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OsSPL14 and OsNF-YB9/YC8-12 subunits cooperate to enhance grain appearance quality by promoting *Waxy* and *PDIL1-1* expression in rice

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ABSTRACT

The identification of seed development-related regulators is critical for the genetic improvement of yield and grain quality in cereal crops. SQUAMOSA PROMOTER BINDING PROTEIN-LIKE14 (OsSPL14) is a well-studied, plant-specific transcription factor; however, its roles in controlling rice grain appearance quality and the underlying molecular mechanisms have not been fully elucidated. In this study, we demonstrate that OsSPL14 positively regulates appearance quality by controlling grain chalkiness in rice. Genetic analysis revealed that knockdown or knockout of *OsSPL14* leads to a chalky grain phenotype, which is associated with significant defects in compound starch granules and notable changes in both starch and protein contents in the endosperm. Transcript analysis identified multiple genes regulated by *OsSPL14*, including the key granule-bound starch synthase gene *Waxy* (*Wx*) and the protein disulfide isomerase-like enzyme-encoding gene *PDIL1-1*. Both *in vitro* and *in vivo* assays demonstrated that OsSPL14 directly binds to the GTAC-box motif in the *Wx* and *PDIL1-1* promoters to enhance their expression. Protein–protein interaction experiments further revealed that OsSPL14 interacts with the nuclear transcription factor Y (NF-Y) heterodimer OsNF-YB9/YC8–12 to promote the transcription of *Wx* and *PDIL1-1*, thereby enhancing rice grain appearance quality. Our findings uncover a novel regulatory pathway controlled by OsSPL14 and provide new insights into the molecular mechanisms underlying rice grain appearance quality, with promising implications for genetic improvement in rice.

Key words: *OsSPL14*, nuclear factor Y, protein–protein interaction, grain chalkiness, *Oryza sativa*

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INTRODUCTION

Rice (*Oryza sativa*) is a staple food source for more than half of the global population. With the continuous improvement of living standards, efforts to develop new elite rice varieties with high quality have created a research hotspot in modern agriculture. Rice grain quality is a complex trait comprising cooking and eating, appearance, nutrition, and milling quality (Zhao et al., 2022). In terms of grain appearance quality, it is characterized by four major properties: grain shape, chalkiness, transparency, and color (Zhao et al., 2022). Chalkiness refers to the opaque regions in the endosperm, which not only result in an undesirable appearance for consumers but also significantly affect the eating and cooking quality (ECQ) of rice (Zhao et al., 2022). Thus, chalkiness reduction is a key focus in rice breeding.

Fully developed rice endosperm is mainly composed of storage starch, proteins, lipids, and minerals; starch constitutes 80%–90% of the dry weight of rice grains and serves as a major contributor to rice grain quality (Zhang et al., 2021; Yan et al., 2024). Storage starch is synthesized in specialized amyloplasts of the endosperm through a complex process involving multiple biosynthetic enzymes, including ADP-glucose pyrophosphorylase, soluble starch synthases (SSs), starch-branching enzymes (SBEs), debranching enzymes, and granule-bound SS (GBSS) (Zhou et al., 2016). Generally, mutations in starch biosynthesis enzymes in rice alter endosperm appearance and storage starch characteristics. For instance, the *Waxy* (*Wx*) gene, which encodes GBSSI, is a key regulator of amylose synthesis in the endosperm and a primary factor influencing the ECQ of rice (Tian et al., 2009; Zhang et al., 2021; Shen et al., 2022; Jin et al., 2023). As a critical gene controlling amylose synthesis, *Wx* has been a target of genome

editing for breeding rice varieties with very low amylose content (AC) (Bello et al., 2019). Since the 1990s, numerous natural alleles—including Wx^{a}, Wx^{b}, *wx*, $Wx^{op/hp}$, Wx^{mq}, Wx^{mp}, Wx^{in}, Wx^{la}, Wx^{lv}, and Wx^{mw}—have been reported (Wang et al., 1995; Sato et al., 2002; Zhang et al., 2019, 2021; Zhou et al., 2021; Cai et al., 2022). These alleles confer diverse GBSSI activities, contributing to regional variations in rice AC and influencing consumer preferences. CRISPR-Cas9-mediated gene knockout has also been used to mutate *Wx* to confer diverse GBSSI activities that affect the AC of rice grains (Huang et al., 2020, 2021; Zeng et al., 2020; Xu et al., 2021). Overall, fine-tuning the expression of *Wx* is essential to improve rice grain quality and is important in rice breeding for products with better quality to meet consumer demand.

Rice seed storage proteins (SSPs) are the second most abundant class of components in the endosperm, representing approximately 8% of the dry weight of rice grains; they influence the nutritional quality, pasting, and textural properties of cooked rice (He et al., 2021). According to their solubility, rice SSPs are generally classified into four categories: glutelin, prolamin, albumin, and globulin (Shewry and Halford, 2002). During seed development, large amounts of disulfide-bond-rich prolamins and glutelins are synthesized on the rough endoplasmic reticulum (rER), then translocated into the rER lumen for oxidative protein folding. Only after this process can these folded proteins be sorted and subsequently stored in the rice endosperm (Wang et al., 2016). Protein disulfide isomerase-like (PDIL) enzymes are the primary catalysts of disulfide-linked protein folding; they assist polypeptide folding in the ER lumen (Kawagoe et al., 2005; Onda et al., 2009; 2011). The rice genome encodes at least 19 PDIL proteins, but only PDIL1-1 has been demonstrated to play an important role in seed development by affecting starch and storage protein synthesis in the rice endosperm (Houston et al., 2005; Satoh-Cruz et al., 2010; Onda et al., 2011; Han et al., 2012; Kim et al., 2012; Xia et al., 2018; Cao et al., 2022; Hori et al., 2022). Mutations in *PDIL1-1* produce floury endosperm with irregularly shaped and loosely packed starch granules, likely caused by ER stress (Han et al., 2012; Cao et al., 2022). Furthermore, suppression of *PDIL1-1* expression can alter the formation of aggregates containing proglutelin through non-native intermolecular disulfide bonds in the rER (Kim et al., 2012). Therefore, *PDIL1-1* is considered to play an important role in starch and protein accumulation, affecting grain appearance quality in rice. However, the upstream regulators of *PDIL1-1* that modulate rice endosperm development remain elusive.

SQUAMOSA PROMOTER BINDING PROTEIN-LIKE14 (*OsSPL14*) encodes a plant-specific transcription factor reported to play critical roles in various developmental processes in rice, such as plant architecture and grain yield (Jiao et al., 2010; Miura et al., 2010; Wang et al., 2015a; 2021; Song et al., 2017; 2022; Zhang et al., 2017; Li et al., 2022; 2023), disease resistance (Wang et al., 2018; Liu et al., 2019; Hui et al., 2023), drought tolerance (Zhu et al., 2022; Chen et al., 2023), seed germination (Miao et al., 2019), salt tolerance (Jia et al., 2022a), and chilling tolerance (Jia et al., 2022b). However, it remains largely unknown whether and how OsSPL14 is involved in controlling rice grain appearance quality. Here, we found that OsSPL14 positively regulates rice grain appearance quality. Knockdown or knockout of *OsSPL14* significantly increased chalkiness, which may be associated with changes in seed storage substances and compound starch granules. Furthermore, OsSPL14 physically interacts with the OsNF-YB9/YC8–12 complex, forming a heterotrimer that acts upstream of *Wx* and *PDIL1-1* to mediate rice grain appearance quality. Overall, our findings provide a comprehensive understanding of the regulatory mechanisms underlying storage starch and protein accumulation by uncovering the function of *OsSPL14* in the rice endosperm, which may have great practical implications for decreasing chalkiness in rice grains.

RESULTS

OsSPL14 is essential for endosperm development in rice

We previously demonstrated that *OsSPL14*, which is cleaved by miR529 and miR156, regulates axillary bud outgrowth and grain size in rice (Li et al., 2022; 2023). Intriguingly, we observed that *OsSPL14*-downregulated plants (including *OsSPL14-RNAi*, *miR156e-OE*, and *miR529a-OE*) displayed a chalky or floury-white endosperm phenotype (Supplemental Figure 1A–1L), with higher percentages of grains with chalkiness (PGWC) and degrees of endosperm chalkiness (DEC) (Supplemental Figure 1M and 1N). Head rice yield/rate, defined as the percentage weight of intact milled grains relative to total grain weight, is an essential yield and quality trait that is negatively associated with chalkiness (Zhao et al., 2022). Our results show that the head rice yield of *OsSPL14*-downregulated plants was significantly decreased compared with that of the wild type (WT) (Supplemental Figure 1O).

To gain insights into the function of *OsSPL14* in rice grain development, we investigated its spatiotemporal expression pattern. Reverse transcription (RT)–quantitative polymerase chain reaction (qPCR) analysis revealed that *OsSPL14* is expressed in developing endosperm, with peak expression at 6 days after fertilization (DAF) (Supplemental Figure 2A). Histochemical assays detected β-glucuronidase (GUS) activity in transgenic plants carrying 1.0 kb of the *OsSPL14* sequence upstream of the translation start site fused with the *gusA* reporter gene. Our results showed that *OsSPL14* is expressed in rice grains; however, its expression gradually decreased with further development and maturation of the grain (Supplemental Figure 2B), suggesting its involvement in rice grain endosperm development.

To further clarify the biological role of *OsSPL14* in rice grain development, we used the CRISPR-Cas9 genome editing system to specifically knock out *OsSPL14* in the Zhonghua11 (ZH11) background (Supplemental Figure 3A and 3B). As expected, two *osspl14-cri* lines exhibited a bushy phenotype similar to that of *OsSPL14-RNAi* transgenic plants (Supplemental Figure 3C; Li et al., 2022; 2023). Additionally, *OsSPL14* overexpression (OE) lines were generated and characterized (Supplemental Figure 3D). Expression of the OsSPL14 protein was confirmed by western blot analysis, validating the successful generation of both the loss-of-function mutant and OE lines (Supplemental Figure 3E). Subsequently, WT, *osspl14-cri*, and *OsSPL14-OE* lines were cultivated under natural field conditions; grain phenotypes were carefully examined. Time-course analysis showed that

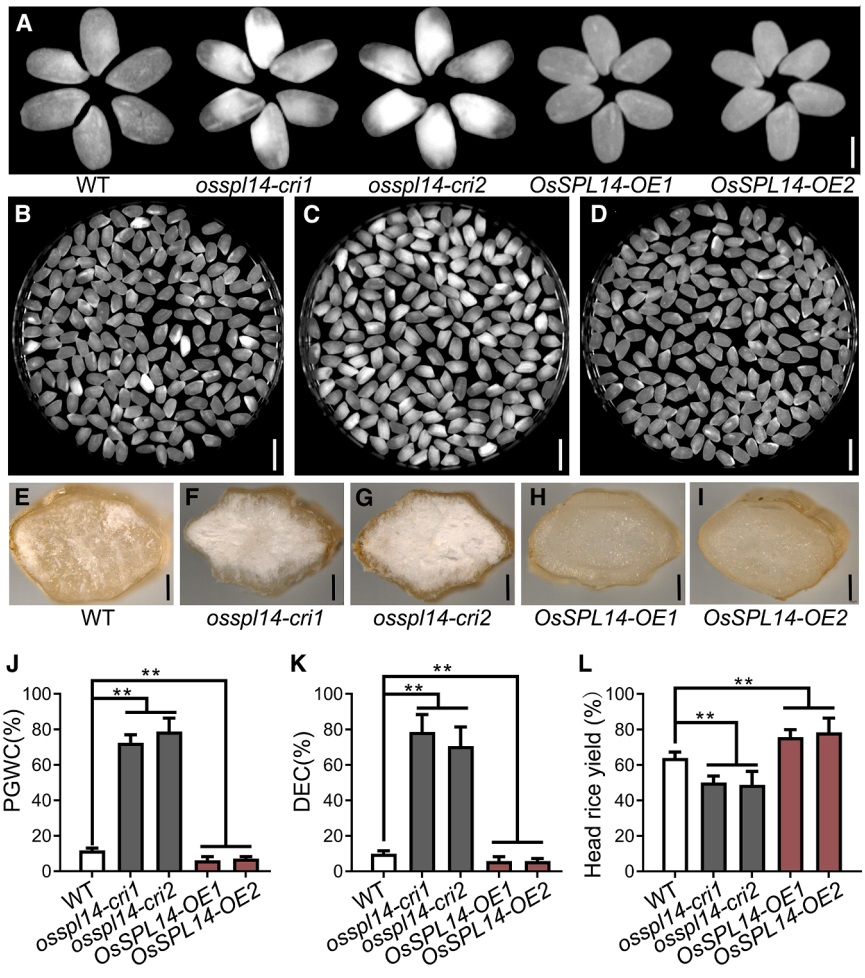

Figure 1. Characterization of grains in *OsSPL14* transgenic lines.

(A) Mature grains of WT, *osspl14-cri*, and *OsSPL14-OE* lines. Scale bars, 3 mm.

(B–D) Images of 200 mature grains of WT, *osspl14-cri*, and *OsSPL14-OE*. Scale bars, 10 mm.

(E–I) Cross-sectional views of WT, *osspl14-cri*, and *OsSPL14-OE* grains. Scale bars, 0.5 mm.

(**J–L**) Comparison of the percentage of grains with chalkiness (PGWC), degree of endosperm chalkiness (DEC), and head rice yield in WT, *osspl14-cri*, and *OsSPL14-OE*. Data are presented as means ± SD from three replicates, each comprising at least 200 seeds. Significant differences between WT and transgenic lines were determined using Student's *t test* (**$p < 0.01$).

osspl14-cri produced considerably opaque endosperm as early as 21 DAF (Supplemental Figure 3F). Additionally, knockout of *OsSPL14* significantly increased grain length, grain width, and 1,000-grain weight, whereas seed setting remained unaffected (Supplemental Figure 3G–3J). At maturity, both *osspl14-cri1* and *osspl14-cri2* exhibited chalky endosperm (Figure 1 A–1C) with higher PGWC and DEC but lower head rice yield relative to the WT control (Figure 1J–1L). In contrast, the OE lines presented more translucent endosperm (Figure 1D) with lower PGWC and DEC but higher head rice yield (Figure 1J–1L). Furthermore, cross-section analysis showed that the endosperm of *osspl14-cri* was floury-white, whereas that of the WT and OE lines was translucent (Figure 1E–1I). Taken together, our results demonstrate that *OsSPL14* plays a critical role in regulating endosperm development and grain appearance quality in rice.

Seed storage substances and compound starch granules are altered in *OsSPL14* transgenic plants

Chalkiness is associated with changes in the content of grain storage components and/or endosperm structure. Mature seeds were easily stained with iodine solution, indicating that all plants accumulated abundant starch in the endosperm at 14 DAF (Supplemental Figure 4A and 4B). To explore intrinsic changes in grains from transgenic plants, we examined the contents of storage substances in mature seeds. Total starch contents and ACs in *osspl14-cri* and *OsSPL14*-downregulated grains were significantly lower than those in the WT; opposite results were observed in *OsSPL14-OE* plants (Figure 2A and 2B; Supplemental Figure 4C and 4D). Additionally, total storage protein content in the endosperm increased by 18.9%–28.3% in *osspl14-cri* mutants but decreased by approximately 16.1%–20.8% in *OsSPL14-OE* lines compared with the WT (Figure 2C). Similar results were observed in *OsSPL14*-downregulated plants (Supplemental Figure 4E). Among the different protein fractions, prolamin content slightly decreased in *osspl14-cri* and *OsSPL14*-downregulated plant grains, whereas glutelin content significantly increased relative to that in the WT (Supplemental Figure 5).

To determine whether starch granules in *osspl14-cri* mutants and *OsSPL14*-downregulated plants were morphologically defective, semi-thin endosperm sections at 14 DAF were analyzed to observe starchy endosperm cell development. The results showed that in WT and *OsSPL14-OE plants,* smaller starch granules initially formed in the amyloplast and gradually filled interior regions to eventually form regularly shaped polyhedrons (Figure 2D and 2F). In contrast, endosperm cells of *osspl14-cri* contained few compound granules and numerous small starch granules that were loosely packed and spread apart, with large empty spaces, implying arrested amyloplast growth in *osspl14-cri* mutants (Figure 2E). A similar result was obtained via transmission electron microscopy (TEM). The *osspl14-cri* endosperm contained abundant single and loosely packed starch granules, in contrast to the polyhedral and tightly packed granules observed in *OsSPL14-OE* and WT endosperm (Figure 2G–2I). Furthermore, scanning electron microscopy (SEM) images of transverse sections of mature grains were used to further examine the structure of compound starch granules. As shown in Figures 2J–2L, starch granules in *osspl14-cri* were small, round, and loosely packed, whereas those in WT and

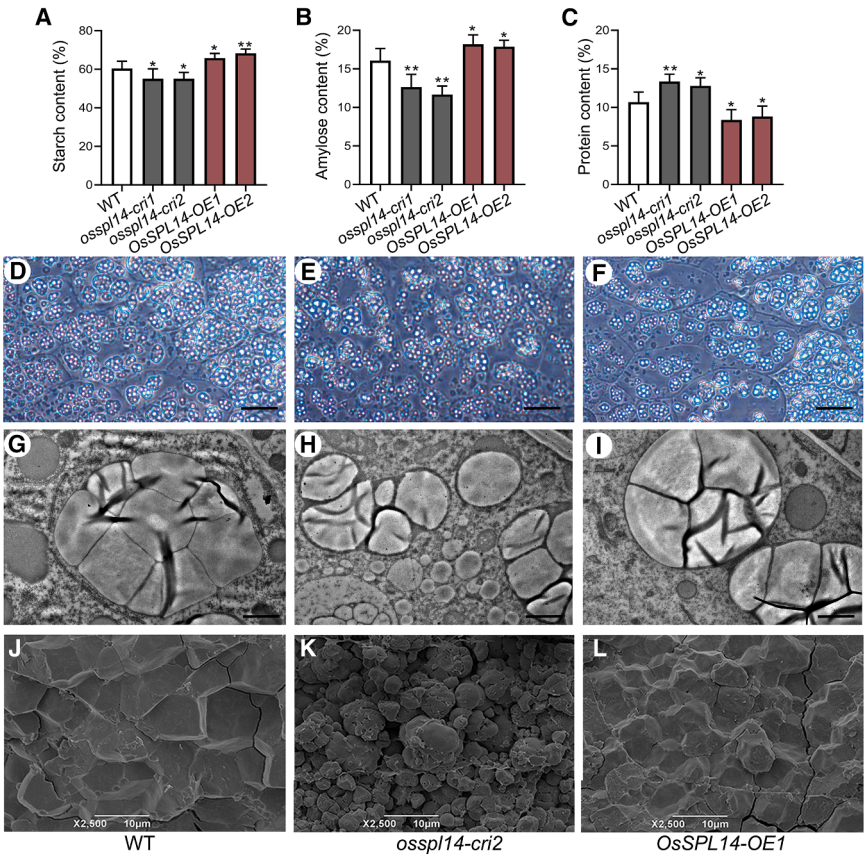

Figure 2. Altered starch properties in *OsSPL14* transgenic lines.

(**A–C**) Quality trait parameters of mature seeds from WT, *osspl14-cri*, and *OsSPL14-OE*. Data are presented as means ± SD (n = 6). Significant differences between WT and transgenic lines were determined using Dunnett's test (**p < 0.01, *p < 0.05).

(**D–F**) Semi-thin sections of endosperm at 10 DAF in WT, *osspl14-cri*, and *OsSPL14-OE*. Scale bars, 10 μm.

(**G–I**) TEM images of compound starch granules in WT, *osspl14-cri*, and *OsSPL14-OE* at 10 DAF. Scale bars, 1 μm.

(**J–L**) SEM images of the central area of mature endosperm in WT, *osspl14-cri*, and *OsSPL14-OE*. Scale bars, 10 μm.

OsSPL14-OE endosperm cells were regularly polyhedral and densely packed. Collectively, these findings suggest that *OsSPL14* plays a vital role in the accumulation of storage substances and in starch granule development during the grain-filling stage.

OsSPL14 regulates the transcription of genes involved in storage starch and protein production

To investigate the underlying reason for changes in storage components, we collected 50 additional genes involved in rice endosperm development and analyzed their expression levels in 6-DAF endosperm (Supplemental Table 1). As shown in Figure 3A, *osspl14-cri* mutants generally exhibited lower expression levels of starch synthesis and α-amylase inhibitor genes—such as *AGPL1-4*, *AGPS1*, *AGPS2a-b*, *GBSSI/Wx*, *SBE1*, *SBE2a-b*, *SSIIc*, and *SSIIIa-b*—but significantly higher expression levels of genes associated with starch decomposition (*Amy3B-E*), relative to the WT and OE. These results suggest that *OsSPL14* promotes starch synthesis while inhibiting starch decomposition by modulating the expression of related genes, ultimately resulting in increased starch accumulation.

Additionally, the expression levels of multiple protein synthesis genes—such as *10KD prolamin*, *13KD prolamin*, *Globulin1-2*, *GluA1-3*, and *GluB1*—were substantially upregulated in *osspl14-cri* endosperm but significantly downregulated in *OsSPL14-OE* endosperm (Figure 3B). Similarly, the expression levels of other reported protein synthesis genes, including *GluB4*, *GluD1*, *RA16*, *RA17*, *RA5b*, *RAG1*, and *RAG2*, showed differential expression patterns in *osspl14-cri* mutants and *OsSPL14-OE* (Figure 3B). Furthermore, the expression levels of certain ER stress-responsive genes, such as *PDIL1-1* and *PDIL2-3*, were noticeably elevated in *OsSPL14-OE* but reduced in *osspl14-cri* mutants (Figure 3B). However, the expression levels of some other genes, such as *GBSSII*, *ISA1-3*, *SS1*, *SSIIa-b*, and certain *PDIL* family members, exhibited irregular or nonsignificant changes in transgenic lines compared with the WT (Supplemental Figure 6). These results indicate that *OsSPL14* participates in a complex regulatory network by modulating the expression of multiple genes involved in seed development.

OsSPL14 directly binds to the promoters of *Wx* and *PDIL1-1*

Wx and *PDIL1-1*, both of which exhibited relatively high transcript abundance in developing panicles and endosperm (Supplemental Figure 7), have been identified as master regulators of amylose and protein accumulation during rice grain filling, respectively (Han et al., 2012; Zhang et al., 2019). Promoter sequence analysis revealed that *Wx* and *PDIL1-1* contain several putative GTAC-boxes within their 2.0-kb promoter regions (Figure 4D and 4E). Additionally, the reduced amylose and increased protein content, as well as the downregulated expression of *Wx* and *PDIL1-1* in *osspl14-cri* mutants, suggested that OsSPL14 regulates the expression of these genes by directly binding to their promoter regions. This hypothesis was first tested using a yeast one-hybrid (Y1H) assay. The results demonstrated that OsSPL14 fused with the GAL4-activation domain (*pJG4-OsSPL14*), but not *pJG4* alone, could bind to the putative regulatory regions of *Wx* and *PDIL1-1* and activate expression of the *LacZ* reporter (Figure 4A). Furthermore, a firefly luciferase transactivation assay in *Nicotiana benthamiana* leaf epidermal cells confirmed that the *Wx* and *PDIL1-1* promoters were specifically recognized by the OsSPL14 protein (Figure 4B and 4C).

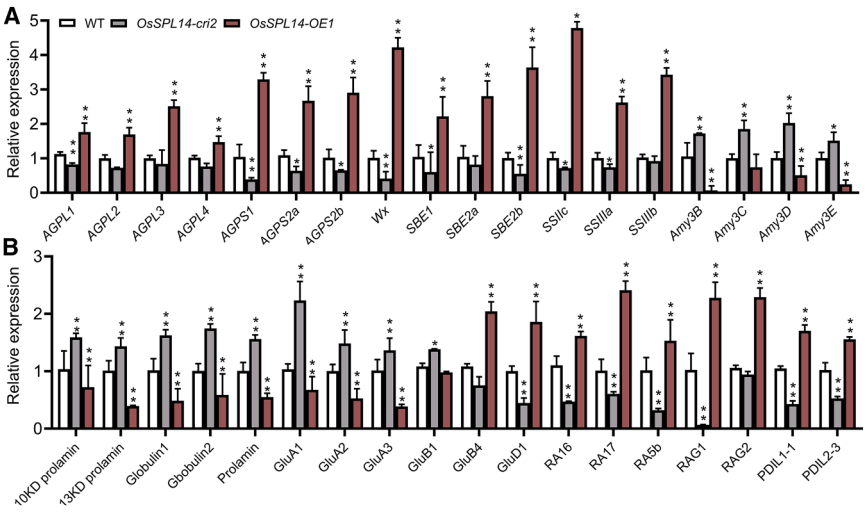

Figure 3. Expression levels of genes involved in the production of storage starch and proteins in WT and transgenic lines.
(A and B) Expression analysis of genes related to storage components in 6 DAF endosperm. Rice *UBIQUITIN5* was used as an internal control. Significant differences between WT and transgenic lines were determined using Student's *t* test (**$p < 0.01$, *$p < 0.05$).

To further confirm that OsSPL14 binds to *Wx* and *PDIL1-1*, we performed chromatin immunoprecipitation (ChIP)-qPCR assays using an OsSPL14-specific antibody in panicles at the grain-filling stage of WT plants. The promoter regions of *Wx* and *PDIL1-1* were divided into several short fragments (P1–P8), which were analyzed by ChIP-qPCR (Figure 4D and 4E). Significant enrichment was detected in the P2–P4 regions of the *Wx* promoter and the P6–P8 regions of the *PDIL1-1* promoter, all of which contain GTAC boxes; little or no enrichment was observed in other regions (Figure 4F and 4G). These results indicate that OsSPL14 directly binds to the promoters of *Wx* and *PDIL1-1* *in vivo*. Furthermore, electrophoretic mobility shift assay (EMSA) results were consistent with the ChIP-qPCR and Y1H findings, showing that OsSPL14 specifically binds to *Wx*-P2 and *PDIL1-1*-P8 (Figure 4H and 4I). Taken together, these results demonstrate that OsSPL14 specifically binds to the promoters of *Wx* and *PDIL1-1* both in *vitro* and in *vivo*.

OsSPL14 interacts with endosperm-preferential OsNF-YB9 and OsNF-YC8–12

To uncover the regulatory network underlying OsSPL14 and chalkiness, we conducted yeast two-hybrid (Y2H) assays to screen for OsSPL14-interacting proteins using a cDNA library of *Arabidopsis* transcription factors (Ou et al., 2011). Nuclear transcription factor Y subunit B6 (AT5G47670), the closest homologue of rice OsNF-YB7 and OsNF-YB9, was identified (Supplemental Figure 8A, 8B, and 8D; Supplemental Table 3). Further analysis focused on OsNF-YB9 because Y2H assays confirmed that OsSPL14 interacts with OsNF-YB9 but not with OsNF-YB7 in yeast (Figure 5A; Supplemental Figure 8C). We then corroborated this interaction using a firefly luciferase complementation (LUC) imaging assay. For the LUC assay, we included internal controls: OsNF-YC11 interacted with OsNF-YB1 (positive control) but not with OsNF-YA8 (negative control) (Supplemental Figure 9), consistent with previous findings (E et al., 2018; Bello et al., 2019). As shown in Figure 5C, the LUC activity signal was detected when OsSPL14 and OsNF-YB9 were co-infiltrated into *N. benthamiana* leaves; it was absent in the negative control. Additionally, a co-immunoprecipitation (Co-IP) assay in rice protoplasts consistently confirmed the interaction between OsSPL14 and OsNF-YB9 (Figure 5D).

OsNF-YB9, a seed-specific transcription factor (Supplemental Figure 10A), has been reported to dimerize with OsNF-YC subunits in the cytoplasm, facilitating its translocation to the nucleus to assemble a functional complex (E et al., 2018). Therefore, five endosperm-preferentially expressed OsNF-YC subunits, including OsNF-YC8, 9, 10, 11, and 12 (Supplemental Figure 10B–10F), were tested for interactions with OsNF-YB9. As expected, OsNF-YB9 and OsNF-YC8–12 formed dimer complexes in yeast and *in planta* (Figure 5B–5D). Furthermore, Y2H, LUC, and Co-IP assays consistently demonstrated that OsSPL14 interacts with OsNF-YC8–12 both *in vitro* and *in vivo* (Figure 5), suggesting that OsSPL14, OsNF-YB9, and OsNF-YC8–12 function as a heterotrimeric complex.

To verify the role of *OsNF-YB9* and *OsNF-YC8–12* in rice grain appearance quality, we generated two *osnf-yb9-cri* mutant lines using CRISPR-Cas9 (Supplemental Figure 11A) and obtained two pentuple mutants of *OsNF-YC8–12* (*osnf-yc8–12*) (Xu et al., 2022). Observation of mature grains showed that *osnf-yb9-cri* and *osnf-yc8–12* mutants exhibited increased chalkiness, with higher PGWC and DEC but lower head rice yield relative to the control (Supplemental Figure 11B and 11E–11G). SEM analysis revealed that the chalky endosperms of *osnf-yb9-cri* and *osnf-yc8–12* were filled with round, loosely packed starch granules, in contrast to the tightly packed and sharp-edged starch granules observed in WT (Supplemental Figure 11C and 11D). Collectively, all biochemical and genetic analyses demonstrated that OsSPL14, OsNF-YB9, and OsNF-YC8–12 directly interact and play critical roles in regulating rice grain appearance quality, particularly grain chalkiness.

OsSPL14 and OsNF-YB9/YC8–12 coordinately regulate the expression of *Wx* and *PDIL1-1*

To elucidate the regulatory mechanism of OsSPL14 on *Wx* and *PDIL1-1*, we quantified the expression levels of *Wx* and *PDIL1-1* in the developing endosperm of WT and transgenic lines at 6 DAF. The results showed that *Wx* and *PDIL1-1* were upregulated in *OsSPL14* OE plants but downregulated in *OsSPL14* knockout and knockdown plants compared with WT (Figure 6A and 6B, Supplemental Figure 12). Additionally, the LUC reporter gene driven by the putative *Wx* or *PDIL1-1* promoter was induced by OsSPL14 in both rice protoplasts and *N. benthamiana* leaves

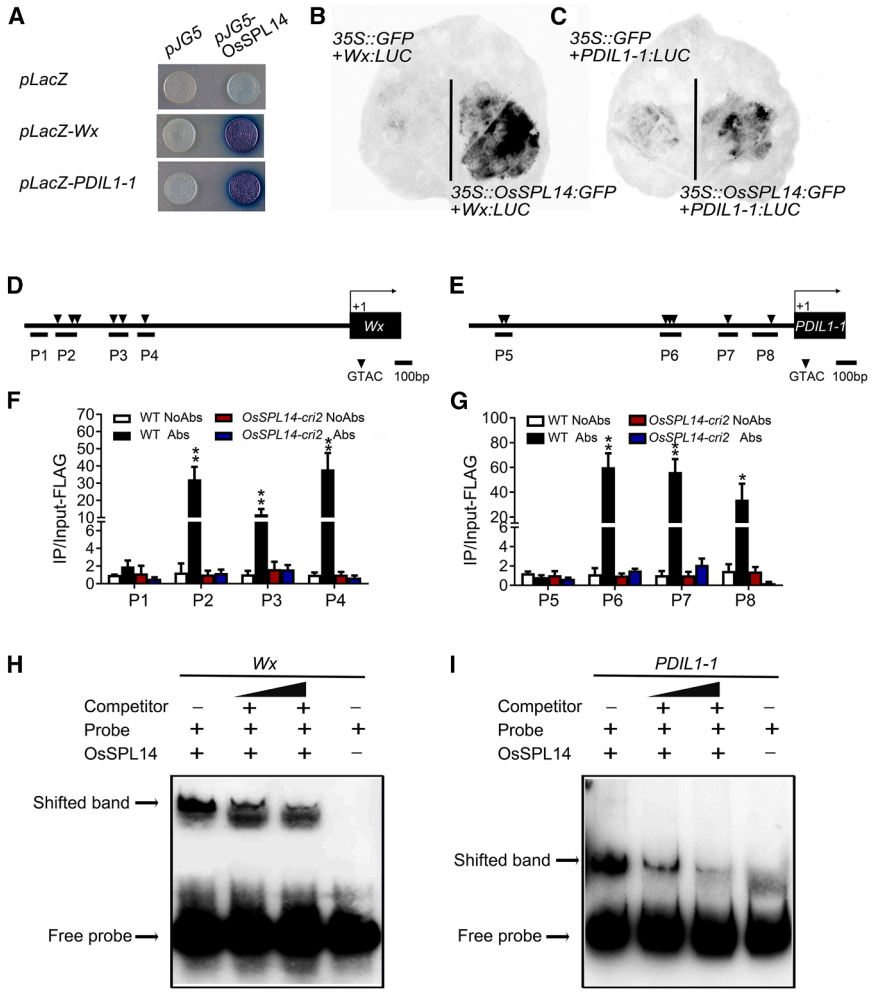

Figure 4. OsSPL14 directly binds to *Wx* and *PDIL1-1 in vitro* and *in vivo*.

(**A**) Y1H assays showing that OsSPL14 binds to the promoters of *Wx* and *PDIL1-1*.

(**B and C**) Interactions between *OsSPL14* and the *cis*-elements of *Wx* and *PDIL1-1* in *N. benthamiana* leaves. OsSPL14 was fused with GFP (*35S::OsSPL14:GFP*) and co-expressed with *Wx:LUC* or *PDIL1-1:LUC* reporter constructs. Empty vector (*35S::GFP*) was used as a negative control.

(**D and E**) Schematic representation of putative OsSPL14 binding sites in the promoters of *Wx* and *PDIL1-1*.

(**F and G**) ChIP-qPCR assays showing *OsSPL14*-binding regions in the *Wx* and *PDIL1-1* promoters. Data are presented as means ± SD (n = 3). $^{**}p < 0.01$.

(**H and I**) EMSA showing *OsSPL14* binding to the *cis*-elements of *Wx* and *PDIL1-1*. Probes corresponding to regions P2 and P8 were used. Equal amounts of protein were loaded in each lane.

(Figure 6E–6G; Supplemental Figure 13). These findings suggest that OsSPL14 functions as a transcriptional activator of *Wx* and *PDIL1-1* via binding to their promoters.

To investigate the regulatory role of OsNF-YB9/YC on *Wx* and *PDIL1-1*, Y1H assays were performed, confirming that OsNF-YB9 and OsNF-YC11 exhibit weak binding to the promoters of *Wx* and *PDIL1-1* (Supplemental Figure 14). Furthermore, RT-qPCR results revealed that the expression levels of *Wx* and *PDIL1-1* were downregulated in *osnf-yb9-cri* and *osnf-yc8–12* mutants (Figure 6C and 6D). Additionally, LUC transient transcriptional activity assays demonstrated that *Wx* and *PDIL1-1* activities were induced in the presence of OsNF-YB9/YC11 compared with the negative control (Figure 6E–6G, Supplemental Figure 13). These results collectively indicate that the OsNF-YB9/YC complex activates *Wx* and *PDIL1-1* expression in rice.

To determine whether the transcriptional activity of OsSPL14 is influenced by the OsNF-YB9/YC heterodimeric complex and vice versa, *ProWx::LUC* or *ProPDIL1-1::LUC* was co-transformed with OsSPL14 and/or OsNF-YB9/YC into rice protoplasts and *N. benthamiana* leaves. The results showed strong activation of LUC upon co-transformation with OsSPL14 and OsNF-YB9/YC11 (Figure 6E–6G; Supplemental Figure 13), indicating that OsNF-YB9/YC11 enhances the binding ability of OsSPL14 to *Wx* and *PDIL1-1* and acts as a partner of OsSPL14 during the regulation of endosperm development in rice. Therefore, our results demonstrate that OsSPL14, interacting with OsNF-YB9/YC8–12 subunits, directly binds to the promoters of *Wx* and *PDIL1-1* and subsequently activates their expression, thereby influencing storage synthesis and grain appearance quality in rice. Based on these findings, we propose the following working model: OsNF-YB9 is imported into the nucleus through heterodimerization with OsNF-YC8–12 to form a transcriptional complex with the transcription factor OsSPL14. The OsSPL14–OsNF-YB9/YC8–12 heterotrimer then directly targets the promoters of *Wx* and *PDIL1-1*, activating their expression and thereby regulating storage synthesis and chalkiness in rice (Figure 7).

DISCUSSION

OsSPL14 activates *Wx* and *PDIL1-1* to affect grain storage starch and protein accumulation

Storage starch and protein, the main components of cereal crop grains, are two major sources of nutrition and energy for humans and livestock. Previously, a series of endosperm-defective mutants revealed that defects in starch and protein biosynthesis can reduce crop grain yield and quality (Zhao et al., 2022). However, little is currently known about the transcriptional regulatory network involved in the production of these storage substances. In this study, we identified a novel function of *OsSPL14* in controlling grain appearance quality in rice. Our genetic analysis demonstrated that loss of *OsSPL14* function resulted in a chalky endosperm phenotype with significant changes in starch granule morphology and storage content

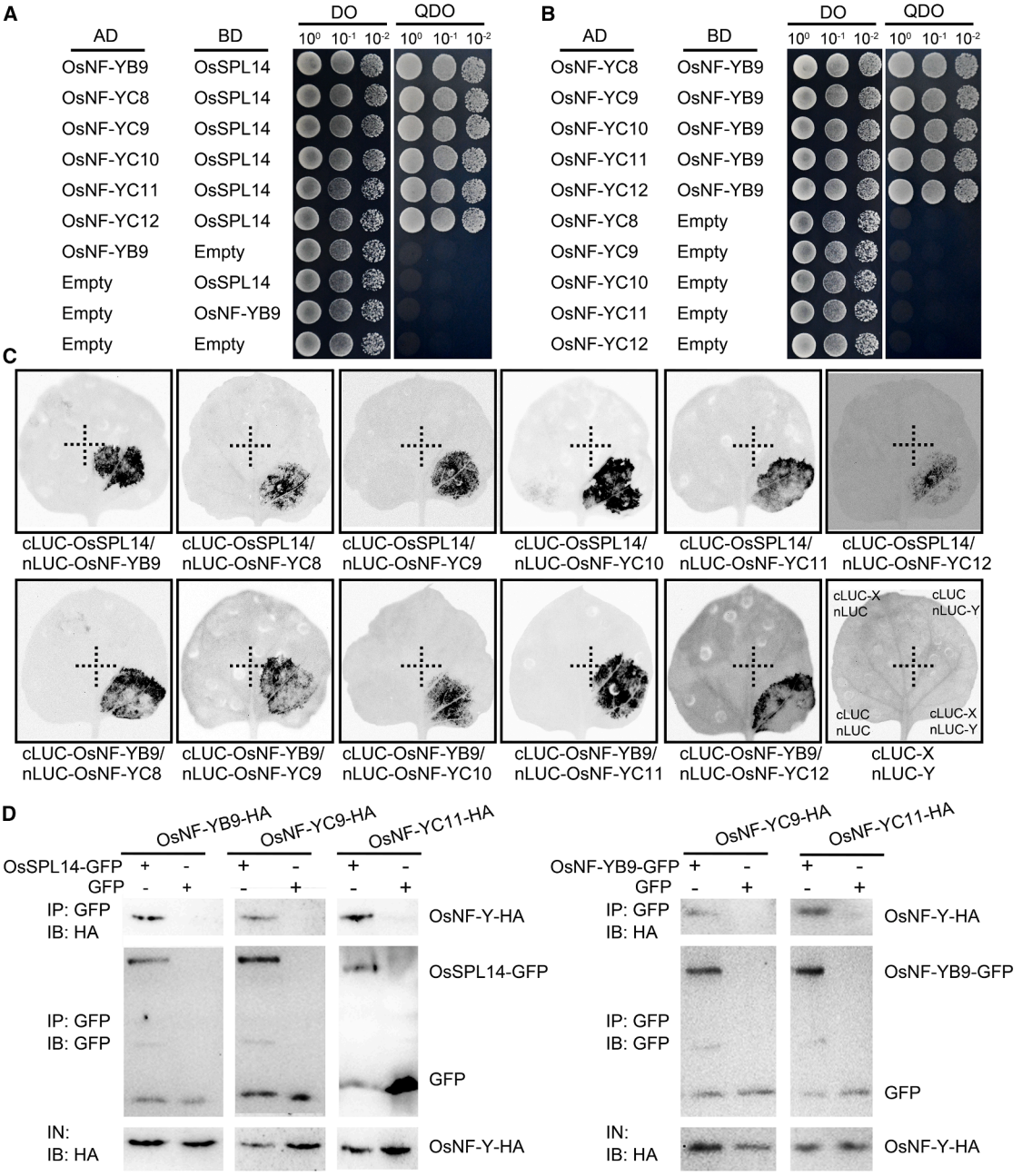

Figure 5. Protein–protein interaction analysis of OsSPL14, OsNF-YB9, and OsNF-YC8–12.

(A) Y2H assays showing interaction between OsSPL14 and OsNF-Y subunits.

(B) Y2H assays showing interactions between OsNF-YB9 and OsNF-YC8–12. Transformed yeast cells were spotted onto a stringent selection medium lacking Trp, Leu, His, and Ade (–WLHA) or a non-selective control medium lacking Trp and Leu (–WL).

(C) LUC assay showing interactions among OsSPL14, OsNF-YB9, and OsNF-YC8–12 in *N. benthamiana*.

(D) Co-IP assay showing interactions among OsSPL14, OsNF-YB9, and OsNF-YC9/11 in rice protoplasts. Total proteins were immunoprecipitated (IP) with anti-GFP, and the immunoblots were probed with anti-GFP and anti-HA antibodies, respectively. '+' or '−' indicate the presence or absence of the protein in each sample. OsNF-YB9/YC9/YC11-HA was detected in the immunoprecipitated GFP–OsSPL14 complex, indicating a physical association between OsSPL14 and OsNF-YB9/YC9/YC11 in rice. Similarly, OsNF-YC9/YC11-HA was detected in the immunoprecipitated GFP–OsNF-YB9 complex, indicating that OsNF-YB9 interacts with OsNF-YC9/YC11 in rice. IB, immunoblot; IN, input.

(Figures 1 and 2). Accordingly, the *osspl14-cri* mutant exhibited considerably lower AC but greater protein accumulation in endosperm compared with the WT or *OsSPL14-OE* (Figure 2A–2C). Consistent with the observed phenotypes, RT-qPCR results indicated that *OsSPL14* is involved in starch and protein metabolism; it regulates the expression of a series of rice starch and protein biosynthetic genes (Figure 3). Among the validated downregulated core genes related to storage starch and protein synthesis, *Wx* is targeted by OsNF-YB1/YC12 or OsNF-YB1/OsMADS14 to regulate storage starch synthesis

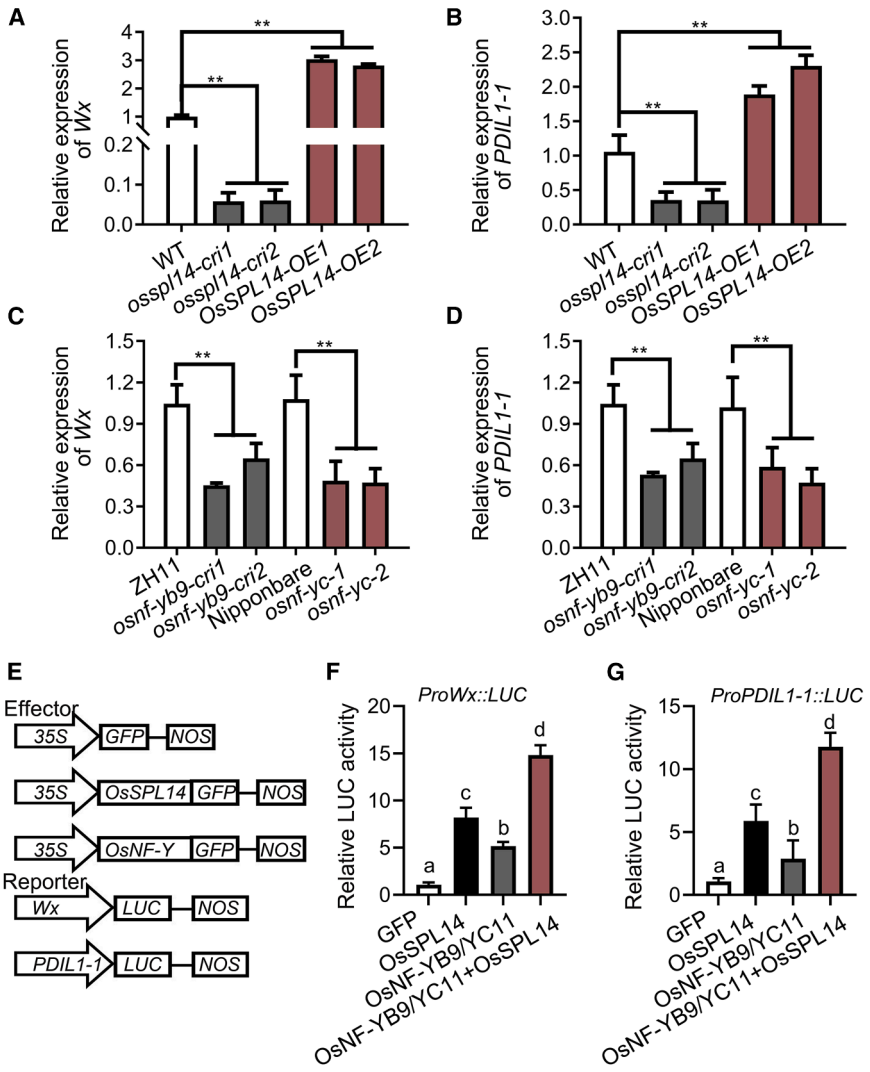

Figure 6. OsSPL14 and OsNF-Y factors positively regulate *Wx* and *PDIL1-1*.

(**A–D**) Expression levels of *Wx* and *PDIL1-1* in 6 DAF endosperm of control, *osspl14-cri*, *OsSPL14-OE*, *osnf-yb9-cri*, and *osnf-yc* plants. Data are presented as means ± SD (n = 3). Rice *UBIQUITIN5* was used as an internal control. $^{**}p < 0.01$.

(**E**) Diagram of reporter and effector constructs used in transcriptional activity assays.

(**F and G**) Transcriptional activity assays in rice protoplasts showing that the OsSPL14–OsNF-YB9/YC11 complex enhances the transcriptional activation of *Wx* and *PDIL1-1*. GFP was used as a negative control. Data are presented as means ± SD (n = 3). Different lowercase letters indicate significant differences according to one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test ($p < 0.01$).

(Bello et al., 2019; Feng et al., 2022), and *PDIL1-1* is reportedly a potential direct target of OsSPL14 (Lu et al., 2013). These studies provided clues that *Wx* and *PDIL1-1* are potential targets of OsSPL14. As expected, DNA binding and transcription assays confirmed that *Wx* and *PDIL1-1* are indeed direct targets of OsSPL14 (Figure 4A–4C). Further experiments demonstrated that OsSPL14 directly binds to the promoters of *Wx* and *PDIL1-1*, thereby positively regulating their expression (Figures 4 and 6).

Wx and *PDIL1-1* are two grain appearance regulators expressed in rice endosperm and involved in the accumulation of storage nutrients (Supplemental Figure 7). For example, in chalky *crnf-yb1* grains, decreased expression of the *Wx* gene led to a significant reduction in AC but an increase in protein content (Xu et al., 2016; Bello et al., 2019). Additionally, it has been reported that changes in amylose and/or protein content affect rice grain quality, which can be attributed to the downregulation of *PDIL1-1*. Failure to express *PDIL1-1* leads to a floury endosperm phenotype. The expression levels of several ER stress-associated genes were significantly higher in mutants than in WT endosperms, suggesting that ER stress—induced by the absence of *PDIL1-1*—likely triggers programmed cell death (PCD) and compromises the normal starch synthesis process, ultimately resulting in a floury endosperm (Han et al., 2012). In the current study, the *osspl14-cri* mutant and *OsSPL14*-downregulated plants (*OsSPL14-RNAi*, *miR156e-OE*, and *miR529a-OE*) also exhibited higher accumulation of total protein and glutelin (Figure 2C; Supplemental Figures 4 and 5). This phenomenon could be attributed to the lack of *PDIL1-1* activation, as previously reported in several *PDIL1-1* mutants (Kawagoe et al., 2005; Han et al., 2012; Kim et al., 2012). Given that *PDIL1-1* is a downstream target of OsSPL14 and is involved in the ER stress response, future research will focus on analyzing ER stress and PCD in the endosperms of WT and *OsSPL14* transgenic lines. Collectively, our findings demonstrate that *OsSPL14* mutations affect storage starch and protein accumulation by modulating the expression of *Wx* and *PDIL1-1* in rice, thus influencing rice appearance quality and economic value (Figure 7). These findings confirm that OsSPL14 acts as a critical regulator of starch and protein biosynthesis; they provide new insights into the molecular and genetic mechanisms of endosperm development in rice.

The OsSPL14–OsNF-YB9/YC complex regulates the synthesis of storage starch and protein

NF-Y is a heterotrimeric transcription factor consisting of three subunits: NF-YA, NF-YB, and NF-YC. In mammals and yeast, each NF-Y subunit is encoded by a single gene, whereas in plants, each subunit is encoded by multiple genes within a gene family (Laloum et al., 2013), making functional studies of these subunits more complex. The rice genome encodes 11 NF-YAs, 11 NF-YBs, and 12 NF-YCs (E et al., 2018). Over the past decade, several NF-Y subunits have been identified as directly or indirectly related to starch synthesis and storage protein accumulation during endosperm development. For instance,

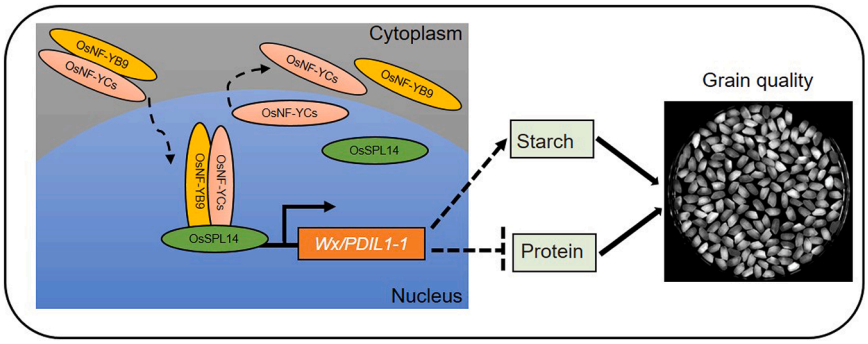

Figure 7. A proposed model of the OsSPL14–OsNF-YB9/YC8–12 complex regulating grain quality by modulating *Wx* and *PDIL1-1* expression during grain filling.
A working model of the OsSPL14–OsNF-YB9/YC8–12 complex regulating appearance quality in rice seeds. OsNF-YB9 binds to OsNF-YC8–12 to form a dimer, which then interacts with OsSPL14 to form a heterotrimer. This trimeric complex activates the transcription of the key starch and protein accumulation genes, *Wx* and *PDIL1-1*, by directly binding to the "GTAC" motif in their promoters, thus regulating grain appearance quality in rice.

NF-YA8, YB1, YB7, YB9, and NF-YC8–12 are predominantly expressed in the endosperm, suggesting that they participate in regulating endosperm development (E et al., 2018). OsNF-YB1 interacts with OsMADS14 to promote *OsAGPL2* and *Wx* expression, thereby regulating storage starch synthesis during grain filling in rice (Feng et al., 2022). Similarly, OsNF-YB9 has been reported to interact with OsbZIP76 or SPK to regulate endosperm development in rice (Niu et al., 2021). Indeed, NF-YB and NF-YC can form a heterodimer, which may further interact with NF-YA or other transcription factors to regulate downstream target genes. For example, the NF-YB1/YC12 heterodimer interacts with the key grain quality regulator OsbHLH144. This trimer recognizes the GCC-box and activates the downstream *Wx* gene to promote endosperm development (Bello et al., 2019). Additionally, proteins such as OsERF115 have been shown to interact with the OsNF-YB1/YC11 (or YC12) heterodimer to form a transcriptional complex that regulates the expression of GCC-box-containing target genes, thereby influencing rice grain quality (Xu et al., 2016).

Here, we revealed the sequential interactions of OsSPL14, NF-YB9, and NF-YC8–12 in yeast and *in planta* using Y2H, LUC, and Co-IP assays (Figure 5). Furthermore, we confirmed that this heterotrimeric complex binds to the promoters of *Wx* and *PDIL1-1*, activating their expression and thereby regulating endosperm development in rice (Figure 6). This hypothesis is also supported by several lines of indirect evidence. First, the chalky phenotype observed in *osspl14-cri*, *nf-yb9-cri*, and *nf-yc8–12* mutants followed consistent trends during seed development. Second, RT-qPCR results showed that transcription levels of the downstream genes *Wx* and *PDIL1-1* were reduced in *osspl14-cri*, *nf-yb9-cri*, and *nf-yc8–12* mutants. Finally, OsNF-YB9/YC subunits were found to enhance the promoter activity of *Wx* and *PDIL1-1* activated by OsSPL14. Taken together, these findings identify a novel non-canonical NF-Y trimeric complex consisting of OsSPL14 and OsNF-YB9/YC8–12, which plays a critical role in regulating endosperm development and rice grain appearance. This finding is consistent with previous reports that NF-YB/YC can interact with proteins other than NF-YA to form heterotrimers (Xu et al., 2016; Bello et al., 2019). Nevertheless, we cannot exclude the possibility that NF-YAs—particularly seed-specific NF-YAs—participate in forming this complex. Further investigation is needed to explore the role of NF-YAs and their possible interactions with the OsSPL14–OsNF-YB9/YC heterotrimer.

OsSPL14 provides a new potential gene resource for yield and quality improvement in rice

Superior grain quality and high yield are important breeding goals in rice. However, these objectives have long been considered negatively associated. One of the primary reasons is the opposing effects of certain individual genes on grain quality and yield. For instance, mutations in *GW2*, *GW8*/*OsSPL16*, and *GS2* result in larger grains and higher yields; such mutations also increase grain chalkiness (Song et al., 2007; Wang et al., 2012; 2015b; Hu et al., 2015). Therefore, the identification of genes that contribute to both high grain yield and superior quality, and elucidation of their molecular mechanisms, can greatly facilitate the breeding of rice varieties with improved overall performance.

OsSPL14 has attracted great attention in recent years. It was found to boost grain yield by shaping ideal plant architecture (Jiao et al., 2010; Miura et al., 2010; Zhang et al., 2017), contribute to yield heterosis between *indica* and *japonica* (Huang et al., 2016), and mitigate the conflict between grain yield and immunity (Wang et al., 2018; Liu et al., 2019), thus substantially impacting rice yield. However, relatively fewer insights have been gained regarding the physiological functions of *OsSPL14* in controlling grain appearance quality in rice. In this study, we found that *OsSPL14* may offer a new approach to addressing the trade-off between grain quality and yield. OsSPL14 directly interacts with the transcription factor OsNF-YB9/YC and enhances the transcriptional activity of *Wx* and *PDIL1-1*, which in turn determines grain appearance quality. Phenotypic and molecular analyses further confirmed that *OsSPL14* positively influences rice grain quality by regulating storage starch and protein accumulation in the endosperm. These results not only reveal a novel molecular mechanism underlying grain chalkiness but also provide a new strategy for breeding rice with both high yield and superior quality. Of course, more field experiments are needed in future studies. Moreover, OsSPL14 regulates plant height, tiller number, stem diameter, and panicle primary branch number in a dose-dependent manner (Li et al., 2023). Therefore, optimization of OsSPL14 expression levels to balance yield- and quality-related traits is essential to achieve the dual breeding goals of high yield and superior grain quality. Furthermore, the identification and functional analysis of additional transcription factors involved in this regulatory network will provide a theoretical foundation for better understanding the synthesis of storage starch and proteins, with substantial implications for crop breeding.

METHODS

Plant materials and growth conditions

The WT rice plants used in this study were *japonica* (*O. sativa* ssp. *geng*) varieties ZH11 and Nipponbare. The plants were cultivated in an experimental field during the normal growing season in Wuhan, Hubei, China (30.5°N, 114.3°E, 15 m above sea level; average daily temperature approximately 28°C).

Plasmid construction and plant transformation

The CRISPR vector was constructed using a CRISPR-Cas9-based genome-editing system (Ma et al., 2015). To generate *OsSPL14* OE lines, full-length coding sequences (CDSs) of *OsSPL14* were amplified and cloned into the *35S::GFP* vector. The plasmids were transformed into ZH11 rice callus. Primers used for plasmid construction are listed in Supplemental Table 2.

RNA isolation and expression analysis

RNA from all tissues except developing endosperm was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA from developing endosperm was obtained using a seed RNA extraction kit (Life Science, Beijing, China). First-strand cDNA synthesis was performed using a Reverse Transcription Kit (Invitrogen). RT-qPCR was conducted using the first-strand cDNA as the template. Endogenous *UBIQUITIN5* (LOC_Os03g13170) transcripts were used to normalize gene expression levels. RT-qPCR was performed on a Bio-Rad CFX384 Real-Time System using SYBR Green. Changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ method. Each experiment was repeated three times. All primers used for expression analysis are listed in Supplemental Table 2.

GUS staining and GUS activity analysis

To investigate the tissue-specific expression of *OsSPL14*, approximately 1.0-kb promoter fragments of OsSPL14 were amplified from genomic DNA and cloned into *DX2181::GUS* to create *DX2181::OsSPL14:GUS*. Whole grains were immersed in GUS staining solution (Biosharp, BL622A) and incubated at 37°C in the dark for 1–5 h, depending on the tissue. After incubation, the tissues were cleared in de-staining buffer (ethanol: acetic acid, 84:16 v/v) at room temperature for 1–4 h. Cleared tissues were washed several times with 70% ethanol and observed under a dissecting microscope. Primers used for plasmid construction are listed in Supplemental Table 2.

Microscopy

For SEM, brown rice seeds were transversely cut with a knife and coated with gold under vacuum conditions. The morphology of starch granules in the endosperm was examined using a scanning electron microscope (JSM-6390LV, JEOL). SEM analysis was based on at least three biological replicates of the mounted specimens. All procedures followed the manufacturer's protocol.

TEM analysis of developing seeds was performed as previously described (Wang et al., 2016) using a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan). For semithin section experiments, transverse sections of developing endosperms were stained with iodine-potassium iodide.

Analysis of grain quality

Threshed seeds were air-dried and stored at room temperature for at least 3 months before testing. Fully filled grains were used to measure grain quality. The percentage of chalky grains among the total number of dehulled grains was used to calculate the grain chalkiness rate. Head rice yield/rate was estimated as the percentage weight of intact milled grains relative to total grain weight. Dehulled rice grains were ground into powder; 0.5-g samples were used to measure total starch, amylose, and protein contents. The content is expressed as a percentage of the total sample weight on an oven-dry basis. Total starch contents and ACs were measured with a Starch/AC Assay Kit (Solarbio, BC0705, BC4265). Protein, glutelin, and prolamin contents in the flour were measured based on previously published methods (Bello et al., 2019).

Y2H and Y1H assays

Full-length cDNAs were subcloned into either the pGBKT7 or pGADT7 vector (Clontech, Dalian, China). The prey and bait plasmids were verified by sequencing and subsequently transformed into yeast strain AH109 using the lithium acetate method. The transformed yeast cells were grown on SD medium lacking Leu and Trp (–WL) at 30°C for 3 days, then spotted onto stringent selection medium lacking Trp, Leu, His, and Ade (–WLHA) to test for possible interactions.

For Y1H analysis, DNA fragments corresponding to the promoters of target genes were independently inserted into the *pLacZ* plasmid. The CDSs of potential trans-activators were fused with the GAL4 AD domain in *pJG4*. These constructs were transformed into *Saccharomyces cerevisiae* strain EGY48 using the Clontech One-Hybrid System (Coolaber, YK3010-50P). Yeast cells were transformed with the indicated plasmids and grown on SD/–Ura/–Trp plates, then transferred to SD/–Ura/–Trp plates containing 2% galactose, 1% raffinose, 1× BU salts, and 80 mg/L X-Gal (Clontech). The interaction was confirmed by the appearance of blue colonies on the medium. Primers used for cloning are listed in Supplemental Table 2.

Luciferase-based transient transcriptional activity assay

For the luciferase-based transient transcriptional activity assay in *N. benthamiana* leaves, the CDS of *OsSPL14* was inserted into the *35S::GFP* vector. The promoters of *Wx* and *PDIL1-1* were fused to the *pFA2300::LUC* vector. The resulting vectors (*35S::OsSPL14:GFP*/*35S::GFP* and/or *Wx:LUC*/*PDIL1-1:LUC*) were transferred into *Agrobacterium GV3101*, then co-infiltrated into *N. benthamiana* leaves using an injection syringe. Approximately 48 h after infiltration, leaves were injected with 1 mM luciferin (Promega, Madison, WI, USA) in 0.1% Triton X-100, and fluorescence was quenched in the dark for several minutes. Luciferase signals were collected using a Chemiluminescence Imaging System (Tanon Science and Technology 5200, Beijing, China).

For the luciferase-based transient transcriptional activity assay in rice protoplasts, the *Wx* and *PDIL1-1* promoters were fused to the *pGreen* vector. The vectors (*35S::OsSPL14:GFP* and/or *Pro::Wx:LUC*/*Pro::PDIL1-1:LUC*) were co-transformed into rice protoplasts and incubated overnight in darkness. Relative LUC activity ($LUC^{Firefly}$/$LUC^{Renilla}$ ratio) was measured using a dual-luciferase reporter assay kit and a GloMax luminometer (Promega) to determine transactivation activity. *35S::GFP* was used as an internal control. Primers used for plasmid construction are listed in Supplemental Table 2.

EMSA

To express the OsSPL14 protein in *E. coli*, the CDS of *OsSPL14* was amplified and cloned into the *pET28a-HIS* expression vector, then introduced into *E. coli* Transetta cells (DE3) (TransGen Biotech, Beijing, China). Recombinant proteins were purified using Ni Singarose 6FF Beads (AOGMA, AGM90046) via affinity chromatography.

Biotin-labeled and unlabeled *Wx* and *PDIL1-1* probes were synthesized (Sangon Biotech, Shanghai, China). For the EMSA assay, biotin-labeled promoters were used as probes, and unlabeled promoters were used as competitors. EMSA was performed according to the manufacturer's instructions (Beyotime, No. GS008, Jiangsu, China). Images were visualized using a Tanon-5200 Chemiluminescent Imaging System (Tanon Science and Technology). Primers used for EMSA are listed in Supplemental Table 2.

ChIP-qPCR assay

To validate the binding of OsSPL14 to *Wx* and *PDIL1-1 in vivo*, a ChIP assay was performed using 6-day-old WT and *osspl14-cri2* grains.

Approximately 2.5 g of tissue was harvested and incubated in cross-linking buffer (0.4 M sucrose, 10 mM Tris-HCl [pH 8.0], 1 mM phenylmethylsulfonyl fluoride [PMSF], 1 mM ethylenediaminetetraacetic acid [EDTA], 1% formaldehyde) for 10 min under a vacuum. Cross-linking was halted by adding 2 M glycine. An OsSPL14 antibody (ABclonal, Woburn, MA, USA; A17556) was used for IP. DNA pulled down by IP was analyzed by RT-qPCR. Enrichment was calculated as the ratio of *osspl14-cri2* to ZH11. Data are presented as means ± SD of three independent experiments. Primers used in the ChIP-qPCR assays are listed in Supplemental Table 2.

LUC complementation imaging assays

The full-length cDNA was in-frame fused with the N-terminal half of luciferase (*nLUC*) or the C-terminal half of luciferase (*cLUC*). The resulting binary expression vectors were transformed into *Agrobacterium* strain *GV3101* (containing the pSoup-p19 plasmid). *Agrobacterium* cells carrying various expression vectors were co-infiltrated into *N. benthamiana* leaves with appropriate controls. After infiltration, plants were incubated at 22°C for 48 h, and fluorescence images were captured using a low-light cooled charge-coupled device imaging system (Tanon Science and Technology 5200). Primers used for plasmid construction are listed in Supplemental Table 2.

Immunoblot analysis and Co-IP

For immunoblot analysis, total protein from rice leaves was extracted in protein extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% NP-40, 1 mM PMSF, and a plant protease inhibitor cocktail [Roche, Basel, Switzerland]). Equal amounts of total protein were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and detected by immunoblotting using anti-actin (Abbkine, Atlanta, GA, USA; catalog: A01050-2) or anti-OsSPL14 (ABclonal, A17556) antibodies.

In vivo Co-IP assays were performed via transient protein expression in rice protoplasts. Hemagglutinin (HA)-tagged OsNF-Y and GFP-tagged OsSPL14, OsNF-YB9, or GFP-empty constructs were co-expressed in rice protoplasts, extracted using the buffer described above, immunoprecipitated with a GFP antibody (MBL, Woods Hole, MA, USA; M192-3), and detected using anti-HA (MBL, catalog: M180) and anti-GFP (MBL, catalog: M047) antibodies. Primers used for plasmid construction are listed in Supplemental Table 2.

ACCESSION NUMBERS

Accession numbers are as follows: *OsSPL14*, LOC_Os08g39890; *OsNF-YB9*, LOC_Os06g17480; *OsNF-YC8*, LOC_Os01g39850; *OsNF-YC9*, LOC_Os01g01290; *OsNF-YC10*, LOC_Os01g24460; *OsNF-YC11*, LOC_Os05g23910; *OsNF-YC12*, LOC_Os10g11580; *Wx*, LOC_Os06g04200; *PDIL1-1*, LOC_Os11g09280.

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AUTHOR CONTRIBUTIONS

Y.L. and W.Y. conceived and designed the research; Y.L. designed and performed the experiments; Y.L. and W.Y. prepared the figures and drafted the manuscript; X.G., J. S., K.X., and T.Q. generated and identified some transgenic plants; X.Z., Z.S., and Y.H. assisted with field experiments; B.Z., H.Z., and H.L. provided helpful comments and revised the manuscript.

SUPPLEMENTAL INFORMATION

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