

Supplementary material

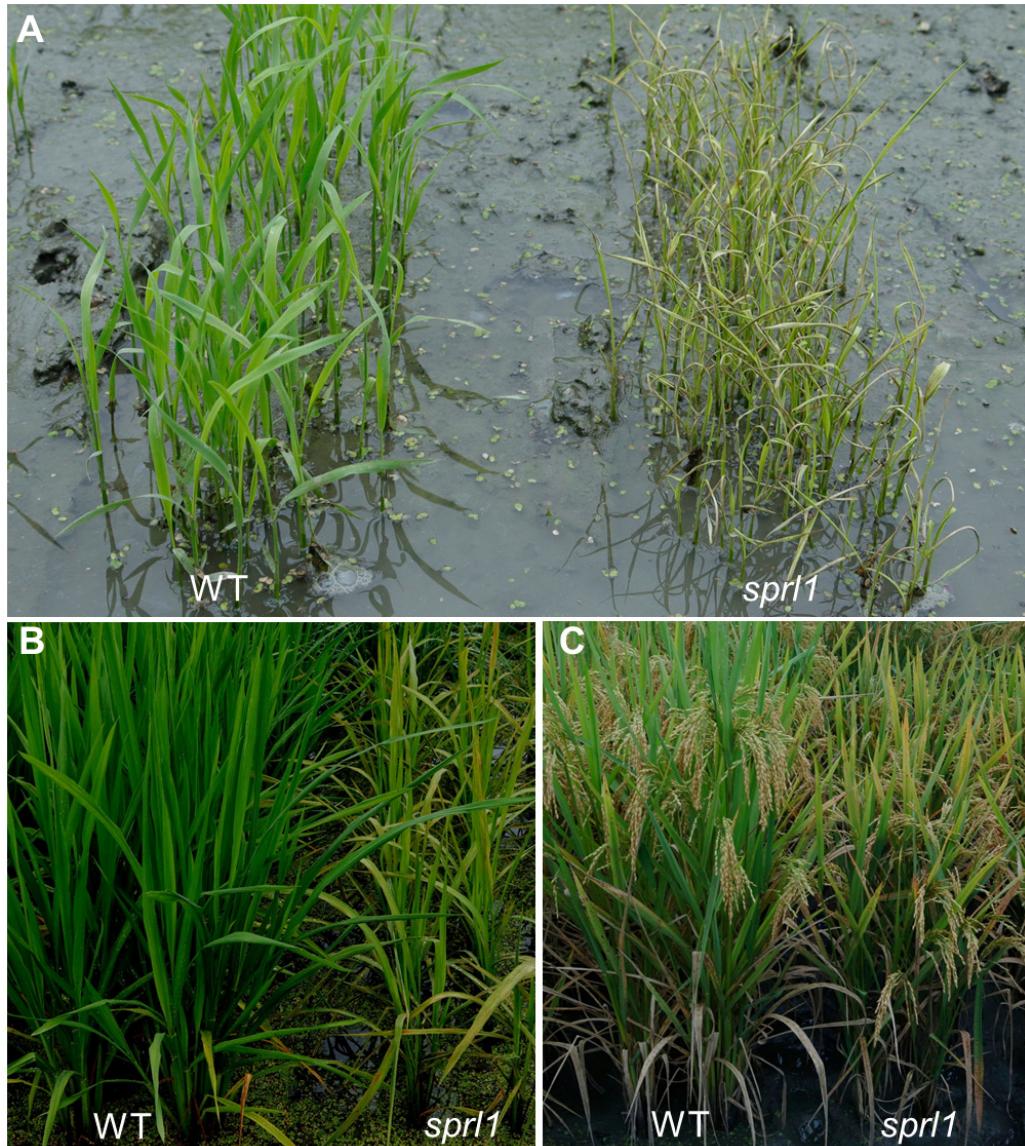


Figure S1. Plant phenotypes of the *sprl1* mutant and its wild type (WT). **(A)** Seedlings at the three-leaf stage. **(B)** Plants at the tillering stage. **(C)** Plants at the mature stage.

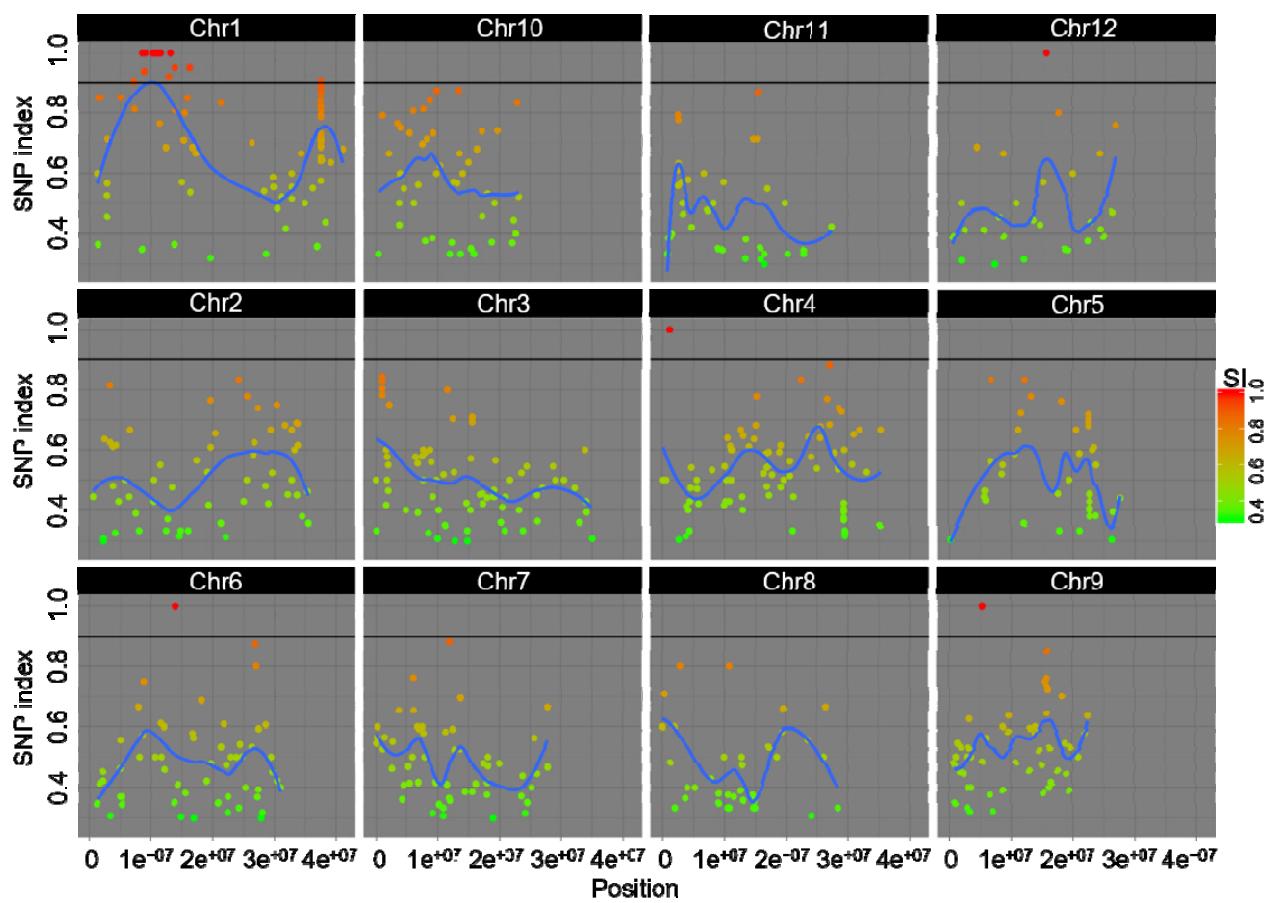


Figure S2. Δ (SNP-index) plot of the whole genome generated using MutMap.

OsPPO1		MAAAAAAMATATSATAAPPLR. IRDAARRTRRRGHVRCAVASG. AAEAPAAPGAR. . VSADCVVGGSISGLCTAQ	72
Zea mays		MVAATATAMATAASPLLNTRTPARLRLHRGLSLVRCAAVAGGAEEAAPASTGAR. . LSADCIVVGGGISGLCTAQ	71
Hordeum vulgare		MAGAGATMATAATAPPLR. . GRVTRRHGVRPRCRAAAGS. ATETPAVGVR. . LSADCIVVGGGISGLCTAQ	66
AtPPO1		MELSLLRPTTOSLLPSFS. KPNLRLNVKPLRLRCRVAGGPTVGSSKIEGGGTTITTDCCIVVGGGISGLCTAQ	73
Nicotiana tabacum	MTTTPIANHPNIFTHQG. .	SSSP. LAFLNRTSFIPFSS. ISKRNSNCNGWTRCSVAKDVTVPSSAVDGG. . PAAEELDCVIVGAGISGLCIAQ	88
Solanum tuberosum	MTTAVANHPSIFTHRSPLPSSSSSPSFLNRTNFIPYFS. TSKRNSNCNGWTRCSVAKDVTVPSEVDGN. . QFPELDCCVVGAGISGLCIAK	97	
Bacillus subtilis		MSDGKK. . HVVILGGGIGLAIAF	22
Homo sapiens		MGRTVVLGGGIGLAASY	19
Mycobacterium tuberculosis		MPTRSYCVGGGISGLTSAY	20
OsPPO1	ALATKHG. . VGDVLVTEARAPGGNI	TTAERAGECYLWEEGPNSFQPSDPV. . LTMAVDGSKDDLVFGD. . PNAPRFVWLWEGKLRRPVPS. .	156
Zea mays	ALATRHG. . VGDVLVTEARAPGGNI	TTVERPEECYLWEEGPNSFQPSDPV. . LTMAVDGSKDDLVFGD. . PNAPRFVWLWEGKLRRPVPS. .	155
Hordeum vulgare	ALATRHG. . VGDVLVTEARAPGGNI	TTVERPEECYLWEEGPNSFQPSDPV. . LTMAVDGSKDDLVFGD. . PNAPRFVWLWEGKLRRPVPS. .	150
AtPPO1	ALATKHDPA. APNLIVTEARDRGGNI	TREE. NGFLWEEGPNSFQPSDPM. . LTMAWDGSKDDLVLDG. . PTAPRFVWLWNGKLRRPVPS. .	157
Nicotiana tabacum	VMSANYP. . NLMVTEARDRAGGNUTI	IVER. DGYLWEEGPNSFQPSDPM. . LTMAWDGSKDDLVLDG. . PNAPRFVWLWKGKLRRPVPS. .	168
Solanum tuberosum	VISANYP. . NLMVTEARDRAGGNUTI	IVER. DGYLWEEGPNSFQPSDPM. . LTMAWDGSKDDLVLDG. . PDAPRFVWLWKDKLRRPVPS. .	177
Bacillus subtilis	YMEKEIKENKLPLELTLYEASPRVGGK	IIVVK. . DGYIILERGPDSFLERKKS. . APQLVKDLGLEHLLVNN. . ATGOSYLVNRTLHPMKGAVM	112
Homo sapiens	HLSRAPCP. . PKVVLVESSERLGGWTSVRGP.	NGAIFIPLGPRGIIRPAGA. GARTLLLVSCLGLDSEVLPVPGDHAAQRFLYVGGAHALP. .	110
Mycobacterium tuberculosis	RLRQAVG. . DDATITLFEPADRLGGVLRTEHIG. . GQPMDFGAELGLSRQLAST. . MPALLAELGLSDRQLAST. . GARPLYSQRLIPLPQTVV	★ ★	107
OsPPO1	PGDPFPFDLMSIPGKLRAGLGALGVRAAPPREESVEDFVRRNLGAEVFERLIEPF	CSGYAGDPSKLSMKAAFHKWRLEDTGGSTIGGSTIKTI	252
Zea mays	KPADLPFFDLMSIPGKLRAGLGALGIRPPPPGPREESVEE	FVRRNLGAEVFERLIEPFCSGYAGDPSKLSMKAAFHKWRLEETCGGSIGGSTIKTI	251
Hordeum vulgare	KPGDPFPFDLMSIPGKLRAGLGALGIRPPPPGPREESVEE	FVRRNLGAEVFERLIEPFCSGYAGDPSKLSMKAAFHKWRLEETCGGSIGGSTIKAI	246
AtPPO1	KLTDLPFFDLMSIPGKLRAGFGALGIRPSPSPGHEESVEE	FVRRNLGAEVFERLIEPFCSGYAGDPSKLSMKAAFHKWRLEETCGGSIGGSTIKAI	253
Nicotiana tabacum	KLTDLPFFDLMSIPGKLRAGFGALGIRPSPSPGHEESVEE	FVRRNLGAEVFERLIEPFCSGYAGDPSKLSMKAAFHKWRLEETCGGSIGGSTIKAI	264
Solanum tuberosum	KLTDLPFFDLMSIPGKLRAGFGALGIRPSPSPGHEESVEE	FVRRNLGAEVFERLIEPFCSGYAGDPSKLSMKAAFHKWRLEETCGGSIGGSTIKAI	273
Bacillus subtilis	GIPTLAFFPVTGLESKGRKAAMDFI. L. LPASKTPKDDDLSGEFFRRRVDEEVNIELPPI	LLGEFVYQTEQKHRSLILGSMKIKTR	211
Homo sapiens	GLRGELRSPPPFSKPLFVAGREL. TKPRGKDEDETHSAQRLGPVEASLDSLCRGVFGANSRELPS. FQAEQTHRSILLSLLGA	205	
Mycobacterium tuberculosis	G. I. TPSAGSMAGIVDDATLARDAEAPRFTWQVSDPAVADLADRFDQDVQVARSVDPILLSGVYAGSAATIGLRAAAPSAAALDRGATSVTAVRQA	205	
OsPPO1	QERGKNPKPPRDRPPIPTPKGQTVASFRKGLTMLPDAITSR	LSKVKLWSWLTSITKSDNKGAYIVYETPEVSVQAKTVVMTFSYVASDILRPSSSDA	352
Zea mays	QERSKNPKPPRDRPPIPTPKGQTVASFRKGLTMLPNAITSS	LSKVKLWSWLTSITKSDNKGAYIVYETPEVSVQAKSVIMTFSYVASNILRPSSSDA	351
Hordeum vulgare	QDKGKNPKPPRDRPPIPTPKGQTVASFRKGLTMLPNAITASR	LSKVKLWSWLTSITKSDNKGAYIVYETPEVSVQAKSVIMTFSYVASNILRPSSSDA	346
AtPPO1	QERKPKNAERDPRPPIPTPKGQTVGSRFKGLRMLPDAISAR	LSKVKLWSWLTSITKLESQGIVHNTYETPDGLVSVQAKSVIMTFSHVASGLLRPSESAA	353
Nicotiana tabacum	KERSSTPKAPRDRPPIPTPKGQTVGSRFKGLRMLPDAISAR	LSKVKLWSWLTSITKSEKGGYHNTYETPECVLSQSRSIVMTVFSYVASNILRPSVAA	364
Solanum tuberosum	KERSSNPKPPRDRPPIPTPKGQTVGSRFKGLRMLPDAICER	LSKVKLWSWLTSITKSEKGGYHNTYETPECVLSRSRSIVMTVFSYVASNILRPSVAA	373
Bacillus subtilis	PQGSQ. . QIATKQQGQFQTLQTLQTLQVEETEKOL	TKLKVYKGTVKTLSSHSCSYSLDNG. MTLDAADVIVIADPHKAAAGMLSEL. . A	299
Homo sapiens	GRTPOP. . DSALIQLAERWSQSLRGCLEMLPQALETHTLT	SRGVSVLVRQGPVCGLSLOAEGRKVSLRD. SSLEADHVISAFAVSLSELLPAEAPL	302
Mycobacterium tuberculosis	LPPGSG. . CPVFAGLDGQYQVLLDGLVRRS. . RVHWMVARVQQLER. GWVDRDETGGR. . WQADAVILAVAPRPLARLVDGJAPRT	284	
OsPPO1	ADALSIFYPPPVAATVSYPKEARKECLIDGELOQFGQI	HPRSQGVETLGTIYSSSLFPNRPAGRVLNNYIGGSTNTGIVSKT. . ESELEVEADR	448
Zea mays	ADALSIFYPPPVAATVSYPKEARKECLIDGELOQFGQI	HPRSQGVETLGTIYSSSLFPNRPAGRVLNNYIGGATNTGIVSKT. . ESELEVEADR	447
Hordeum vulgare	ADALSIFYPPPVAATVSYPKEARKECLIDGELOQFGQI	HPRSQGVETLGTIYSSSLFPNRPAGRVLNNYIGGSTNTGIVSKT. . ESDLVEADR	442
AtPPO1	ANALSKFYPPPVAATVSYPKEARKECLIDGELOQFGQI	PRKGLKGFFQIPTQGVETLGTIYSSSLFPNRPAGRVLNNYIGGSTNTGILSKS. . EGELVEADR	449
Nicotiana tabacum	ANALSNFYPPPVAATVSYPKEARKECLIDGELOQFGQI	PRKGLKGFFQIPTQGVETLGTIYSSSLFPNRPAGRVLNNYIGGAKNPEILSKT. . ESQLVEADR	460
Solanum tuberosum	ADALSKFYPPPVAATVSYPKEARKECLIDGELOQFGQI	HPRSQGVETLGTIYSSSLFPNRPAGRVLNNYIGGATNTEIVSKT. . ESQLVEADR	469
Bacillus subtilis	1SHLKMNHSTSIVANALGEGPEGSVQ. . MEHESTGFVI	SRNSDFAITACTWTNKWWHAPEBGTILLRAYEVKAGDESIVDLS. . DNDININMLE	389
Homo sapiens	ARALSAITAVSVAVLNQYQGAHLP. . VQFGHIVPSSEDPGVLGIIVYDSVAFPEQDGSPPGLRLTVMLGGSWLQTEASGCVLSQEFQQRQ	394	
Mycobacterium tuberculosis	HAAARQIVSASSAVIALAVPGTAFP. . HCSGVIVAGDESPHAKITLSSRKWGRG. . DVALRLSICRFGDEPALTAS. . DDQLLAWAAD	370	
OsPPO1	DLRKMLINPKAVDPLVLGVRVIPQAIPQFLI	CHLDLLEAKAKSAIYGKGGYDLEIIGCNIVAVALGRCEGAYESASQISDYLTKYAYK	536
Zea mays	DLRKMLINSTAVDPLVLGVRVIPQAIPQFLVGHLDLLEAKAKAALDGRGGYDGL	IIGGNYVAIVALGRCEGAYESASQISDYLTKYAYK	535
Hordeum vulgare	DLRKMLINPRAADPLALGVVRVIPQAIPQFLI	IIGGNYVAIVALGRCEGAYESASQISDYLTKYAYK	530
AtPPO1	DLRKMLIKPNSTDPLKLGVVRVIPQAIPQFLVGHFDR	DLTAKSSLTSSGYEGLIIGGNYVAIVALGRCEGAYETASIVNNFISRYAYK	537
Nicotiana tabacum	DLRKMLIKPKAODPLLVGVVRVIPQAIPQFLVGHLDLITSTAKAAMNDNGLEGL	IIGGNYVSGVALGRCEGAYEVASEVTGEISRYAYK	548
Solanum tuberosum	DLRKMLIKPKAODPLLVGVVRVIPQAIPQFLVGHLDLITGTAKTALSNDNLGDIIGGNYVSGVALGRCEGAYEVASEVTGEISQYAYK	557	
Bacillus subtilis	DLKKVMM. . INCEPEMCVTRI	HESMPQVYVGHKQRIKELREALAS. AYPGVYTGASFEGVPGDCIDQCKAAVSDALTYFS. .	470
Homo sapiens	EAAATQLG. LKEMPSHCILVHLHKNCI	PQYTLGHWOKLESARQFTAHRLP. LTIAAGASYEVAVNDCEESGRQAAIVSVLGTEPNS. .	477
Mycobacterium tuberculosis	DLTVFG. . VAVDPDVVRVRI	EAAMPQGPGHADVAELRAGFPP. . TLAAGASYLDGIVGPACVGAAGRAVTSVIEADQAVAR	452

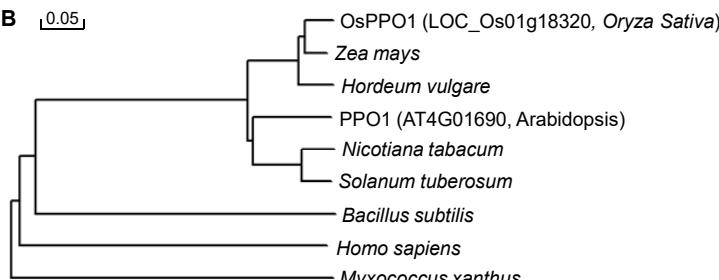


Figure S3. Sequence alignment and phylogenetic analysis of OsPPO1 and its homologues. **(A)** Sequence alignment. Identical residues are highlighted in blue, similar residues ($\geq 75\%$ identical) are highlighted in black. A black underline shows the putative chloroplast signal peptides. The red arrow indicates the mutant site of *sprl1*. The black asterisks shows the Gly-rich motif. **(B)** Phylogenetic tree representing alignment of *OsPPO1* homologs. The unrooted neighbor-joining tree using percentage identities was constructed based on a multiple sequence alignment produced with the program DNAMAN. Accession numbers for the respective sequences are as follows: rice (OsPPO1, LOC_Os01g18320), Zea mays (AAF26417.1), Hordeum vulgare (KAE8789512.1), Arabidopsis (PPO1, AT4G01690, BAA11820.1), Nicotiana tabacum (CAA73865.1), Solanum tuberosum (NP_001275224.1), *Bacillus subtilis* (AOT51497.1), *Homo sapiens* (BAA07538.1), *Myxococcus xanthus* (WP_003413871.1). Scale represents percentage substitution per site.

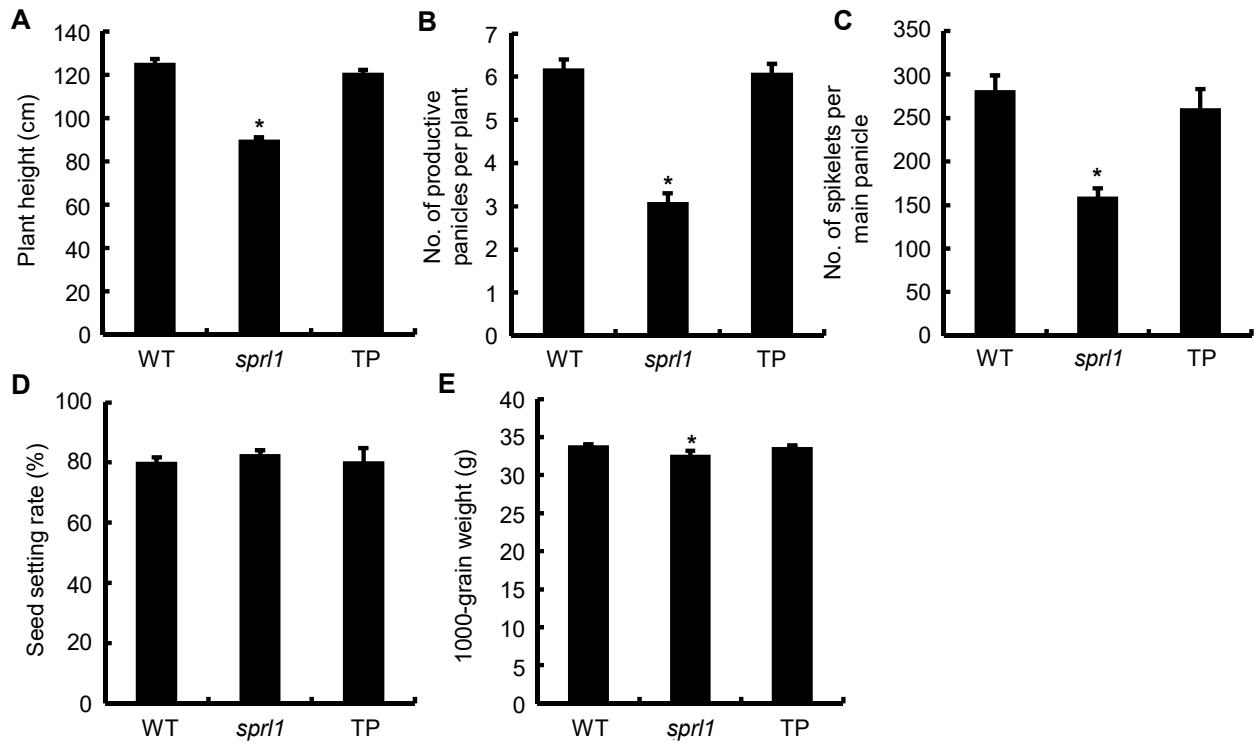


Figure S4. Major agronomic traits of complementation lines. Error bars represent standard errors of three independent experiments. The asterisk indicates statistically significant differences compared with the wild type at * $p < 0.05$. WT, wild type; TP, transgenic plants.

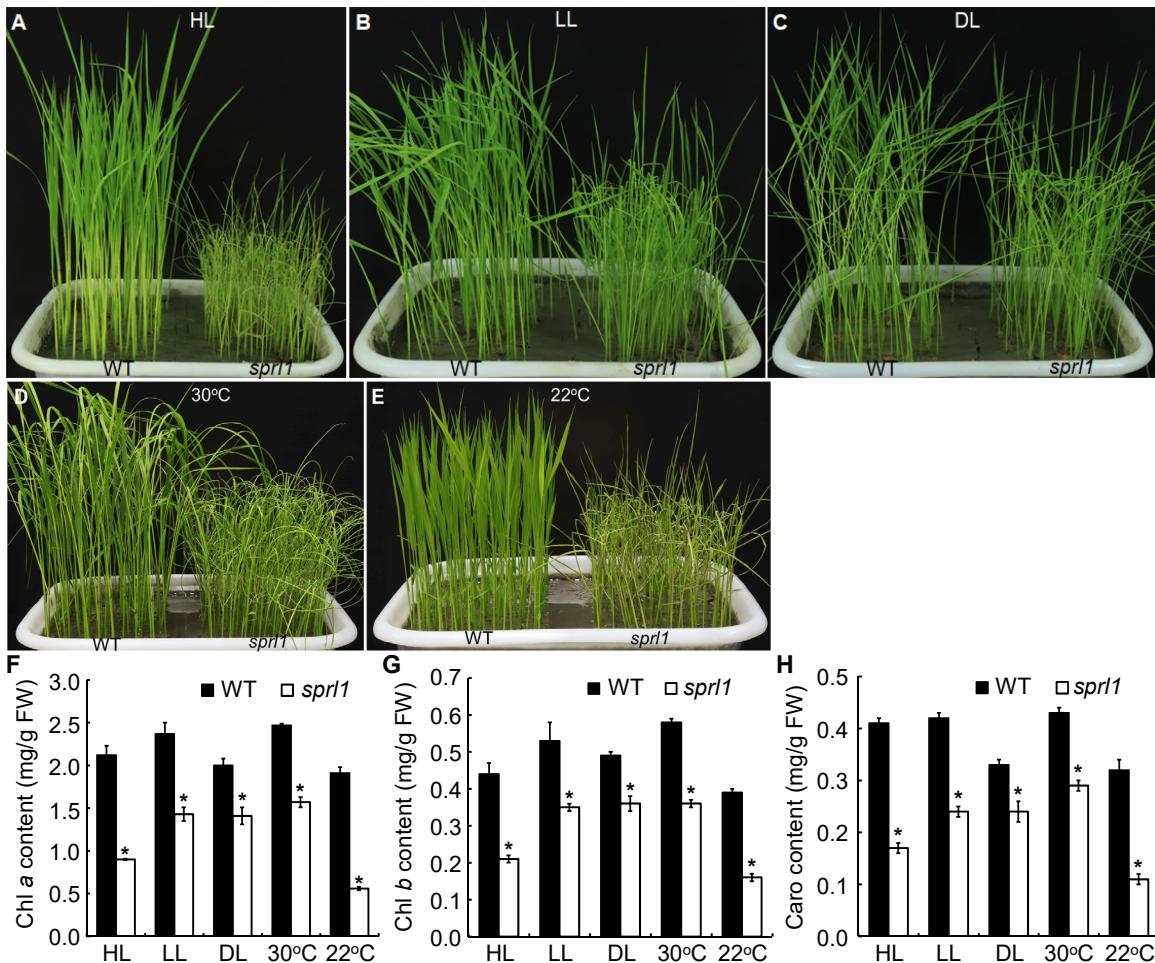


Figure S5. Phenotypic characterization of the *sprl1* mutant and WT grown in the growth chamber under various light intensity and temperature conditions. **(A,B,C)** 12-day-old seedlings grown under 12 h of high light (HL, 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$), low light (LL, 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and dim light (DL, 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) conditions at 28°C respectively/12 h of darkness at 26°C. **(D,E)** 12-day-old seedlings grown under constant 30°C and 22°C conditions respectively under 12 h of low light (LL, 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$)/12 h of darkness. **(F,G,H)** Contents of Chl *a*, Chl *b* and Caro respectively in WT and *sprl1* under various light intensity and temperature conditions. Data are means \pm SD of three independent experiments. Asterisks indicate statistically significant between WT and *sprl1* mutant at * $p < 0.05$ by Student's *t* test.

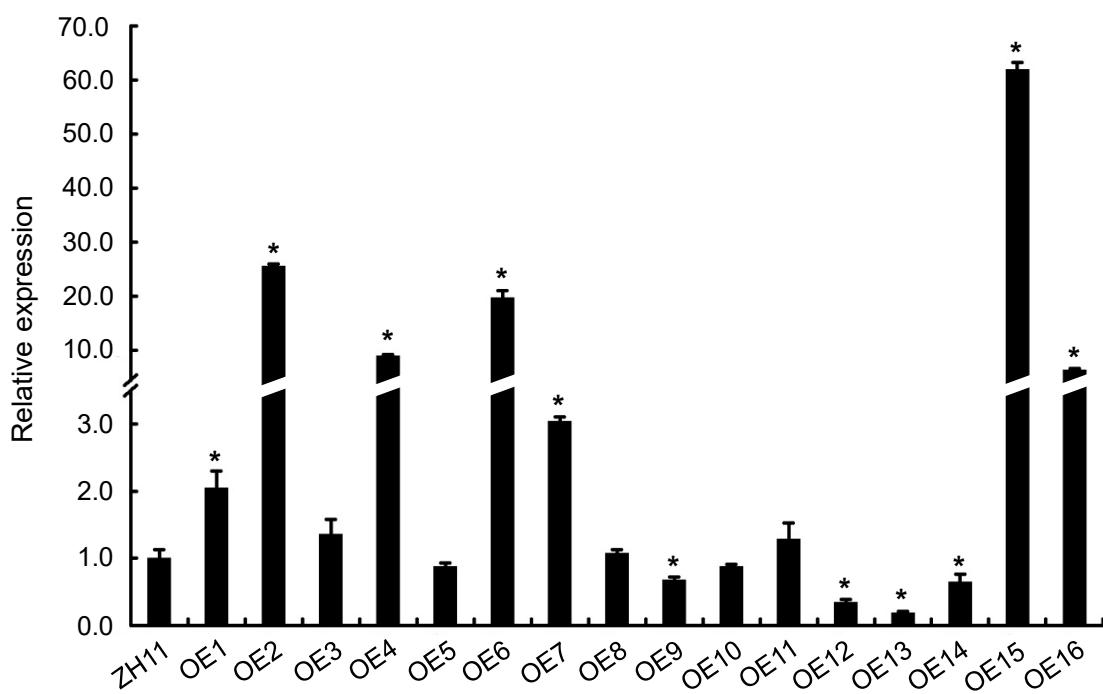


Figure S6. Relative expression levels of *OsPPO1* gene in the over-expressing lines. OE1-OE16 indicate transgenic lines overexpressing *OsPPO1* gene. The expression level in the control ZH11 was set to 1.0, and those in the over-expressing lines were calculated accordingly. Error bars represent standard errors of three independent biological replicates. Asterisk indicates statistically significant difference compared with the control ZH11 at * $p < 0.01$.

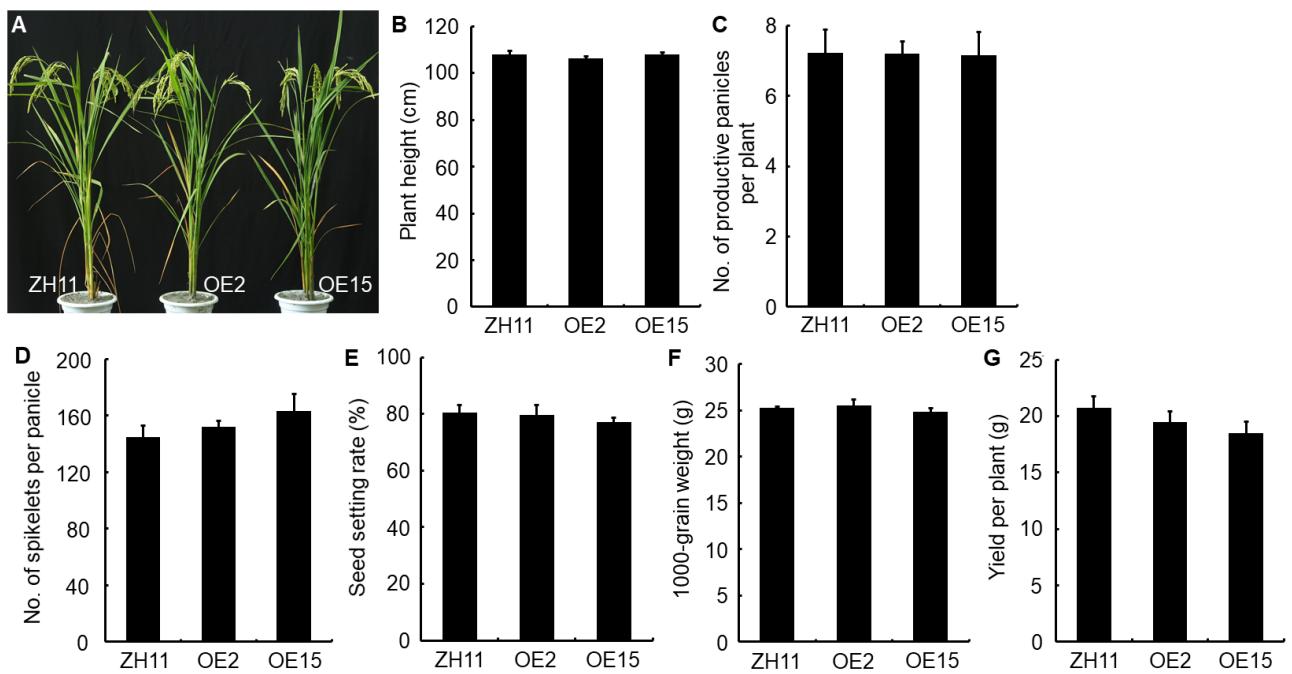


Figure S7. Comparison of major agronomic traits between the over-expressing lines and the control ZH11. Error bars represent standard errors of three independent experiments. The statistically significant differences were performed by Student's *t* test. * $p < 0.05$.

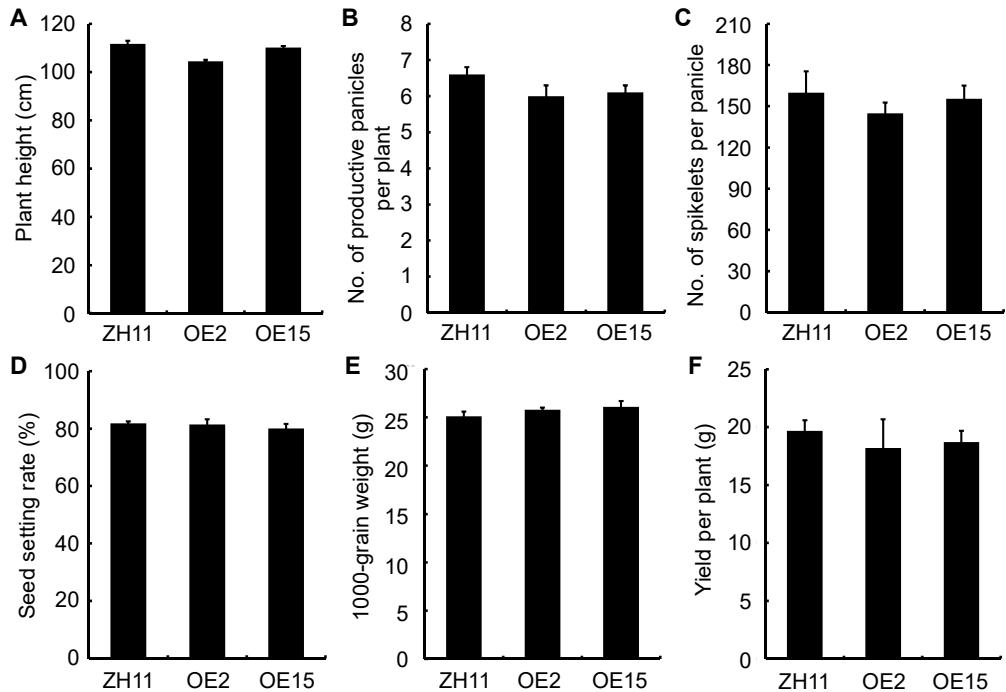


Figure S8. Comparison of major agronomic traits between the over-expressing plants with oxyfluorfen treatment and the control ZH11 without oxyfluorfen treatment. The 3-week-old seedling overexpressing *OsPPO1* gene were sprayed with 125 μ M of oxyfluorfen, and wild-type ZH11 plants were sprayed with water as control. Error bars represent standard errors of three independent experiments. The statistically significant differences were performed by Student's *t* test. * $p < 0.05$.

Table S1. Primers used in vector construction and detection

Marker	Forward primer (5'-3')	Reverse primer (5'-3')
<i>OsPPO1</i> -Com F/R	GAGCTCGGTACCCGGGGATCCAGGG	CAGGTCGACTCTAGAGGATCCGTCTGG
	CTGAGGCTGACATAACGG	GAAGAGTCTAACAG
<i>OsPPO1</i> -Tra F/R	TCGTATGTTGTGTAAT	CGTCCAATATGCTGCAGT
<i>OsPPO1</i> -OE F1/R1	GGACAGGGTACCCGGGGATCCATGG	AGAGCCCTGGCATGCCTGCAGTCAC
	CCGCCGCCGCCAGCC	GTAGGCGTACTTGGT
<i>OsPPO1</i> -OE F2/R2	ATCCACCAGTTGCTGCTG	AATCAGTAAATTGAACGG
<i>OsPPO1</i> -Gfp F/R	CGGGGATCCTCTAGAGTCGACATGG	CACCATGGTACTAGTGTGACCCACTT
	CCGCCGCCGCCAGCC	GTAGGCGTACTTGGT

Table S2. Primers used in qRT-PCR

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
<i>OSPO1</i>	AGGTGTGTATGCTGGTGA	AGCATAGTCAGACCCTTC	
<i>rbcL</i>	CTTGGCAGCATTCCGAGTAA	ACAACGGGCTCGATGTGATA	Su et al., 2012
<i>rbsS</i>	CAGCAATGGCGGCAGGAT	AGGGCACCCACTTGGAACG	Li et al., 2015
<i>psaA</i>	GGAGGTGGCGAGTTAGTA	GATTGCTTATCGGGTAT	Li et al., 2015
<i>psbA</i>	TATGGTCTGAGTGGGA	TTATGCTCTGCCCTGGAAT	Li et al., 2015
<i>FC1</i>	GGTCAACAGGGGTGAAAGAG	GTGCATCCAAGAGCTGGAAC	Inagaki et al., 2015
<i>FC2</i>	CTTGCTTATGTTGGTGCTA	AGCCCCACTCCCACAGTC	Inagaki et al., 2015
<i>HEMA1</i>	CGCTATTCTGATGCTATGGGT	TCTTGGGTGATGATTGTTGG	Su et al., 2012
<i>GSAM</i>	TGGACGTAAGGACATCAT	GTCCAAGTAATCGTAGGT	Wang et al., 2021
<i>HEMB</i>	ACCTGGATTGCCATACTT	TTCGTCGATCATGTTCACTG	Wang et al., 2021
<i>CHLD</i>	CAAGGGTCGCCAAGGTAAA	CTTCAGGTCCGAGAGTCAG	Li et al., 2019
<i>CHLH</i>	CCAATCGTAACCGAAGGT	CAATAATTGGCGCTCTCAA	Inagaki et al., 2015
<i>CHLI</i>	TTCGACAGGGATCCAAAGGC	ACAGCACCAAGGTTACTCCG	Li et al., 2019
<i>DVR</i>	CCATTGCCAGTTCTTGGTG	AATTGAATGGCTAATGGCGT	Inagaki et al., 2015
<i>PORA</i>	TGTACTGGAGCTGGAACACAA	GAGCACAGCAAAATCCTAGACG	Su et al., 2012
<i>YGL</i>	GGCACTGCTAGGACTCAC	CCCAAGACGAAGAACGGT	Li et al., 2019
<i>CAO1</i>	GATCCATACCGATCGACAT	CGAGAGACATCCGGTAGAGC	Su et al., 2012
<i>CatA</i>	CAACCGAACGTCGACAACCTCTT	TTCACCGGCAGCATCAGGTAGTTT	Yang et al., 2020
<i>CatB</i>	GCTGCTTCTGCCAGCGATAAT	AAATAGTTGGCCAAGACGGTGC	Yang et al., 2020
<i>APX1</i>	AGGTGCCACAAGGAAAGATCTGGT	TCAGCAGGGCTTGTCACTAGGAA	Yang et al., 2020
<i>APX2</i>	TGGGAAGATGCCACAAGGAGAGAT	TCCGCAGCATATTCTCCACCAGT	Yang et al., 2020
<i>POD1</i>	ACGTCGGGTCGCCAACAAC	CGAACTCGTCCACCGACGCC	Yang et al., 2016
<i>AOX1a</i>	CTTCGCATCGGACATCCATTA	TCCTCGGCAGTAGACAAACATC	Yang et al., 2020
<i>AOX1b</i>	CCTGCTCAGTTCATCACCATCA	GCATAAAACGGAGTGACAATAGC	Yang et al., 2020
<i>ROC5</i>	ACGAGCTCTCTCGTTGGG	GCAAAACACCCACGGCGTAAA	
<i>ZHD1</i>	CAGTTCTGTGACGAGGTCGG	ATGCATTGCTCCGGTCAT	
<i>REL1</i>	GCACCTCAGCTTGGAACTCT	CTGAGTCAGTGATCGGAGG	
<i>REL2</i>	TTGCCTTGTGGAGTTGGT	TGCACTTCACAGGACAAGCA	
<i>SRL1</i>	TCCTCATCTCCTGCCCTCCTC	GAACCAAGGGTTGGGAGAG	
<i>Actin 1</i>	TGTATGCCAGTGGTCGTACCA	CCAGCAAGGTCGAGACGAA	Li et al., 2015

Table S3. Segregation of F₂ populations from the crosses between *sprl1* and its wild-type parent 188R

Combination	No. of total plants	No. of normal plants	No. of mutant plants	Expected ratio	χ^2	$\chi^2_{0.05}$
<i>sprl1</i> × 188R	453	345	108	3:1	0.27	3.84

Table S4. Insertion/deletion (InDel) markers used for mapping of the *sprl1* locus

Marker	Forward primer (5'-3')	Reverse primer (5'-3')
L1	CAGAAGCCGCTTACTC	AAACCCAAATTCCAACAC
L2	GATTCATGGTAGGGTTT	TACAGCGAGTGAGGTCTT
L3	GTTGGAGATGGCATTAGA	AAGGTAGACGGCAGTGAG
L4	GGTGTACCTACAAGCCC	TCTGCTCAGATCCTGCGA
L5	TGGTGAAAGGCACCTAGC	TTGCTCCCACACCTGTCG