nature genetics

OsSPL14 promotes panicle branching and higher grain productivity in rice

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Identification of alleles that improve crop production and lead to higher-yielding varieties are needed for food security. Here we show that the quantitative trait locus *WFP* (*WEALTHY FARMER'S PANICLE*) encodes OsSPL14 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14, also known as IPA1). Higher expression of *OsSPL14* in the reproductive stage promotes panicle branching and higher grain yield in rice. *OsSPL14* controls shoot branching in the vegetative stage and is affected by microRNA excision. We also demonstrate the feasibility of using the *OsSLP14*^{WFP} allele to increase rice crop yield. Introduction of the high-yielding *OsSPL14*^{WFP} allele into the standard rice variety Nipponbare resulted in increased rice production.

Rice is a staple food for nearly half of the world's population, with more than 10,000 rice varieties providing almost one-quarter of the global per capita dietary energy supply[1]. Grain number, grain size, panicle size and branch number in a panicle are directly associated with rice productivity. Several genes have been shown to influence these rice traits: *GN1A* and *APO1* regulate grain number[2,3]; *GS3, GW2* and *qSW5* regulate grain size[4–6]; *DEP1* controls panicle size[7]; and *FZP* and *LAX1* (ref. 8,9) control branch number per panicle. However, identification of other genes that regulate these traits would help us further understand the molecular mechanisms that regulate rice productivity and would also facilitate breeding of new varieties that increase rice crop yield.

To investigate genes that regulate rice grain productivity, we selected two rice lines, Nipponbare and ST-12 (from the Stocked rice collections of Togo field and Nagoya University-12), which differ in number of grains per panicle. The typical *japonica* rice variety, Nipponbare, has approximately 152 grains in the main panicle; in contrast, ST-12 has approximately 475 grains in the main panicle (**Fig. 1a–c**). Nipponbare and ST-12 have 10.5 and 28.9 primary branches in the main panicle, respectively, and thus the difference in grain number between the two lines is primarily due to the difference in the number of their primary branches (**Fig. 1b,d**).

To identify the gene responsible for the increased number of primary panicle branches in ST-12, we produced an F_2 population derived from a cross between Nipponbare and ST-12 plants and observed the number of primary branches in 192 of these plants (**Supplementary Fig. 1**). Quantitative trait locus (QTL) analysis of F_2 plants with 118 molecular markers detected two major QTLs with a $\log_{10}$ odds (LOD) score greater than 3.0 on chromosomes 1 (LOD score = 4.952, additive effect = 3.022) and 8 (LOD score = 13.229, additive effect = 4.275) (**Supplementary Table 1**). Because the QTL on chromosome 1 included the *GN1A* locus, we sequenced and detected the same mutation as the dominant Habataki allele[2]. We concluded that the detected QTL on chromosome 1 in this study might be affected by *GN1A*.

To further understand the molecular mechanisms of the production of primary panicle branches, we focused on the positional cloning of the QTL detected on chromosome 8, named here *WFP* (*WEALTHY FARMER'S PANICLE*), which acts in a semidominant manner (**Supplementary Fig. 2**). Analysis of 3,000 F_2 plants narrowed the candidate region to between RM223 and RM264 (**Fig. 1e**). Further high-resolution mapping using F_3 and F_4 recombinant lines narrowed the candidate region down to a 2.6-kb area (**Fig. 1e** and **Supplementary Figs. 3** and **4**). The Rice Annotation Project Database (RAP-DB) has predicted that the gene at the Os08g0509800 locus encodes a hypothetical protein in this region (RAP-DB; see URLs and **Fig. 1e**). We compared the sequence of the 2.6-kb candidate region between the Nipponbare and ST-12 lines but found no difference in nucleotide sequence. We performed expression analysis of the gene at Os08g0509800 but did not detect expression in either Nipponbare or ST-12 plants (data not shown). We could find no evidence of transcripts from the gene at Os08g0509800 in available databases, suggesting Os08g0509800 may contain a pseudogene.

Analysis of neighboring genes revealed that the candidate 2.6-kb region was upstream of Os08g0509600 (also predicted as LOC_Os08g39890 in the Rice Genome Annotation Project; see URLs and **Fig. 1e**), which encodes the plant-specific transcription factor OsSPL14 (ref. 10). Phylogenetic analysis indicated that the *OsSPL* gene at Os08g0509600 is categorized as *OsSPL14* and is conserved in sorghum, wheat, maize and *Arabidopsis thaliana* (**Supplementary Fig. 5a,b**)[10]. Sequence analysis revealed that there was no difference in the coding region of *OsSPL14* between Nipponbare and ST-12 plants.

Quantitative RT-PCR analysis of *OsSPL14* detected a difference in expression between Nipponbare and ST-12 in the shoot apices and

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Received 1 March; accepted 21 April; published online 23 May 2010; doi:10.1038/ng.592

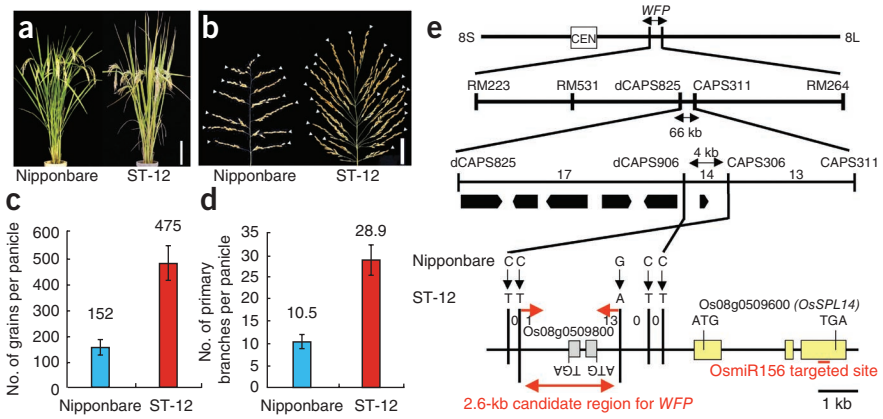

Figure 1 Characterization and cloning of the *WFP* QTL. (**a**) The gross morphologies of Nipponbare and ST-12. Scale bar, 20 cm. (**b**) The panicle morphologies of Nipponbare and ST-12. Arrowheads indicate primary branches. Scale bar, 5 cm. (**c**) Comparison of grain number per main panicle between Nipponbare and ST-12. (**d**) Comparison of primary branch number per main panicle between Nipponbare and ST-12. (**e**) The *WFP* locus was detected between RM223 and RM264 on chromosome 8. Positional cloning narrowed the *WFP* locus to a 66-kb region between dCAPS825 and CAPS311, and six genes are predicted to be located in this region by RAP-DB. Further analysis used 14 recombinant plants to narrow the candidate region to 2.6 kb. Numbers on the map indicate the number of recombinants. Predicted open reading frames and sequence differences between Nipponbare and ST-12 around the *WFP* candidate region are shown. Values are means, with bars showing s.d. ($n = 40$ plants in **c**,**d**).

young panicles, with higher expression in ST-12 than in Nipponbare (**Fig. 2a**). Because primary branches begin differentiation in the early stages of panicle development, we investigated *OsSPL14* expression level at several developmental stages of the panicle. The expression of *OsSPL14* at the 1–2-mm and 2–5-mm stages was about ninefold higher in ST-12 than in Nipponbare (**Fig. 2b**). We also assessed the expression pattern by *in situ* hybridization in young panicles (**Fig. 2c–j**). By this means we detected *OsSPL14* expression around the branch meristems in Nipponbare and ST-12 (**Fig. 2c–e**,**g–i**), and again, we detected higher *OsSPL14* expression in young panicles of ST-12 (**Fig. 2c–e**,**g–i**).

To investigate whether *OsSPL14* is the gene underlying the *WFP* QTL, we cloned 11-kb genomic fragments of *OsSPL14*, including the 2.6-kb candidate region, five SNPs around the candidate region and the coding region, from both Nipponbare and ST-12 (*pNip::OsSPL14* and *pST-12::OsSPL14*; **Supplementary Fig. 6a**,**b**); we then transformed each genomic fragment into Nipponbare. Both transgenic plant lines showed a higher primary branch number than Nipponbare plants transformed with a vector control (**Fig. 2k**,**l**). These results confirmed that *OsSPL14* functions in the regulation of primary branch number, and thus we concluded that the *WFP* QTL encodes OsSPL14.

Heritable differences in gene expression not due to DNA sequence changes are defined as epigenetic alleles[11]. Epigenetic alleles have been reported in *Arabidopsis*[12–17] and rice[18]. We considered whether heritable epigenetic marks in the endogenous *OsSPL14* promoter may be related to different expression levels of *OsSPL14*. To test this, we performed bisulfite sequencing to compare DNA methylation levels of the 2.6-kb candidate region in Nipponbare and ST-12; overall, there was no significant difference in total DNA methylation in the

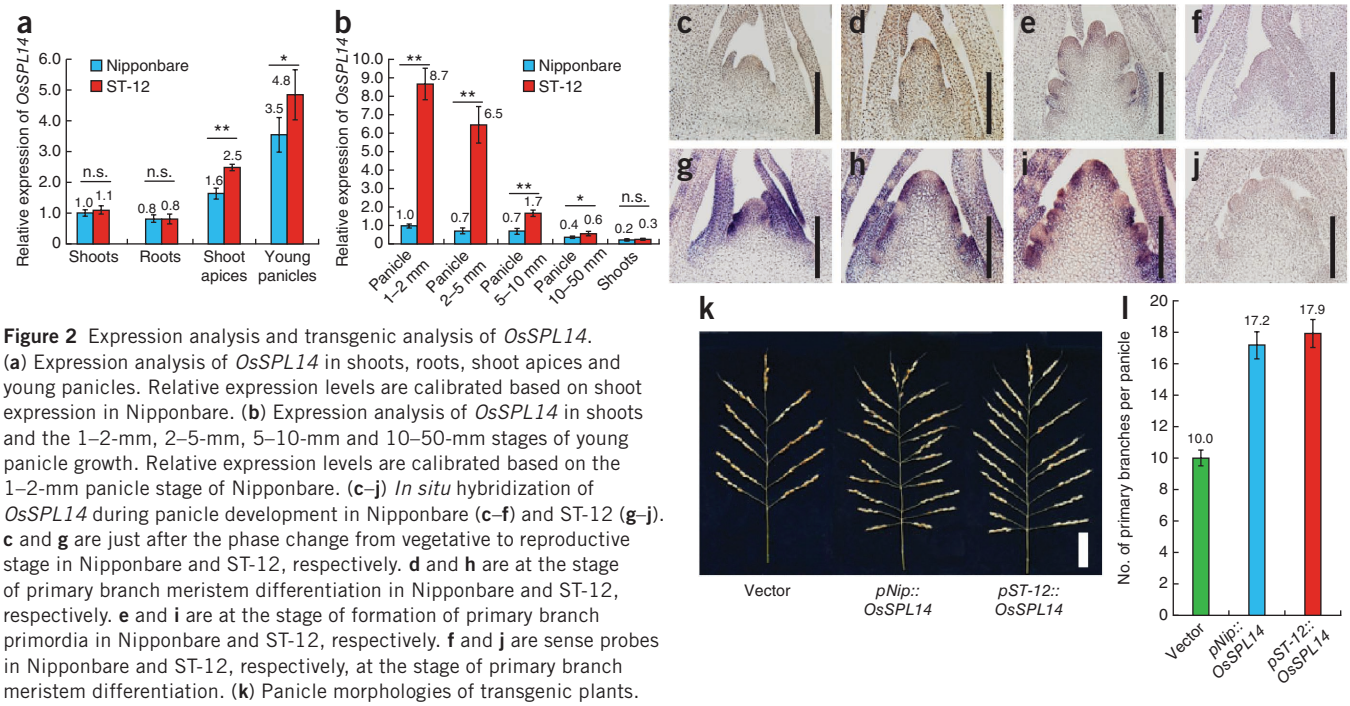

Figure 2 Expression analysis and transgenic analysis of *OsSPL14*. (**a**) Expression analysis of *OsSPL14* in shoots, roots, shoot apices and young panicles. Relative expression levels are calibrated based on shoot expression in Nipponbare. (**b**) Expression analysis of *OsSPL14* in shoots and the 1–2-mm, 2–5-mm, 5–10-mm and 10–50-mm stages of young panicle growth. Relative expression levels are calibrated based on the 1–2-mm panicle stage of Nipponbare. (**c–j**) *In situ* hybridization of *OsSPL14* during panicle development in Nipponbare (**c–f**) and ST-12 (**g–j**). **c** and **g** are just after the phase change from vegetative to reproductive stage in Nipponbare and ST-12, respectively. **d** and **h** are at the stage of primary branch meristem differentiation in Nipponbare and ST-12, respectively. **e** and **i** are at the stage of formation of primary branch primordia in Nipponbare and ST-12, respectively. **f** and **j** are sense probes in Nipponbare and ST-12, respectively, at the stage of primary branch meristem differentiation. (**k**) Panicle morphologies of transgenic plants. *OsSPL14* driven by the Nipponbare and ST-12 promoter, indicated as *pNip::OsSPL14* and *pST-12::OsSPL14*, respectively. Vector, the TAC7 vector control. Scale bar, 5 cm. (**l**) Comparison of primary branch number per main panicle of transgenic plants. Values are means, with bars showing s.d. ($n = 3$ times in **a**,**b**; $n = 40$ plants in **l**). Scale bars in **c–j** indicate 200 μm. **Significant at 1% level; *significant at 5% level; n.s., not significant.