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The *OsPSI-F* gene regulates growth and development in rice by modulating photosynthetic electron transport rate

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Abstract

Key message *Ds* insertion in rice *OsPSI-F* gene results in semi-dwarf plants with reduced tiller number and grain yield, while genetic complementation with *OsPSI-F* rescued the mutant phenotype.**Abstract** Photosynthetic electron transport is regulated in the chloroplast thylakoid membrane by multi-protein complexes. Studies about photosynthetic machinery and its subunits in crop plants are necessary, because they could be crucial for yield enhancement in the long term. Here, we report the characterization of *OsPSI-F* (encoding *Oryza sativa* PHOTOSYSTEM 1-F subunit) using a single copy *Ds* insertion rice mutant line. The homozygous mutant (*ospsI-f*) showed striking difference in growth and development compared to the wild type (WT), including, reduction in plant height, tiller number, grain yield as well as pale yellow leaf coloration. Chlorophyll concentration and electron transport rate were significantly reduced in the mutant compared to the WT. *OsPSI-F* gene was highly expressed in rice leaves compared to other tissues at different developmental stages tested. Upon complementation of the mutant with *proUBI::OsPSI-F*, the observed mutant phenotypes were rescued. Our results illustrate that *OsPSI-F* plays an important role in regulating proper growth and development of rice plants.**Keywords** Chlorophyll fluorescence · Electron transport rate · Antenna function · Photosystem 1 · Rice plant growth · Tillering mutant

Abbreviations

OsPS1-F	<i>Oryza sativa</i> PHOTOSYSTEM 1-F subunit
TAIL-PCR	Thermal Asymmetric Interlaced PCR
<i>Ac/Ds</i>	<i>Activator/Dissociation</i> transposable elements
F_m	Maximum yield of fluorescence

F_v	Variable fluorescence
F_v/F_m	Maximal photochemical efficiency of PSII

Introduction

Productivity and yield in plants are regulated by photosynthesis. Understanding the molecular basis of this regulatory mechanism would help to improve productivity and yield in crop plants such as rice. Various studies have proposed different approaches to increase yield and productivity by altering different parameters of photosynthesis [e.g., improving photosynthetic efficiency (Ambavaram et al. 2014; Zhu et al. 2010), and by genetic manipulation of photoprotection (Kromdijk et al. 2016)]. However, the key players linking antenna function (light-harvesting) of photosystems and photosynthetic electron transport are not well studied in rice.

In higher plants, light reaction of photosynthesis is driven by two photosystems, namely, PSI and PSII (Ben-Shem et al. 2003; Chitnis 1996, 2001). Electron flow across these two photosystems (PSII to PSI) leads to NADPH and ATP generation, which are subsequently utilized to reduce carbon

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dioxide to carbohydrate. In higher plants, the core of PSI is composed of 14 polypeptides (PSI-A to L and PSI-N to O). All of them are encoded by nuclear genes except PSI-A to C, I, and J whose genes are localized in plastid DNA. PSI-C, D, E, and H are peripheral and found in the stromal side of the thylakoid membrane. In contrast, two large subunits (PSI-A and PSI-B) along with PSI-F polypeptide and others are on the luminal side of the thylakoid (Busch and Hippler 2011; Chi et al. 2012; Chitnis 1996; Järvi et al. 2007). Different subunits present in PSI have distinct functions, which are coordinated in an organized manner for this photosystem to work. The functions of a few subunits of PSI have been reported in *Arabidopsis* (Haldrup et al. 2000; Ihnatowicz et al. 2004; Jensen et al. 2000). However, such studies are lacking in rice. A few studies showed the effect of loss of chlorophyll *b* molecules on regulation of photosynthesis in rice (Terao and Katoh 1996; Terao et al. 1996). In addition, various agronomic traits of rice have been reported to be related to the photosynthetic machinery (Ambavaram et al. 2014; Makino 2011; Murchie et al. 2002).

Psa-F is the ortholog of *PSI-F* in *Chlamydomonas*. Using *Psa-F* deficient mutant of the green alga, it has been shown that *Psa-F* regulates the interaction between plastocyanin and PSI without affecting its photoautotrophic growth (Farah et al. 1995). However, down-regulation of *PSI-F* was found to affect photoautotrophic growth in the dicotyledonous plant *Arabidopsis* (Haldrup et al. 2000). These findings suggest differences in the function of the gene between algae and higher plants. Rice being a monocotyledonous plant, its *PSI-F* may exhibit functional variation. Therefore, further studies are warranted to elucidate the function of *PSI-F* gene in rice.

Here, we report functional characterization of *OsPSI-F* and its role in regulating growth and development in rice. The importance of *OsPSI-F* was studied using a *Ds* insertion mutant line (*osps1-f*), which has reduced plant height, tiller number, and grain yield. The mutant plants have lower chlorophyll content and electron transport rate compared to the wild type (WT). Genetic complementation with *proUBI::OsPSI-F* rescued the developmental defects of the mutant. Hence, our findings demonstrate that *OsPSI-F* plays a critical role in regulating plant growth and development.

Materials and methods

Plant materials and growth conditions and phenotypic screens

The *Ds* insertion lines were generated in rice (*Oryza sativa* ssp *japonica* cv. Nipponbare (Kolesnik et al. 2004) and WT Nipponbare was used as control for all experiments). All the plants including WT, *Ds* insertion lines, and transgenic

plants were grown in the greenhouse located in the National University of Singapore under natural light and temperature conditions. Phenotypic screening was done as reported in Ramamoorthy et al. (2011).

Ds copy number determination by Southern blot analysis

Genomic DNA preparation and Southern blot analyses were carried out according to Ramamoorthy et al. (2011). Around 5–6 µg of genomic DNA was digested with three different restriction enzymes (*Cla*I, *Eco*RI and *Hind*III) and fractionated on 1.0% agarose gel and transferred onto a Hybond-N⁺ membrane (GE Healthcare Life Sciences Cat. No. RPN119B) and fixed by UV-cross linking. This membrane was hybridized with digoxigenin (DIG)-labeled *GUS* probe in DIG Easy Hyb solution (Roche Cat. No. 11603558001) at 42 °C. Detection was carried out according to the manufacturer's protocol using DIG Wash and Block Buffer Set and chemiluminescent substrate CDP-StarTM (Roche Cat. No. 11685627001). Hybridization signals were visualized by autoradiography.

Thermal asymmetric interlaced PCR (TAIL-PCR)

The FSTs of *Ds* insertion lines were obtained by TAIL-PCR (Liu and Whittier 1995) using the *Ds* specific primers and arbitrary degenerated (AD) primers. *Ds* terminal outward primers (at 5' and 3') were designed and AD primers were selected from Kolesnik et al. (2004). TAIL-PCR comprised of three steps, primary PCR products have nonspecific products in high amount and low level of desirable specific products. In subsequent secondary and tertiary PCR nonspecific primers got diluted and concentration specific products increased. *Ds* primer and AD primers ratio was (4 picomole/30 picomole) in PCR mix. Tertiary TAIL-PCR products were sequenced using *Ds* terminal primers and FSTs were obtained by BLAST search.

Molecular cloning of *OsPSI-F* and construct preparation for genetic transformation

The coding sequence of *OsPSI-F* was amplified by PCR using rice WT genomic DNA as template with gene or promoter-specific (for promoter cloning) primers using KAPA HiFi PCR Kit (Cat. No. KR0368). The primer sequences are listed in Online Resource 1. PCR was carried out as per manufacturer's protocol. PCR products were purified by Promega Wizard[®] SV Gel and PCR Clean-Up System (Cat. No. A9282) and then cloned into pJET1.2/blunt vector provided with CloneJET PCR Cloning Kit (ThermoFisher Scientific, Cat. No. K1232). The cloned gene and promoter were confirmed by restriction digestion and

Sanger sequencing. Selected clones were sub-cloned into appropriate destination vector pCAMBIA1300 Ti-derived binary vector (pCAMBIA; <http://www.cambia.org.au>) for *Agrobacterium*-mediated genetic transformation. The maize *UBIQUITIN* promoter-driven *OsPSI-F* construct was used for EX and complementation analyses. The *Agrobacterium*-mediated genetic transformation was carried out according to Hiei et al. (1994) and Ramamoorthy et al. (2011). *OsPSI-F* promoter-driven *GUS* gene construct was used for promoter *GUS* analysis. Cloning strategies and construct details are given as Online Resource 2.

RNA extraction and quantitative polymerase chain reaction (qPCR) analysis

Total RNA was isolated using the TRIZOL[®] Reagent (Life Technologies, Cat. No. 15596-026) from leaf samples of either young (6 weeks) or mature plants (2 months). One μ g total RNA was reverse transcribed using Maxima First-Strand cDNA Synthesis Kit (ThermoFisher Scientific, Cat. No. K1671), as per the manufacturer's protocol. The qPCR was carried out using *OsPSI-F* gene-specific primer pair and rice *ACTIN1* primer pair was used for normalization (Online Resource 1). Three technical replicates were used for each independent qPCR reaction.

Chlorophyll content, F_v/F_m ratio, and electron transport rate measurements

The chlorophyll concentration was measured in the fully expanded leaves by a non-disruptive method using SPAD-502m (Konica-Minolta, Japan). About 50 readings were taken for each plant and the mean and standard error of data are presented. In addition, chlorophyll content was measured as described previously (Arnon 1949). Different leaf samples (100 mg each) were homogenized in liquid nitrogen and extracted with 1 ml of 80% (v/v) acetone. The absorbance was measured using DU[®]730 Life Science UV/Vis Spectrophotometer (Beckman Coulter, USA) at 645 and 663 nm. Four samples were measured for each line and three measurements were taken for each sample and the average values were used to calculate the chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b* ratio, and total chlorophyll contents (Arnon 1949). The chlorophyll fluorescence and electron transport rates were measured by a non-disruptive method using a portable chlorophyll fluorometer PAM-210 (Walz, Germany) with the default setting of slow kinetics mode. The 2-month-old plants were shifted to Percival (Percival Scientific Inc, USA) plant growth chamber (10 h light/14 h dark, 30 °C day/25 °C night temperature and 55% of relative humidity) and dark adapted for overnight and measurements were taken at several timepoints after an hour of illumination. The F_v/F_m ratio and the electron transport rate

were shown as mean and standard error. Relative electron transport rate was calculated using this formula $ETR = Yield (\Phi PSII) \times PAR \times 0.5 \times ETR \text{ Factor } (0.84)$ by PAM-210. Here, yield is PSII effective quantum yield at actinic light, and PAR is photosynthetically active radiation. The factor 0.5 is taking into account that about 50% of all absorbed quanta reach PSII. The ETR factor is defining that the fraction of incident light estimated to be absorbed by the sample default value is 0.84.

Amino acid sequence alignment and phylogenetic analysis of OsPS1-F

The derived amino acid sequence alignment and phylogenetic trees were constructed using selected sequences from algae, dicotyledonous, and monocotyledonous plants. Phylogeny.fr online software (http://www.phylogeny.fr/version2.cgi/simple_phylogeny.cgi) was used with one click mode, where MUSCLE, Gblocks, PhyML, and TreeDyn programs were used for multiple alignment, curation, tree building, and tree rendering, respectively (Dereeper et al. 2008).

Statistical analyses

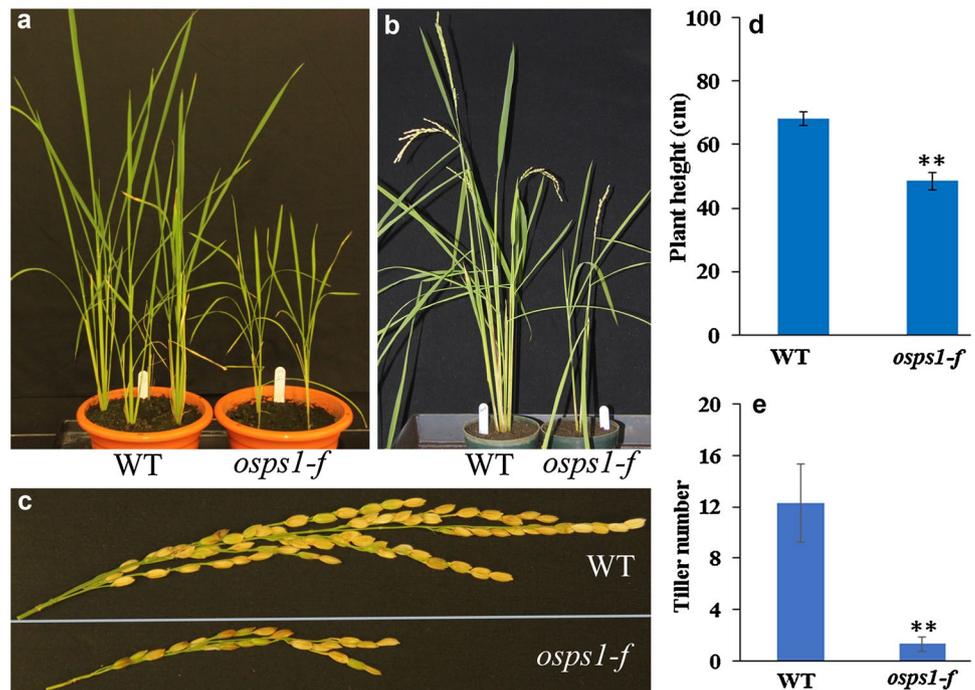
All statistical analyses were performed by two-tailed *t* test using Microsoft[®] Office Excel 2010 and data with *P* value < 0.05 are shown as significant.

Results

osps1-f is a novel semi-dwarf *Ds* insertion mutant

A large pool of *Ds* insertion lines using *Ac/Ds* transposable element system was generated previously (Jiang et al. 2007; Kolesnik et al. 2004). Based on a phenotypic screen, a mutant showed that light green leaves, reduced plant height, tiller number, and grain yield compared to Nipponbare WT rice was selected for further study (Fig. 1). The height difference was observed throughout the growth stages from seedling to mature plant (Fig. 1a, b). At the beginning of tillering stage, *osps1-f* mutant plant height was about 30 cm (60% of WT), and at mature stage, it was two-thirds of the WT (Fig. 1b, d). In addition, *osps1-f* had significantly lower tiller number, with only one tiller observed per plant until booting stage (Fig. 1a), but at maturity, one or two more tillers were produced (Fig. 1b, e). Panicle length, floret, and grain number were also significantly reduced in *osps1-f* compared to the WT (Fig. 1c, Online Resource 3).

Fig. 1 Phenotypic characterization of *osps1-f*. Comparison of height between WT and *osps1-f* plant at (a) young and (b) mature stage (c) main panicle from WT (top) and *osps1-f* (bottom). Comparison of plant height (d) and tiller number (e) of WT and *osps1-f* (significantly different from the WT as per Student's *t* test $**P < 0.01$, $n = 10$). Error bars denote standard deviation, scale bar 10 cm



Ds element was inserted into photosystem I subunit F

For further molecular analysis, *Ds* flanking sequence of mutant was obtained by thermal asymmetric interlaced (TAIL)-PCR. The flanking sequence analysis showed that *Ds* is inserted in the 5'-UTR of the gene encoding PSI-F (*OsPSI-F* for *Oryza sativa* PHOTOSYSTEM 1-F subunit, Fig. 2a), located in rice chromosome 3. The *Ds* insertion position was further confirmed by PCR analysis of seven different mutant siblings and WT as a control. The PCR result showed that the *Ds* element was present in the above-mentioned position and the mutant was homozygous in nature (Fig. 2b). A genomic Southern blot analysis showed that the selected *osps1-f* mutant had a single copy of *Ds* insertion in its genome (Fig. 2c). Quantitative PCR (qPCR) carried out using primer pair for the coding sequence of *OsPSI-F* with total RNA from leaves demonstrated that negligible amount of transcript levels were detected in the *osps1-f* mutant compared to the WT (Fig. 2d).

Genetic complementation of *osps1-f* mutant and ectopic expression of *OsPSI-F*

To confirm that observed mutant phenotype was due to *OsPSI-F* disruption, *osps1-f* mutant was complemented with *proUBI::OsPSI-F*. Ten independent transgenic lines were generated and all of them showed different degrees of rescue of the mutant phenotype. We selected one line that showed

full phenotype rescue (Fig. 3a) and with ~3:1 segregation ratio (WT-like:mutant, indicating single copy transgene insertion) in the next two generations (data not shown). Furthermore, qPCR analysis showed that this line has elevated level of *OsPSI-F* transcripts (Fig. 3b), which confirmed that the mutant phenotype was due to loss of function of *OsPSI-F*. To test whether ectopic expression (EX) of *OsPSI-F* will lead to increased photosynthetic rates, *proUBI::OsPSI-F* was introduced into the WT background. Out of 20 lines generated, three independent lines were selected (with high, medium, and low transgene expression levels) for further analysis. The EX lines generated (with elevated transcript level of *OsPSI-F*) did not exhibit any significant changes in phenotype compared to the untransformed WT (Fig. 3c, d).

OsPSI-F was strongly expressed in leaves compared to other WT tissues

OsPSI-F expression was compared at different developmental stages using young and mature leaves, young and mature panicles, young and mature roots, and 2-week-old WT seedlings. Our qPCR results showed that the expression of *OsPSI-F* was higher in young and mature leaves than in other tissues at different developmental stages (Fig. 4a). To further validate this result, we generated transgenic plants harboring *proOsPSI-F::GUS* gene construct. The GUS staining analysis of T0 plantlets revealed that *OsPSI-F* was highly expressed in the leaves, which correlates with its important function in photosynthesis (Fig. 4a, b).

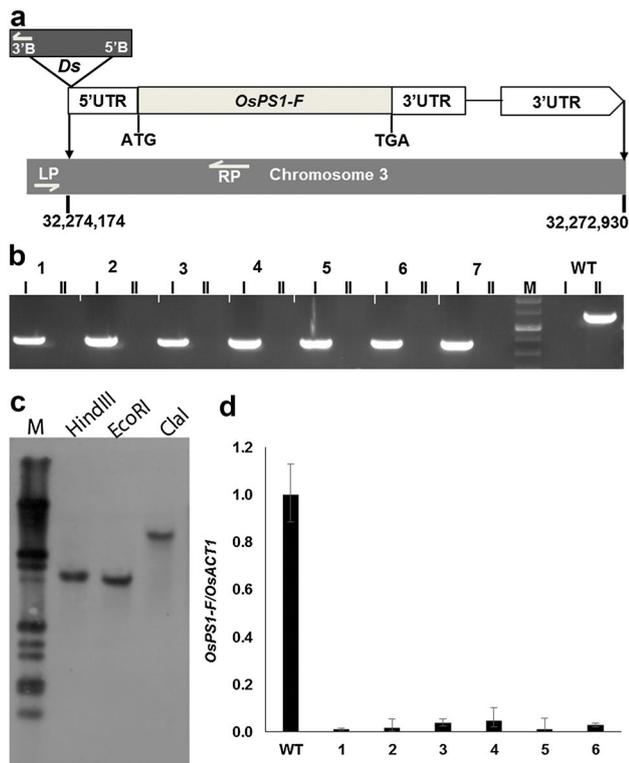


Fig. 2 Molecular characterization of *osps1-f*. **a** Genomic organization of the *osps1-f* showing the location of *Ds* insertion in the 5'-UTR, 70 nucleotides upstream from the start codon ATG. Arrows show the position of the primers used for the characterization of the insertion mutant. **b** Zygosity determination of seven different siblings of *osps1-f* and WT by PCR using three primers, first one 3' border primer (3'B) of *Ds*, second primer was designed before the 5' of insertion (LP), and third was after the 3' of insertion (RP). I and II denote amplification with primer pairs 3'B, LP and LP, RP, respectively. **c** Determination of *Ds* copy number by Southern blot hybridization using genomic DNA of *osps1-f* digested with three different restriction enzymes (*HindIII*, *EcoRI* and *Clal*). **d** qPCR analysis with cDNAs from six different homozygous *osps1-f* seedlings and a WT seedling. Error bars denote standard deviation

osps1-f mutant showed reduced chlorophyll content, F_v/F_m ratio, and electron transport rate

The light green coloration of leaves in mutant plants suggests reduced chlorophyll content in these plants. Indeed, chlorophyll concentration was significantly reduced (by about 35%) in fully expanded leaves of *osps1-f* mutant compared to WT (Fig. 5a). The complementation and EX transgenic lines showed no significant difference in chlorophyll level compared to WT by the non-disruptive method (Fig. 5a). Similarly, measurement of chlorophyll content by acetone extraction revealed that total chlorophyll and chlorophyll *a/b* ratios were significantly lower in the *osps1-f* mutant and there was no significant difference in complementation and EX lines compared to WT (Fig. 5b; Table 1). Furthermore,

chlorophyll fluorescence measured using PAM210 showed that the maximum quantum yield (F_v/F_m) of *osps1-f* mutant was significantly lower (0.68) than that of the WT (0.74) (Fig. 6a) and the representative slow kinetics graphs of chlorophyll fluorescence are given in the Online Resource 4. In addition, the *osps1-f* mutant showed about 40% reduction in electron transport rate, whereas an increase was seen in the complementation and EX lines (P value < 0.01) compared to the WT (Fig. 6b).

Discussion

Photosynthetic machinery is highly complex, which makes it one of the important components that may be manipulated by plant biotechnology for crop improvement. It consists of two important multi-component photosystems, namely, PSI and PSII among others (Ben-Shem et al. 2003; Chitnis 1996, 2001). *PSI-F* encodes 1 of the 14 polypeptides forming the core of PSI. It has been reported that Psa-F polypeptide (PSI-F ortholog) is involved in the docking of plastocyanin to the PSI reaction center and hence in electron transport chain in *Chlamydomonas* (Farah et al. 1995). Based on the amino acid sequence analysis of PSI-F and its orthologs from algae and higher plants, it was observed that PSI-F is highly conserved among plants. At the same time, it is less conserved between algae and higher plants (Online Resource 5), which suggests that there might be functional divergence between the genes from the algae and higher plants. This opens up the possibility of functional differences among divergent groups of higher plants such as dicotyledonous versus monocotyledonous species. Such studies are facilitated by functional genomics and analysis of selected mutants. Findings from such studies can be useful for crop improvement efforts.

Insertional mutagenesis using T-DNA or transposons (Jiang et al. 2007) is an important strategy used for gene function analysis. In this study, we have investigated the role of a photosynthetic machinery subunit, *OsPS1-F*, using *osps1-f* rice mutant generated by *Ds* insertion. The resultant mutant showed several growth defects in plant height, tiller number, and grain yield (Fig. 1, Online Resource 3), suggesting that the gene is critical for maintaining growth and development in rice. Our findings of severely compromised *OsPS1-F* expression (Fig. 2d) in the *osps1-f* mutant combined with the fact that the phenotypic defects could be rescued by complementing the mutant with *OsPS1-F* (Fig. 3a, b, Online Resource 3) confirmed that the observed phenotypes are caused by the loss of function of the tagged gene. Similar results were reported earlier in *Arabidopsis* when reduction of *PSI-F* transcript levels was caused by antisense suppression (Haldrup et al. 2000). The same study reported that the *Arabidopsis* plants could not survive beyond the

Fig. 3 Analyses of *OsPSI-F* complementation (CO) lines and ectopic expression (EX) lines. **a** Phenotype of 2-month-old WT, *osps1-f* and CO32 line, **b** qPCR expression analysis of *OsPSI-F* in WT, *osps1-f* and CO32 line, **c** Phenotypes of 2-month-old WT, and EX lines, and **d** qPCR expression analysis of *OsPSI-F* in WT and EX lines. Error bars denote standard deviation (significantly different from the WT as per Student's *t* test $**P < 0.01$), scale bar 10 cm

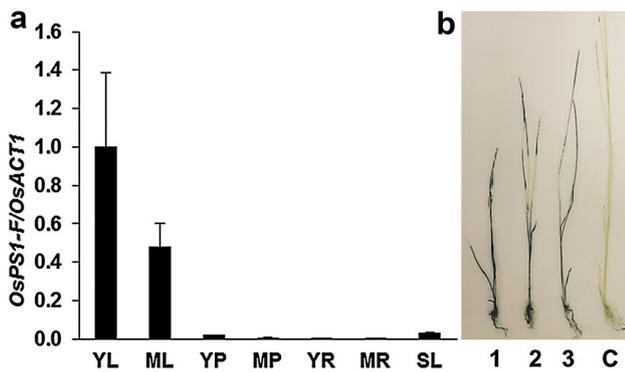
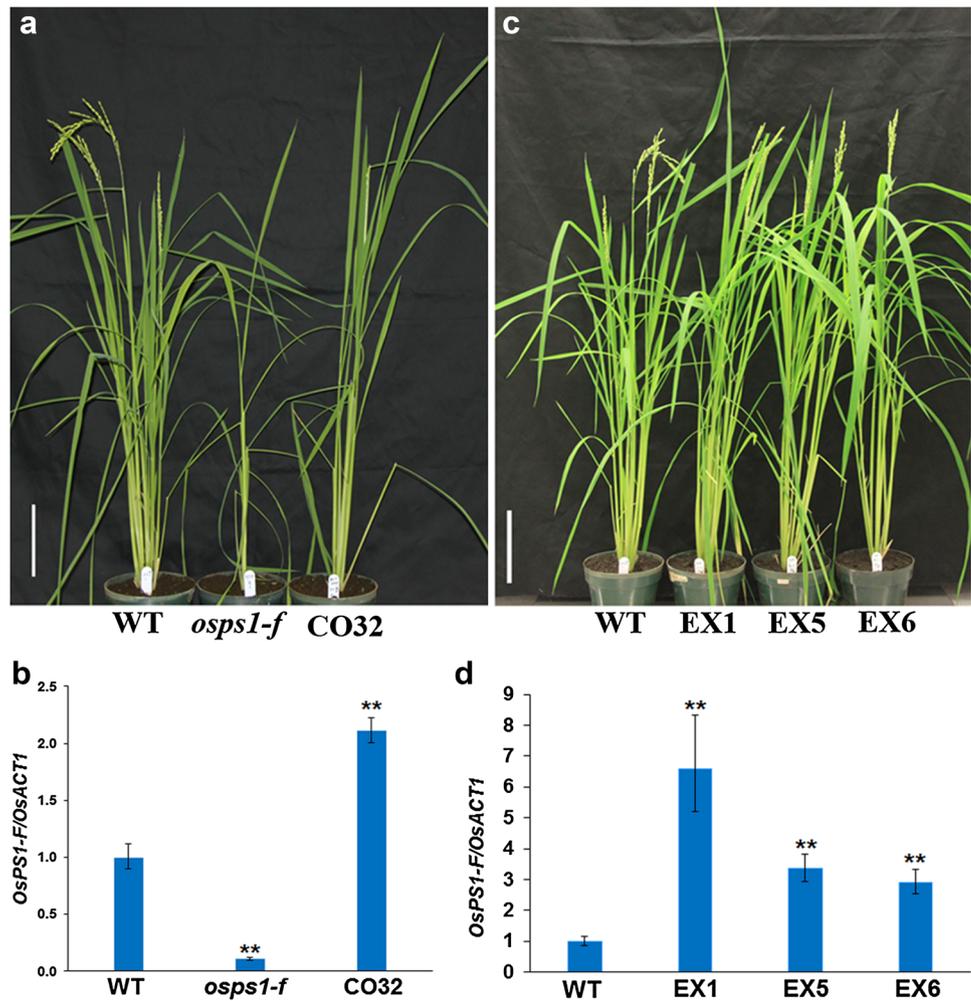


Fig. 4 Spatiotemporal expression of *OsPSI-F* in rice. **a** Expression pattern of *OsPSI-F* in various tissues (YL young leaf, ML mature leaf, YP young panicle, MP mature panicle, YR young root, MR mature root, SL seedlings). **b** Expression patterns of *OsPSI-F* revealed by GUS staining of *proOsPSI-F::GUS* seedlings (1–3) and control (C)

seedling stage when the gene expression level was below 3% that of WT. However, in our study, despite insignificant

levels of expression of the gene in *osps1-f* mutant, the plants survived, albeit with severe reduction in their growth and biomass accumulation compared to the WT. There were no adverse or positive effects on plant phenotype in the EX lines, which appeared similar to the normal WT plants (Fig. 3c, d, Online Resource 3). This could be due to the fact that ectopic expression or overexpression of a subunit from multi subunit protein complex might not be adequate for altering the phenotype (Semple et al. 2008). Likewise, ectopic expression of one gene from a multi-gene family also might not produce any obvious phenotypes in rice, for example, SU(VAR)3–9 homolog (SUVH) genes *SDG703*, *SDG704*, *SDG709*, *SDG713*, *SDG714*, *SDG715*, *SDG726*, and *SDG728* (Ding et al. 2007; Qin et al. 2010), and *YAB3* (Dai et al. 2007). In some cases, such gene alterations showed no visible changes in phenotype unless induced by salt treatment (Liu et al. 2014). Similarly, it is not unreasonable to speculate that overexpressing one subunit of a specific protein complex is not enough to produce any noticeable enhancement of growth and development. However, it is important to analyze the functions of various components of

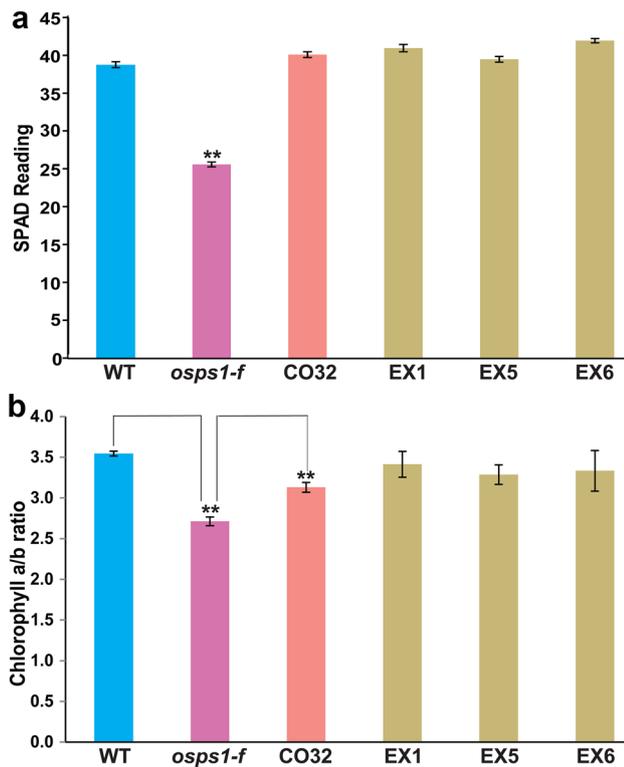


Fig. 5 Chlorophyll content and chlorophyll *a/b* ratio in mutant and transgenic lines. **a** Chlorophyll content of different rice lines. **b** Chlorophyll *a/b* ratio in different rice lines [WT, *osps1-f*, complementation (CO) lines and ectopic expression (EX) lines]. Error bars denote standard error (**significantly different as per Student's *t* test $P < 0.01$)

such complexes or multi-gene families, which might help to reveal a combination that can lead to pronounced enhancement in plant growth in the long term.

Although OsPS1-F is not directly involved in the chlorophyll fluorescence, because there was no significant change observed when *OsPS1-F* was ectopically expressed, it appears to have an indirect influence on photosynthetic

electron transport rate. Earlier observations in *Arabidopsis* indicated that PS1-F plays a major role in the later steps of photosynthesis such as electron transport (Farah et al. 1995; Haldrup et al. 2000). Likewise, the PSII electron transport rate was significantly reduced in *osps1-f* mutant (Fig. 6b) and which is consistent with the earlier findings in *Arabidopsis* and algae (Farah et al. 1995; Haldrup et al. 2000). In addition, a significant reduction in chlorophyll *a/b* ratio in *osps1-f* mutant (Fig. 5) indicates a reduced PSI/PSII ratio which is consistent with the restricted PSI electron transport in the *PsaF*-suppressed plants (Haldrup et al. 2000). This could be the primary reason for the reduced growth of plants observed in our study. Alternatively, the lack of PS1-F may cause a structural alteration in PSI that might be brought about due to changes in the protein complex within PSI, which will be ultimately manifested as reduced overall plant biomass. Collectively, these data suggest that disruption of *OsPS1-F* gene expression could affect the PSII function as well, which can explain the reduction in the overall antenna function as was also reported in *Chlamydomonas* (Farah et al. 1995) and higher plants (Haldrup et al. 2000). The significant increase in the PSII electron transport rate (P value < 0.05 by *t* test) in the EX lines that showed higher *OsPS1-F* expression (Figs. 3d, 6b) further supports this. In addition, this indicates that increasing the photosynthetic electron transport rate alone is not adequate to elicit growth enhancement. As OsPS1-F is a part of the multi subunit protein complex, there may also be some unknown feedback regulation to control the functions of different subunits.

In conclusion, our results suggest that OsPS1-F modulates chlorophyll content and photosynthetic electron transport rate. Along with the previous studies, these findings indicate that PS1-F is one of the major factors that affect plant growth and development, which has a profound influence on crop productivity. Further investigations with other PSI protein subunits may lead to a better understanding of the function of this protein complex.

Table 1 Chlorophyll contents of different genotypes

Genotype	Chlorophyll <i>a</i> ($\mu\text{g/g}$)	Chlorophyll <i>b</i> ($\mu\text{g/g}$)	Total chlorophyll ($\mu\text{g/g}$)
WT	64.84 \pm 7.33	18.29 \pm 2.05	83.13 \pm 9.37
<i>osps1-f</i>	39.18 \pm 6.56*	13.54 \pm 1.65*	52.72 \pm 8.00*
CO32	72.38 \pm 12.08	23.13 \pm 3.94	95.51 \pm 15.99
EX1	80.73 \pm 6.49	23.65 \pm 1.33	104.38 \pm 7.60
EX5	84.66 \pm 3.87	25.79 \pm 1.70	110.45 \pm 5.39
EX6	68.81 \pm 24.27	20.88 \pm 8.40	89.69 \pm 32.59

Data are mean \pm SD of three biological replicates, each with three technical replicates; subjected to Student's *t* test (*significantly different from the rest of the values in each column at P value < 0.05 , the other values are not significantly different from each other). Different genotypes are WT, *osps1-f*, complementation (CO) lines and ectopic expression (EX) lines

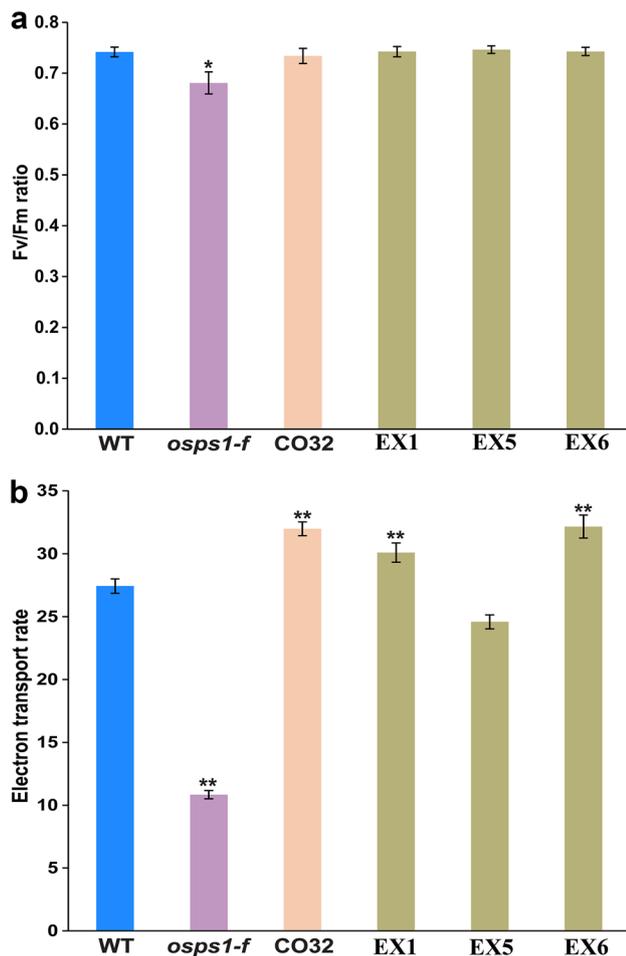


Fig. 6 F_v/F_m ratio of different rice lines. **a** F_v/F_m ratio and electron transport rate in mutant and transgenic lines. **b** Electron transport rate of different rice lines [WT, *osps1-f*, complementation (CO) lines, ectopic expression (EX) lines]. Error bars denote standard error (significantly different as per Student's *t* test * $P < 0.05$, ** $P < 0.01$)

Author contribution statement RR and BV planned and carried out the experiments and data analysis and wrote the manuscript draft. SR provided the mutant line and contributed to manuscript writing. PK conceptualized the project and contributed to data analysis and manuscript preparation. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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