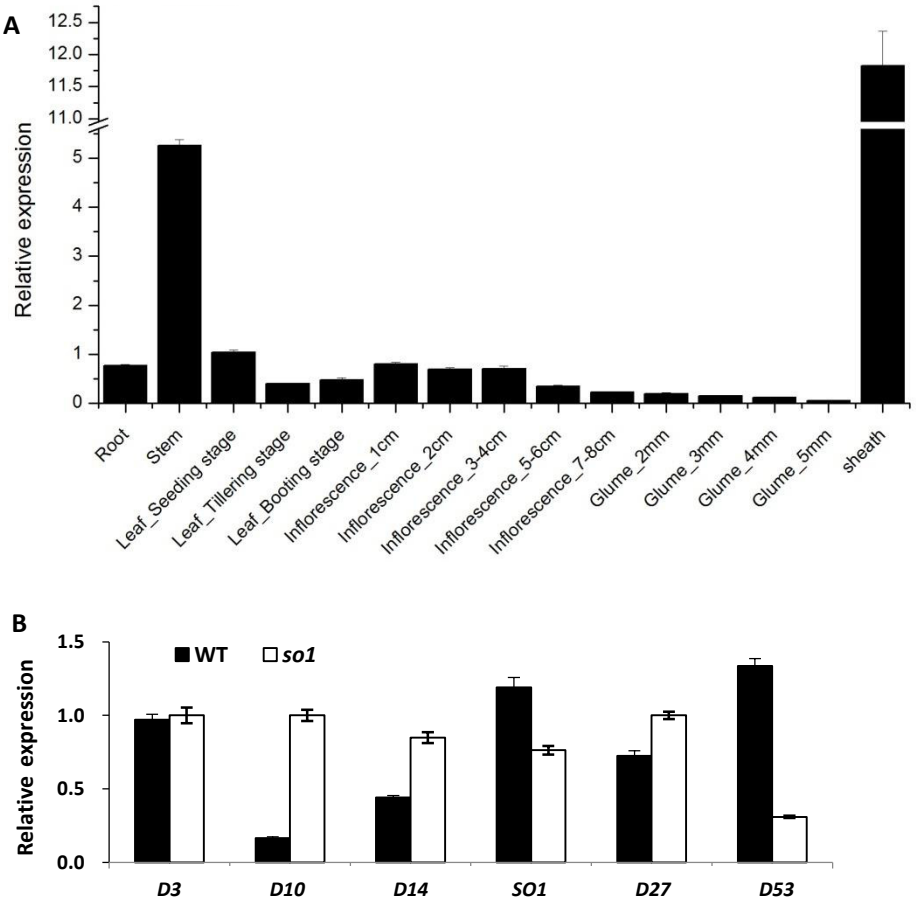

Fig.5 (A) Expression pattern of *SO1* among various tissues. (B) Expression analysis of genes related SL biosynthesis and signal perception between WT and *so1*.

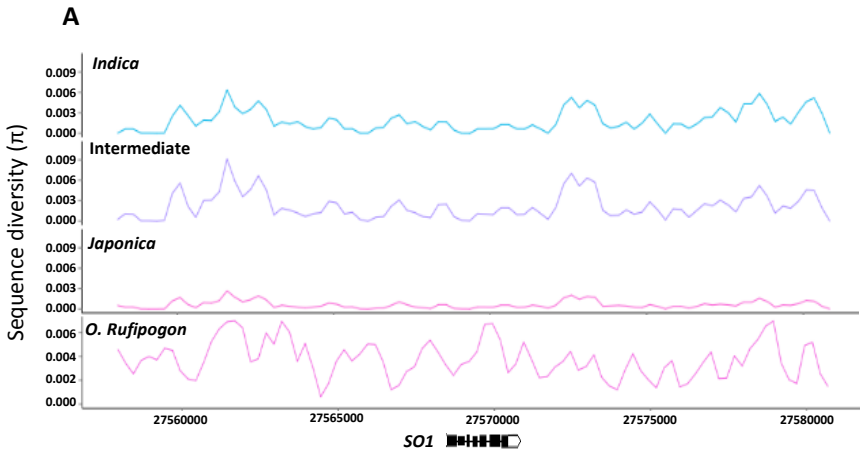

Fig.6 Sequence diversity surrounding *SO1/HTD1* among *Indica*, *Intermediate*, *Japonica* and O.*Rufipogon* (A).