Lose-of-Function of a Rice Nucleolus-Localized Pentatricopeptide Repeat Protein Is Responsible for the *floury endosperm*14 Mutant Phenotypes

Mengyao Xue1, Linglong Liu1, Yanfang Yu1, Jianping Zhu1, Hao Gao1, Yihua Wang1 and Jianmin Wan1,2*

Abstract

**Background:** The endosperm of rice (*Oryza sativa*) has been usually used for the study of starch synthesis. Although several related factors have been revealed, other unknown members remain to be identified, given that starch synthesis is a complicated and sophisticated process.

**Results:** Here, we identified and characterized a new rice seed mutant, *floury endosperm*14 (*flo14*), which showed chalked endosperm and seed-lethal phenotypes. Map-based cloning indicated *FLO14* encodes a novel P-family PPR protein which contains ten PPR motifs. Afterwards the gene was named *OsNPPR3*. Subcellular localization showed *OsNPPR3* was targeted to nucleolus. Quantitative RT-PCR analysis demonstrated that *OsNPPR3* was universally expressed in various tissues, with pronounced levels during rice endosperm development. Molecular analysis further suggested that *OsNPPR3* was involved in the regulation of expression levels and splicing of a few genes in mitochondria.

**Conclusion:** The study demonstrates that the nucleolus-localized PPR protein is responsible for the *flo14* mutant phenotypes through affecting nuclear and mitochondrial gene expression and splicing.

**Keywords:** Rice (*Oryza sativa*), *flo14* mutant, *OsNPPR3*, PPR protein, Nucleolus-targeted

Background

Crop plants accumulate large amounts of starch in storage tissues, such as the endosperm in rice (*Oryza sativa*) and maize, as the main carbon sources for human and livestock (Burrell, 2003). Starch biosynthesis is major in the amyloplast, a kind of specialized plastid in the endosperm cell (Martin and Smith, 1995). Although some related factors involved in starch biosynthesis have been reported in rice (Long et al., 2017), there still existed a large number of unknown genes related to starch synthesis in rice. Floury endosperm (*flo*) mutants are ideal genetic materials for studying the mechanism of starch biosynthesis and amyloplast development.

The first floury endosperm mutant (*flo1*) was previously reported to locate on chromosome 5, and the causative gene was not yet identified (Satoh and Omura, 1981). The *flo2* locus was revealed to encode a nuclear-localized TPR-binding protein, which influenced starch synthesis potentially via interaction with transcription factors such as bHLHs to positively regulate expression of starch synthesis-associated genes (She et al., 2010). The *flo3* mutant showed floury endosperm, accompanied by a low level of the 16-kDa globulin (Nishio and Iida, 1993). The opaque endosperm mutant *flo4* was due to an insertional mutation in the *OsPPDKB* (cytosolic pyruvate orthophosphate dikinase) gene. Further study showed the *OsPPDKB/FLO4* gene could act as an important modulator of carbon flow for starch and lipid biosynthesis during grain filling (Kang et al., 2005). Later on, the *OsSSSIIIa* mutation was identified as the cause of *flo5* mutant. The *OsSSSIIIa/FLO5* protein played an important role in generating relatively long chains in rice endosperm (Ryoo et al., 2007). Recently, a series of
rice flo mutants were identified, including flo6 (Peng et al., 2014), flo7(Zhang et al., 2016), flo8 (Long et al., 2017), flo12 (Zhong et al., 2019), and flo15(You et al., 2019). These mutants provided useful basis to understand the starch regulatory mechanism in rice.

Pentatricopeptide repeat (PPR) proteins are a large family of RNA-binding proteins in higher plants, with more than 600 members in Arabidopsis (Arabidopsis thaliana) (Cheng et al., 2016), and over 400 members in rice (Lurin et al., 2004; Gutierrez-Marcos et al., 2007). Increasing evidence indicates that PPR genes play an important role in plant post-transcriptional regulation, such as RNA editing (Takenaka, 2010; Barkan et al., 2012; Li et al., 2018), RNA splicing, RNA stability, RNA maturation, and translation initiation (Barkan et al., 2012). PPR proteins localize primarily in mitochondria and chloroplasts, and are essential for the normal biological activities of the both organelles (Zhang et al., 2017). Given only limited nucleus-localized PPR proteins have been identified in rice, the information of PPRs that regulate seed development and coordinate gene expression between the organelles and nucleus remains fragmentary.

In this study, we screened and obtained another flo mutant (flo14), which showed abnormal starch biosynthesis and seed-lethal phenotypes. Map-based cloning and complementation test revealed that FLO14 locus encodes a nucleolus-targeted P-subfamily PPR protein, named OsNPPR3. Expression analysis indicated that the transcription and splicing of several nuclear-encoded and mitochondria-encoded genes were markedly altered in flo14 relative to the wild type. Our results provide the first evidence that OsNPPR3 is involved in starch biosynthesis and seed vigor.

**Results**

**Phenotypic Characterization of the flo14 Mutant**

A stably inherited mutant (named flo14) was obtained from a mutant library induced by N-methyl-N-nitro-sourea. The mutant library was produced by the National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University. The flo14 mutant was selected due to the chalky endosperm phenotype and aimed to study the function of starch-related genes. The flo14 mutant was backcrossed twice with background parent to exclude the possibility of other gene variants, and the mutant seeds could only be collected from heterozygous individuals. At the mature stages, flo14 mutant seeds showed floury endosperms in contrast to the transparent endosperm of wild type (Fig. 1a, b). Vertical-sections of imbibed seeds showed that wild-type embryos were well developed with established coleoptiles and shoot apical meristems, whereas only incomplete coleoptile structures were observed in the flo14 embryos (Fig. 1c). The tetrazolium staining revealed that none of the flo14 mutant seeds were stained red, indicating that the seed viability of the mutants was severely reduced (Fig. 1d). The seed germination test showed the flo14 mutant produced no complete shoots and roots, and died about 10 days after germination (Fig. 1e), suggesting the embryogenesis of flo14 was compromised. Consistent with the floury endosperms, thousand kernel weight of flo14 seeds was 10% reduced relative to the wild type (Fig. 1f).

**Starch Granule Development Is Defected in flo14 Mutant**

To determine the morphologic details of the mutant seeds, we performed scanning electron microscope (SEM) examinations. The results indicated that the starch granules of flo14 mutant were loosely packed. In contrast, wild-type ones were equal-sized and densely arranged (Fig. 2a-d). Besides, semi-sectioning was conducted to observe starch granules in developing endosperm at 12 days after flowering (DAF). In the center of wild-type endosperm, the amyloplast was composed of several mature granules that were in large qualities and closely arranged (Fig. 2e, f, red arrowheads). Nevertheless, smaller, immature and more scattered starch granules were observed in the mutant, and they were separated from each other, and a great variety of gaps appeared in the cytoplasmic space (Fig. 2g, h, red triangular arrowheads).

In the flo14 mutant, total starch content was significantly lower than in the wild type. Correspondingly, the amylose content was slightly reduced but not obviously (Fig. 2i, j). Meanwhile, we compared the swelling volume of wild-type and flo14 mutant starch in different urea concentrations from 0 M to 9 M (Additional file 1: Figure S1a, b). We found wild-type starch began to dissolve in 4 M urea solution, while flo14 mutant started to dissolve in 5 M urea. Furthermore, the pasting properties of endosperm starch were analyzed by rapid visco analyzer (RVA; Additional file 1: Figure S1c). The viscosity pattern of the flo14 pasting starch was not obviously different from that of the wild type. Together, the results showed both the fine structure and physicochemical properties of the starch granules had been changed in the flo14 mutant.

**Map-Based Cloning of the Gene Responsible for the flo14 Phenotypes**

Genetic analysis was conducted in the offspring derived from a single plant containing the heterozygous flo14 gene. The numbers of seeds with normal (transparent endosperm and normal seed vigor) and mutant phenotypes (chalky endosperm and seed lethality) were counted separately. Normal: mutant seeds = 188: 67, \( \chi^2 = 0.212 < \chi^2_{0.05,1} = 3.842 \) (Additional file 2: Table S1), indicating that the mutant phenotypes is controlled by a single recessive gene.
Fig. 1 Phenotypic characterization of the flo14 mutant. 

a. Comparison of wild-type (WT) and flo14 mutant (flo14) seeds.
b. Cross sections of wild-type and flo14 mutant seeds.
c. Vertical-sections of imbibed embryos of wild type and flo14 mutant.
d. Tetrazolium assay of wild-type and flo14 mutant seeds.
e. Young seedlings of wild type and flo14 mutant at 5 days after germination.
f. Thousand kernel weight of wild-type and flo14 mutant seeds. Data indicate means ± SD (from at least three independent samples) and was compared with wild type by Student’s t-test (*P < 0.05, **P < 0.01). Scale bars: 1 mm in (a and b), 1 cm in (c and d), 500 μm in (e).

Fig. 2 Starch granules development in the flo14 mutant. 

a-d. Scanning electron microscope (SEM) analysis of the endosperm in wild-type (WT) (a, c) and flo14 mutant (flo14) (b, d) seeds.
e-h. Semi-thin sections of wild-type and flo14 mutant seeds. The central part of 12 days after flowering (DAF) in the wild-type endosperm cells (e-f) and the central part of mutant flo14 endosperm cells (g-h).
i. Starch content of wild type and flo14 mutant (n = 3 each). Data are shown as means ± SD (from at least three independent samples) and was compared with wild type by Student’s t-test (*P < 0.05). NS: no obviously changed. Scale bars: 0.5 mm in (a, b), 15 μm in (c, d), 100 μm in (e, g), 200 μm in (f, h).
To map the mutant gene locus, we generated an F2 population from a cross between the flo14 mutant and indica cultivar N22. Ten individuals containing floury white endosperm were chosen from the F2 progeny, and the gene locus was roughly mapped on the long arm of chromosome 3 between the markers RM168 and RM3199. Then we used additional eight hundred recessive individuals from the same F2 population and narrowed it to a 165-kb region, which contained two Bacterial Artificial Chromosomes (BACs) and fifteen open reading frames (Fig. 3a). Comparison of the sequences between the wild type and flo14 mutant revealed a single nucleotide replacement occurred at the only exon of Os03g0728200, leading to a premature termination of transcription at amino acid residue 1633 of a PPR protein. Afterwards the candidate gene was named OsNPPR3. The wild-type PPR protein was composed of 1806 amino acid residues and contained a tandem repeat of 10 PPR motifs, and was thus classified as P- subclass (Fig. 3b).

To verify OsNPPR3 was responsible for the flo14 mutant phenotypes, we cloned a wild-type genomic fragment including the entire OsNPPR3 coding sequence into the pCUbi1390 binary vector, and then introduced the over-expression gene construct into the flo14 mutant. The expression levels of positive transgenic complementary lines were significantly higher than the control in mature seed endosperms (Fig. 3c). The seeds harvested from the transgenic positive T1 lines (FLO14+/+) were transparent (Fig. 3d) and could germinate and grow normally (Additional file 3: Figure S2), indicating that the developmental defects of embryo and endosperm in flo14 were completely rescued. Meanwhile, semi-thin sectioning indicated the starch granules of the transgenic complementary lines were also restored to the wild-type structure (Fig. 3e). Besides, OsNPPR3 knockout plants generated by the CRISPR/Cas9 system exhibited similar phenotypes as the mutant (Fig. 4). Collectively, the results suggested the P-type PPR is the causative gene of the flo14 mutant.

Expression Pattern Analysis of OsNPPR3 and Genes Associated with Starch Synthesis

Phylogenetic analysis indicated OsNPPR3 shared a high similarity with its homologs in other plants, including Zea mays, Sorghum bicolor, Setaria italic, Brachypodium distachyon, Aegilops tauschii subsp and Ananas comosus (Fig. 5a, Additional file 4: Figure S3). At the same time, temporal and spatial expression analyses showed that OsNPPR3 was universally expressed in various tissues (including roots, panicles, leaves and leaf sheaths) with the highest level in leaves. OsNPPR3 transcripts were accumulated gradually during endosperm development and peaked at 12 DAF (Fig. 5b). To confirm the results, we transformed a vector with the GUS reporter gene driven by the OsNPPR3 promoter into the rice. Histochemical analysis of GUS activity in independent transgenic plants corroborated that OsNPPR3 presented a constitutive expression pattern (Fig. 5c).

To determine whether the defective OsNPPR3 affected starch biosynthesis, we examined the expression profiles of major endosperm starch synthesis-related genes in developing endosperm by quantitative RT-PCR (qRT-PCR). Compared to the wild type, the expression levels of the tested genes were mostly changed in flo14 mutant. Remarkably, the expression levels of SS IIa, BE IIa, BE IIb, PHOL, and AGPS 2b were significantly down-regulated, whereas ISA I and ISA II was obviously up-regulated in the flo14 mutant (Fig. 5d). These results indicated that the starch accumulation was largely affected during endosperm development in the flo14 mutant.

Subcellular Localization of OsNPPR3

OsNPPR3 was predicted as a nucleus-targeted protein by the online tools TargetP (http://www.cbs.dtu.dk/services/TargetP/). To experimentally identify the predicted subcellular localization and the length and position of the nucleus-targeting signal in OsNPPR3 protein, a green fluorescent protein (GFP) fusion construct driven by the cauliflower mosaic virus 35S (CaMV-35S) promoter was generated, which contained a full-length OsNPPR3 coding region in front of the GFP protein and was named as OsNPPR3-GFP. Meanwhile, RPBF (Rice Prolamin Box Binding Factor) was employed as a nucleus-localized marker (Kawakatsu et al., 2009) and RPL23aB (r-Protein family member) as a nucleolus-targeted marker (Degenhardt and Bonham-Smith, 2008). All constructs were transiently expressed in both rice protoplasts (Fig. 6a, b) and tobacco leaves (Fig. 6c-e). The free GFP signals were diffused in the cytoplasm (Fig. 6a, c). By contrast, OsNPPR3-GFP fusion protein was localized in nucleus (Fig. 6b, d), and more accurately, in nucleolus because the signals were more similar to RPL23aB than RPBF (Fig. 6e). These results confirmed that OsNPPR3 protein is targeted to the nucleolus.

OsNPPR3 Affects Expression of Mitochondrial Genome-Encoded Genes and Mitochondrial Ultrastructure

PPR proteins are important for mitochondrial-encoded protein synthesis and mitochondrial function (Liu et al., 2013). The seed lethal phenotype of homozygous mutant suggested that the flo14 mutant might have defects in gene expression relating to mitochondria functions. For validation, expression analysis performed by qRT-PCR using RNA samples prepared from 12 DAF endosperms. Of 13 mitochondrial genome-encoded electron transport chain genes, three (including rps2, cyt c and ccmFc) were significantly up-regulated, while the others showed no difference between
the flo14 mutant and its wild type (Fig. 7a). Further, mitochondrial electron transport chain (ETC) complex proteins were isolated from developing endosperm and analyzed by western blotting. The flo14 mutant displayed an increase abundance of both the rps2 and cyt c bands, but a slight decrease in the ccmFc band compared to the wild-type profile (Fig. 7b), indicating that the accumulation of ETC components is disturbed in the flo14 mutant.
When an electron transport is defective in the cytochrome c pathway, alternative oxidases (AOXs) are generally activated to maintain the tricarboxylic acid cycle and electron transport (Vanlerberghe and Ordog, 2002). The expression of AOX genes in the flo14 mutant showed that an AOX (AOX 1a) was up-regulated 5-fold, whereas other two AOXs (AOX 1b and AOX 1c) demonstrated no significant change relative to the wild type (Fig. 7c), suggesting that the alternative respiratory pathway was activated to compensate for the mitochondrial dysfunction in the flo14 mutant.

Activation of the ETC is essential for the proper formation of the inner envelope cristae in mitochondria (Logan, 2006). Next, we performed transmission electron microscopic analysis to detect any morphological changes in mitochondria of developing endosperm cells. In contrast to the wild-type mitochondria that formed clear inner envelope cristae surrounded by a dense matrix, a large portion of mutant mitochondria lacked obvious cristae internal structure and the mitochondria matrix was extremely porous. Besides, the appearance of
the mutant mitochondria seemed irregular and more dilated than the wild type (Fig. 8).

The flo14 Mutant Is Defective in the Splicing of Mitochondrial Genome-Encoded Genes

Accumulating evidence shows that a large group of nuclear encoded PPR proteins were required for RNA splicing, editing, stability, maturation, and translation (Pfalz et al., 2009; Sosso et al., 2012; Li et al., 2014; Xiu et al., 2016; Wang et al., 2017). To determine whether the flo14 mutation affected RNA splicing of mitochondria genes, we carried out RT-PCR analysis using primers spanning exons of 27 mitochondrial genome-encoded transcripts and compared the lengths of the amplified products between the wild type and flo14 mutant plants. We found two transcripts, including nad 1–2 and nad 2,
were spliced with much reduced efficiency in the flo14 mutant compared to the wild type. In contrast, splicing of the other transcripts showed no significant difference between the flo14 mutant and its wild type (Fig. 9). This result illustrated that the flo14 mutation partially affected splicing of mitochondrial genome-encoded transcripts.

**Discussion**

Endosperm is a storage organ in which a massive amount of storage starch and storage proteins are accumulated during seed development (She et al., 2010). In rice, several key enzymes involved in starch biosynthesis have been well studied, including AGPase (ADP glucose pyrophosphorylase) (Muller-Rober et al., 1992), GBSS (granule-bound starch synthase) (Caballero et al., 2008), SS (soluble starch synthase) (Hirose and Terao, 2004), and starch branching enzyme (BE) (Mizuno et al., 1993). Through a series of endosperm defective mutants, such as floury, white-core or opaque kernel (Satoh and Omura, 1981), several regulatory genes associated with starch synthesis have been isolated. For example, FLO6 encodes a CBM containing protein, and its mutant showed decreased starch content and altered starch physicochemical features (Peng et al., 2014). FLO12 encodes an alanine aminotransferase, and the flo12 mutant exhibited loosely packed starch granules and a lower thousand kernel weight compared to its wild type. Semi-thin sections revealed that compound starch grains in flo12 interior endosperm cells were developed abnormally and amylose content was also decreased (Zhong et al., 2019). Besides, the rice FLO15 encodes a glyoxalase I. A growth arrest of the flo15 mutant amyloplasts was observed during the early grain-filling stage, causing the generation of abnormal amyloplasts lacking compound starch grains (You et al., 2019). In our study, the flo14

| A | B | C | D | E |
|---|---|---|---|---|
| GFP | Marker | Bright | Merged |
| GFP | Marker | Bright | Merged |
| OsNPPR3-GFP | Marker | Bright | Merged |
| OsNPPR3-GFP | Marker | Bright | Merged |
| OsNPPR3-GFP | Marker | Bright | Merged |

*Fig. 6* Subcellular localization of OsNPPR3. a-e Transient expression of 35S: OsNPPR3-GFP fusion protein located in the nucleolus of rice protoplasts (a, b) and Nicotiana tabacum protoplasts (c-e). Nucleus marker was used as a nucleus indicator (a, d). Nucleolus marker was used as a nucleolus indicator (e). RPBF (Rice Prolamin Box Binding Factor) and RPL23aB (r-Protein family member) were employed as nucleus-targeted and nucleolus-targeted marker, respectively. Scale bars: 1.6 μm in (a), 5 μm in (b, c), 10 μm in (d, e)
Fig. 7 Expression analysis of OsNPPR3 and genes associated with mitochondria. a Real-time RT-PCR analysis of the mitochondrial gene expression in endosperms of wild type (WT) and flo14 mutant (flo14) at 12 days after flowering (DAF). b Immunoblotting analysis of mitochondria related proteins in developing endosperm of wild type and flo14 mutant. c Real-time RT-PCR analysis of the gene expression in the wild type and flo14 mutant. Data are shown as means ± SD from three independent biological replicates and compared by Student’s t-test (* P < 0.05, ** P < 0.01). Actin1 was used as an internal control. rpl2: ribosomal protein L2, rpl6: ribosomal protein L6, rpl7: ribosomal protein L7, rps2: ribosomal protein S2, rps13: ribosomal protein S13, cox1: cytochrome c oxidase subunit 1, cyt c: cytochrome c, ccmF: cytochrome c biogenesis B, ccmFn: cytochrome c biogenesis Fn, ccmFc: cytochrome c biogenesis Fc, nad4: NADH dehydrogenase subunit IV, nad9: NADH dehydrogenase subunit XI, AOX 1a: alternative oxidase 1a, AOX 1b: alternative oxidase 1b, AOX 1c: alternative oxidase 1c

Fig. 8 Transmission electron micrographs of mitochondria. Transmission electron micrographs of mitochondria from 12 days after flowering (DAF) wild-type (WT) (a) and flo14 mutant (flo14) (b) endosperms. Scale bars: 1 μm in (a) and (b)
mutant showed a similar chalky endosperm phenotype to the former endosperm mutants (Fig. 1). The SEM and semi-sectioning results showed that starch granules in the grains of the mutant were compromised (Fig. 2a-h). Meanwhile, total starch content of flo14 mutant was significantly lower than that in the wild type and the amyllose content was slightly reduced (Fig. 2i, j). These results indicated that mutation of flo14 dramatically affects starch synthesis in rice endosperm.

PPR is a large protein family implicated in organellar gene expression, including RNA editing, intron splicing, RNA processing, RNA maturation, RNA stability, and translation initiation (Rivals et al., 2006). Previous studies isolated and characterized numerous PPR genes such as THYLAKOID ASSEMBLY 8 (Khrouchtchova et al., 2012), PPR4 (Schmitz-Linneweber et al., 2006) and PPR5 (Beick et al., 2008), and they were required of splicing of ycf3–2, rps12 and trnG-UCC precursor, respectively. By map-based cloning, we found that the single nucleotide replacement occurred at the exon of Os03g0728200, leading to a premature termination of transcription (Fig. 3a, b). Os03g0728200 encoded a P-type subfamily PPR protein with ten PPR motifs and its function was not yet revealed before. Seed harvested from independent complementary positive T1 lines showed similar phenotypes to the wild type (Fig. 3d, e), whereas lose-of-function of OsNPPR3 by CRISPR technology mimicked the mutant grain phenotypes (including the chalky endosperm and seed lethality). Considering the mutagenesis sites were near to the start codon, the protein of CRISPR/Cas9 mutant was supposed to be functionally missed in the plant. Meanwhile, little transcript was detected in the flo14 mutant. The results suggested the flo14 mutant might also carry a functionally defective OsNPPR3 protein, just like the CRISPR/Cas9 mutants. Collectively, these results strongly supported that OsNPPR3 plays a crucial role in starch granules development and seed vigor in rice.

Up to now, only limited PPR proteins have been reported to localize in nucleus or nucleolus. In Arabidopsis thaliana, GRP23 (Glutamine-Rich Protein 23) is a nucleus-localized PPR protein that functions as a potential regulator of gene expression during early embryogenesis.

![Fig. 9 The flo14 mutant is defective in the splicing of mitochondria genes. Gene transcripts are labeled at the left. Spliced (S) and unspliced (U) transcripts are shown at the right. RNA were extracted from 12 days after flowering (DAF) endosperms of wild type (WT) and flo14 mutant (flo14). The splicing genes are tagged with red boxes. UBQ was used as a quantitative control. Nad 1–1: NADH dehydrogenase subunit 1–1, nad 1–2: NADH dehydrogenase subunit 1–2, nad 2: NADH dehydrogenase subunit 2, nad 2–1: NADH dehydrogenase subunit 2–1, nad 2–2: NADH dehydrogenase subunit 2–2, nad 3: NADH dehydrogenase subunit 3, nad 4–1: NADH dehydrogenase subunit 4–1, nad 4–2: NADH dehydrogenase subunit 4–2, nad 5: NADH dehydrogenase subunit 5, nad 6: NADH dehydrogenase subunit 6, nad 7: NADH dehydrogenase subunit 7, nad 9: NADH dehydrogenase subunit 9, atp 1: ATP synthase F0 subunit 1, atp 4: ATP synthase F0 subunit 4, cox1: cytochrome c oxidase subunit 1, cox 2: cytochrome c oxidase subunit II, cox 3–1: cytochrome c oxidase subunit 3–1, cox 3–2: cytochrome c oxidase subunit 3–2, ccmFc: cytochrome c biogenesis Fc (two pairs of primers were used), rpl 2: 50S ribosomal protein L2, rpl 5: ribosomal protein L5, rps 2: 30S ribosomal protein S2, rps 3: 30S ribosomal protein S3, orf X: hypothetical protein, cyt b: cytochrome b.](image-url)
sions of three mitochondrial-genome encoded genes were tightly related to mitochondrial function, including RF5. Moreover, a number of other PPR proteins were reported in maize (Dai et al., 2018). These studies promoted us to study mitochondrial genes in rice, as well as the mutant with defective PPR protein OsNPPR1 (encoding by FLOURY AND GROWTH RETARDATION1 [FGRI1] locus) was revealed to affect splicing of six nuclear genes, many of which were related to mitochondrial functions (Hao et al., 2019). Similar to flo14, the osnppr1/fragr1 mutant produced an opaque grain appearance with loosely arranged starch grains, decreased starch and amylose contents in endosperm. Likewise, osnppr1 also displayed reduced grain weight and weaker seed germination vigor relative to its wild type. However, the seedlings from homologues osnppr1 seeds gradually recovered and grew into normal adult plants. Although the osnppr1 plants exhibited significantly slower grain filling after fertilization, they could produce fertile seeds. In addition, OsNPPR1 was revealed to be nucleus-localized (Hao et al., 2019), instead of nucleolus-localization as shown in OsNPPR3 (Fig. 6). These results suggested OsNPPR1 and OsNPPR3 might play divergent functions for seed development.

Loss of OsNPPR3 function impairs mitochondria structure and is lethal for the embryo. Mitochondria are semi-autonomous organelles that have arisen from an endosymbiotic event (Gray et al., 2001). Although mitochondria have a complete gene expression machinery, the vast majority of mitochondrial proteins are encoded in the nucleus and have to be imported into mitochondria from the cytosol (Burger et al., 2003). Thus, mitochondrial biogenesis relies heavily on the coordinated expression of nuclear encoded genes (Hammani et al., 2011). Meanwhile, mitochondria are cytoplasmic organelles functioning as the factory for energy processing. Seed-lethal and chalky endosperm (a kind of poor grain filling) phenotypes implied some problems might happen in cell energy provision. In addition, the flo14 phenotypes mutant was very similar to rice agr1 (opaque and growth retardation1) mutant with defective PPR protein (Kim et al., 2009). Moreover, a number of other PPR proteins were reported to tightly related to mitochondrial function, including RF5 (RESTORER-OF FERTILITY GENES, Hu et al., 2006) and RF6 (RESTORER-OF FERTILITY GENES6, Huang et al., 2015) in rice, as well as DEK37 (DEFECTIVE KERNEL 37) in maize (Dai et al., 2018). These studies promoted us to study mitochondrial genes in flo14. We found the expressions of three mitochondrial-genome encoded genes were significantly altered in the flo14 mutant (Fig. 7a). These results suggested that mitochondrial genes might be impaired in the mutant due to the mutation of OsNPPR3 gene. Similar phenotypes were observed in PpPPR_71 mutant, with the gene encoded a polypeptide of 833 amino acid with 17 PPR motifs and the C-terminal DYW domain (Tasaki et al., 2010). In the flo14 mutant, both rps2 and Cyt c gene expressions and their encoded proteins were consistently up-regulated. Intriguingly, the gene expression level of ccmFc was down-regulated, while its protein abundance was obviously accumulated compared with the wild type (Fig. 7b). This discrepancy may be due to a feedback regulation or post-transcriptional modification.

RNA splicing is a process of removing introns between neighboring exons during translation (Wu et al., 2012). Several RNA binding factors have important roles in RNA splicing in higher plants including PPR proteins and other nuclear-encoded factors (de Longevialle et al., 2010). A small number of PPR proteins have been discovered to be involved in RNA splicing in mitochondria. In maize, dek2 (defective kernel 2) is a classic mutant with small kernels and delayed development (Qi et al., 2016). Mitochondrial transcript analysis indicated that dek2 mutation caused reduced splicing efficiency of mitochondrial nad1 intron 1. Mitochondrial complex analysis in dek2 immature kernels showed severe deficiency of complex I assembly. EMP10 (EMPTY PERICARP10) is specifically required for the cis-splicing of mitochondrial nad2 intron 1, embryogenesis and endosperm development (Cai et al., 2017). Likewise, DEK37s involves in cis-splicing of mitochondrial nad2 intron 1 and is required for mitochondrial function and seed development in maize (Dai et al., 2018). In our study, the flo14 mutant caused the defects in the splicing of nad 1–2 and nad 2 (Fig. 9), similar to the abovementioned mutants. The NAD 1 and NAD 2 are two of the nine complex I proteins encoded by the mitochondria genome (Brandt, 2006). The splicing defect of certain nad mRNAs was reported to result in partial or complete loss of ETC complex I activity as well as an obvious disturbance of growth and development (Cai et al., 2017). It is plausible that OsNPPR3 might be associated with the fine-tuning of transcript levels in the nucleus for mitochondrial biogenesis. Further studies will be needed to address this hypothesis in the future.

Conclusions

We identified a mutant of flo14, which exhibited a floury endosperm and embryo-lethal phenotypes. Map-based cloning revealed that OsNPPR3 encodes a nucleolus-localized P-family PPR protein that was validated by transgenic complementation and CRISPR experiments. The role of OsNPPR3 in rice seed development was tightly related with mitochondrial ultrastructure as well as RNA splicing of mitochondrial genome-encoded genes. Our research results provide useful information
for the future research to uncover the regulatory mechanism of PPR proteins during rice seed development.

**Methods**

**Plant Material and Growth Conditions**
The flo14 mutant was selected from a chemical mutagenic bank of japonica variety Dianjingyou1. An F2 population was produced from a cross between the flo14 mutant and indica cultivar N22 for mapping. The mutagenic bank was produced by the National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University. Plants were grown under natural and greenhouse conditions.

**Scanning Electron Microscope**
Wild-type and flo14 mutant seeds were transversely cut and examined with a HITACHI S-3400 N scanning electron microscope (http://www.hitachi-hitec.com). Scanning electron microscopy was performed as described in the previous article (Kang et al., 2005).

**Light Microscope**
For the observation of compound granules, transverse sections of developing endosperms (approximately 1 mm in thickness) from the wild-type and flo14 mutant seeds were fixed overnight in 0.1 M phosphate buffer (pH 7.2) with 2% (v/v) glutaraldehyde and 2% (w/v) paraformaldehyde. After dehydration in an ethanol series, samples were embedded in LR White resin (London Resin, Berkshire, UK, http://www.2spi.com/), followed by sectioning with an ultramicrotome (Leica UC7; http://www.leica-microsystems.com). Semi-thin sections were stained with I2-KI for 5 s and subsequently examined under a light microscope (80i, Nikon, http://www.nikon.com).

**Transmission Electron Microscope**
Wild-type and flo14 mutant endosperm samples were obtained from 12 DAF developing plants, which were grown under natural conditions. Endosperm sections were fixed in 2.5% glutaraldehyde in a phosphate buffer at 4 °C for 4 h, then washed and incubated in 1% OsO4 at 4 °C for 12 h. After dehydration in a gradient ethanol series, samples were embedded in Spurr's resin prior to ultrathin sectioning. Sections were stained with uranyl acetate and examined with a Hitachi-H7650 transmission electron microscope.

**Analysis of the Starch Characters of Endosperm**
The starch content of the rice flour was measured with a starch assay kit (Megazyme, Wicklow, Ireland, http://www.megazyme.com/), according to the manufacturer's protocol. Amylose content was assessed following the method described by Liu et al. (2009).

**Mapping Cloning of flo14**
To identify markers linked to OsNPPR3, we used flo14 mutant, N22, 10 F2 plants with homozygous mutant alleles and 100 polymorphic SSR markers. OsNPPR3 was mapped to a 165-kb region with newly developed markers (Additional file 5: Table S2). We obtained the genomic sequences of candidate gene Os03g0728200 from both the mutant and wild-type genome DNAs.

**Complementation Test**
We cloned a wild-type genomic fragment including a 1.5 kb native promoter and entire OsNPPR3 coding sequence into the pCUbi1390 binary vector, and then introduced the complementary gene construct into the flo14 mutant calli. The primers were listed in Additional file 6: Table S3. The transgenic plants were grown in a greenhouse.

**CRISPR/Cas9 System**
One target site that was 20 bp upstream of the protoscaler-adjacent motif sequence (PAM) was designed for Os03g0728200. The combined target sequences were introduced to the gRNA-U3 and gRNA-U6 vectors, followed by two rounds of nested PCR with primers listed in Additional file 6: Table S3. The PCR products were subsequently linked to a CRISPR/Cas9 vector, and the whole construction was verified by PCR and sequencing. Calli derived from japonica variety Dianjingyou1 were used for Agrobacterium-mediated transformation. The original chromatogram files have been shown in Additional files 7, 8 and 9: Chromatograms S1 to S3. The knockout lines were grown in the greenhouse.

**Total RNA Extraction and Real-Time RT-PCR Analysis**
Total RNA was extracted using an RNA prep pure Plant kit (TIANGEN Biotech, Beijing, China, http://www.tiangen.com/en/). A 1-μg portion of total RNA was reverse-transcribed by priming with oligo (dT18) in a 20 μg reaction based on the Prime Script Reverse Transcriptase kit (TaKaRa, http://www.takara-bio.com). The value of Actin1 mRNA (accession number AK100267) was used as an internal control. The primers were listed in Additional file 10: Table S4 for detecting starch synthesis-related genes, Additional file 11: Table S5 for genes in mitochondria, and Additional file 12: Table S6 for genes associated with splicing in mitochondria.

**Histochemical Staining of GUS Expression**
For GUS-staining assay, plant tissues were cut into small segments of approximately 2 cm2. GUS-staining assay was performed as described by Jeon et al. (Jeon et al., 2000). After staining, all samples were fixed in formalin-alcohol-acetic acid (FAA) fixative solution and embedded in paraffin (Kang et al., 2002). The samples were
sectioned to a 12 thickness and observed with the microscope under dark-field illumination.

Subcellular Localization
The OsNPPR3 gene sequences were cloned in frame in front of the GFP coding region in the PAN580-GFP vector and pCMBIA1305 binary vector to create OsNPPR3-GFPs under the control of the CaMV 35S promoters. The constructs were introduced into rice protoplasts separately and Nicotiana tabacum protoplasts. GFP fluorescent signals were detected using a confocal laser scanning microscope (Zeiss LSM710). The methods refer to the literature reported before (Chen et al., 2006).

Protein Extraction and Western Blotting Analysis
Proteins were extracted from 0.5 g samples of developing seeds (12 DAF). Tissues were separately homogenized with a mortar and pestle in an extraction buffer of 100 mM Tris–HCl (pH 7.0), 2% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, and 10% (v/v) glycerol. The resultant extracts were heated at 100°C for 5 min, then centrifuged at 13,000 g for 20 min, and then recover the supernatants. The proteins were fractionated by SDS-PAGE and electrophoresis onto a nitrocellulose membrane using a buffer system consisting of 25 mM Tris-base, 144 mM glycine, 20% (v/v) methanol, and 0.1% (v/v) SDS. The membrane was incubated with a rabbit anti OsNPPR3 serum. OsNPPR3 was then detected on X-ray film using an HRP-conjugated mouse anti rabbit IgG secondary antibody and ECL western blotting detection reagents according to the manufacturer’s protocol (Kang et al., 2005).

Statistical Analysis
All statistical analyses were conducted using Excel 2010 (Microsoft Corp., Redmond, WA, USA).

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12284-019-0359-x.

Additional file 1: Figure S1. Comparison of starch physicochemical features.
Additional file 2: Table S1. The genetic analysis of flo14 mutant.
Additional file 3: Figure S2. Young seedlings of wild type and the complementation of the flo14 mutant.
Additional file 4: Figure S3. Comparison of amino acid sequences of the Os03g0728200 protein with its close species.
Additional file 5: Table S2. Markers used for OsNPPR3 mapping in this study.
Additional file 6: Table S3. Primers used for vector construction.
Additional file 7: Chromatogram S1. The chromatogram for the wild type of target site.
Additional file 8: Chromatogram S2. The chromatogram for the transgenic line CR9–1 of target site.

Additional file 9: Chromatogram S3. The chromatogram for the transgenic line CR9–2 of target site.
Additional file 10: Table S4. Primers used for gene expression associated with starch synthesis.
Additional file 11: Table S5. Primers for gene expression in mitochondria.
Additional file 12: Table S6. Primers for genes associated with splicing in mitochondria.

Abbreviations
AGPase: ADP glucose pyrophosphorylase; AGPL: ADP-glucose pyrophosphorylase large subunit 2; AGL2: ADP-glucose pyrophosphorylase large subunit 2; AGP2: ADP-glucose pyrophosphorylase 2b; AOX1a: Alternative oxidase 1a; AOX1b: Alternative oxidase 1b; AOX1c: Alternative oxidases; ATP synthase F0 subunit 1; ATP synthase F0 subunit 4; BAC: Bacterial artificial chromosomes; BE1a: Branching enzyme 1a; BE1b: Branching enzyme 1b; BE: Branching enzyme; CaMV-35S: Cauliflower mosaic virus 35S; ccmB: cytochrome c oxidase; ccmF: cytochrome c oxidase; ccmG: cytochrome c oxidase; Cox: cytochrome c oxidase subunit 1; Cox1: cytochrome c oxidase subunit 2; cto: Cytochrome c oxidase; cyb: cytochrome b; DAF: Days after flowering; DEK2: DEFECTIVE KERNEL2; DEK3: DEFECTIVE KERNEL3; EMP10: EMPTY PERICARP 10; ETC: Electron transport chain; flo: floury endosperm; GBSS: Granule-bound starch synthase; GFP: Green fluorescent protein; GP23: Glutamine-Rich Protein 23; ISA1: Isoamylase I; ISA2: Isoamylase II; ISU1: Isu 1; AT1G09100: Cytochrome c oxidase subunit 1; ISU2: Isu 2; ATP synthase F0 subunit 1; ISU3: Isu 3; ATP synthase F0 subunit 4; ISU4: Isu 4; NADH dehydrogenase subunit; ISU5: Isu 5; NADPH dehydrogenase subunit; ISU6: Isu 6; OAR: Oat4; OAT4: Oat4; OAT6: Oat6; OAT8: Oat8; P013: Phytochrome B; PAM: Protospacer-adjacent motif; PHOL: Phyllocholine phosphatase 1; PNC1: Protein Norman localization to the nucleus and mitochondria; PPD2: Pyruvate phosphate dikinase 2; PPD2: Pyruvate phosphate dikinase 2; PPD8: Pyruvate phosphate dikinase 8; PPR: Pentatricopeptide repeat; PUL: Pulvinula; qRT-PCR: quantitative RT-PCR; RFR1: RESTORER-OF FERTILITY GENE 1; RFR2: RESTORER-OF FERTILITY GENE 2; RFR3: RESTORER-OF FERTILITY GENE 3; RPS10: Rice Protein Box Binding Factor; rpl: ribosomal protein; RVA: Rapid visco analyzer; SEM: Scanning electron microscope; SS: Soluble starch synthase; SUS: UTP-glucose-1-phosphate-uridylyl-transferase; WT: Wild type.

Acknowledgements
This research was supported by Key Laboratory of Biology, Genetics and Breeding of Japonica Rice in Mid-lower Yangtze River, Ministry of Agriculture, P. R. China, Jiangsu Collaborative Innovation Center for Modern Crop Production.

Authors’ Contributions
JW, YW and LL provided the mutant and made the overall design and supervision of this study. MX, YY and LZ performed the experiments. MX drafted the manuscript and LL overhauled the manuscript completely. All authors read and approved the manuscript.

Funding
This research was supported by the grants from The National Key Research and Development Program of China (2017YFD0100401), a project (2016ZX08001006) from the Ministry of Agriculture of China for transgenic research, Jiangsu Science and Technology Development Program (BE2017368), and Key project for Jiangsu Agricultural New Variety Innovation (JZC201701), NIFSC (31571639) and the Fundamental Research Funds for the Central Universities (KYZZ201601).

Availability of Data and Materials
The materials used and/or analyzed during the current study are available from the corresponding author on request.

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
Not applicable.
Competing Interests
The authors declare that they have no competing interests.

Received: 22 July 2019 Accepted: 19 December 2019
Published online: 30 December 2019

References
Barkan A, Rojas M, Fuji S, Yap A, Chong YS, Bond CS, Small I (2012) A combinatorial amino acid code for RNA recognition by pentatricopeptide repeat proteins. PloS Genet 8:e1002910
Beck S, Schmitz-Linneweber C, Williams-Carrier R, Jensen B, Barkan A (2008) The pentatricopeptide repeat protein PPR5 stabilizes a specific tRNA precursor in maize chloroplasts. Mol Cell Biol 28:5337–5347
Brandt U (2006) Energy converting NADH: quinone oxidoreductase (complex I). Annu Rev Biochem 75:69–92
Burger G, Gray MW, Lang BF (2003) Mitochondrial genomes: anything goes. Trends Genet 19:709–716
Burrell MM (2003) Starch: the need for improved quality or quantity—an overview. J Exp Bot 54:451–456
Caballero L, Bancel E, Debton C, Branlard G (2008) Granule-bound starch synthase gene family in rice (Oryza sativa). Plant Physiol 147:1281–1295
Dai DW, Luan SC, Chen XZ, Wang Q, Feng Y, Zhu CG, Qi WW, Song RT (2018) Mitochondrial function and mitochondrial pyruvate orthophosphate dikinase gene (OsPPDKB). Plant J 92:1091–1109
Kawakatsu T, Yamamoto MP, Tourou SM, Yasuda H, Takaiwa F (2009) Compensation and interaction between RIS2E1 and RPBF during grain filling in rice. Plant J 59:908–920
Khrouchtcheva A, Monde RA, Barkan A (2012) A short PPR protein required for the splicing of specific group II introns in angiosperm chloroplasts. RNA 18:1197–1209
Kim SR, Yang JH, Moon S, Ryu CH, An K, Kim KM, Yim J, An G (2009) Rice OGR1 encodes a pentatricopeptide repeat-DWy protein and is essential for RNA editing in mitochondria. Plant J 59:738–749
Li XJ, Zhang YZ, Hou MM, Sun F, Shen Y, Xiu ZH, Wang XM, Chen ZL, Sun SSM, Small I, Tan BC (2014) Small kernel 1 encodes a pentatricopeptide repeat protein required for mitochondrial nad7 transcript editing and seed development in maize (Zea mays) and rice (Oryza sativa). Plant J 79:797–809
Li XL, Huang WL, Yang HH, Jiang RC, Sun F, Wang HC, Zhao J, Xu CH, Tan BC (2018) EMP18 functions in mitochondrial apr6 and cot2 transcript editing and is essential to seed development in maize. New Phytol 212:896–907
Liu LL, Mu XD, Liu SJ, Zhu CL, Jiang L, Wang YH, Shen Y, Ren YL, Dong H, Chen LM, Liu X, Zhao ZG, Zhai XH, WM JN (2009) Identification and characterization of a novel Waxy allele from a Yunnan rice landrace. Plant Mol Biol 71:699–706
Liu YJ, Xiu ZH, Meeley R, Tan BC (2013) Empty Pericarp5 encodes a pentatricopeptide repeat protein that is required for mitochondrial RNA editing and seed development in maize. Plant Cell 25:886–888
Logan DC (2006) The mitochondrial compartment. J Exp Bot 57:1225–1243
Long WH, Dong BN, Wang YH, Pan YW, Wang YL, Liu LL, Chen XL, Liu X, Liu SJ, Tan YL, Chen LM, Wan WM (2017) FLOWERING LOCUS ENDOPERM6 encodes the UDP-glucose pyrophosphorylase 1, affects the synthesis and structure of starch in rice endosperm. J Plant Physiol 205:513–522
Lurin C, Anders C, Aubourg S, Bellauoi M, Bittton F, Bruey C, Caboche M, Debast C, Gualberto J, Hoffmann B, Ret ML, Martin-Magniette M, Mireau H, Peeters N, Renou J, Szurke T, Taconnat L, Small I (2004) Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. Plant Cell 16:2089–2103
Martin C, Smith AM (1995) Starch biosynthesis. Plant Cell 7:971–985 Review
Mizuno K, Kawasaki T, Shimada H, Sato H, Kobayashi E, Okumura S, Arai Y, Baba T (1993) Alteration of the structural properties of starch components by the lack of an isoform of starch branching enzyme in rice seeds. J Biol Chem 268(25):19084–19099
Muller-Rober B, Sonnewald U, Willmitzer L (1992) Inhibition of the ADP–glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. EMBO J 11:1229–1238
Nishio T, Iida S (1993) Mutant having a low content of 16-kDa allergenic protein in rice (Oryza sativa L.). Theor Appl Genet 83:445–453
Peng C, Wang YH, Liu F, Ren YL, Zhou RN, Lv J, Zheng M, Zhao SL, Zhang L, Wang CM, Jiang L, Zhang X, Guo XP, Bao YQ, Wan JM (2014) FLOWERING ENDOSPERM6 encodes a B48 domain-containing protein involved in compound granule formation and starch synthesis in rice endosperm. Plant J 77(6):917–930
Pfalz J, Bayraktar OA, Priekyl J, Barkan A (2009) Site-specific binding of a PPR protein defines and stabilizes S′ and 3′ mRNA termini in chloroplasts. EMBO 28:2042–2052
Qi WW, Yang Y, Feng XZ, Zhang ML, Song RT (2016) Mitochondrial function and maize kernel development requires Dek2, a Pentatricopeptide repeat protein involved in nad1 RNA splicing. Genetics 201(2):239–249
Rivals E, Bruyere C, Toffano-Nioche C, Lecharny A (2006) Formation of the Arabidopsis pentatricopeptide repeat family. Plant Physiol 141:825–835
Ryoo N, Yu C, Park CS, Baik MY, Park IM, Cho MH, Bho SH, An G, Hahn TR, Jeon JS (2007) Knockout of a starch synthase gene OsSSIIIa/IIIb causes white-corneous endosperm in rice (Oryza sativa L.). Plant Cell Rep 26:1083–1090
Sato H, Omura T (1981) New endosperm mutations induced by chemical mutagen in rice, Oryza sativa L. Breed 31:316–326
Schmitz-Linneweber C, Williams-Carrier RE, Williams-Voelker PM, Kroeger TS, Vickas A, Barkan A (2006) A pentatricopeptide repeat protein facilitates the
trans-splicing of the maize chloroplast rps12 pre-mRNA. Plant Cell 18:2650–2663
She KC, Kusano H, Koizumi K, Yamakawa H, Hakata M, Imamura T, Fukuda M, Naito N, Tsumuraki Y, Yaeshima M, Tsuge T, Matsumoto K, Kudoh M, Itoh E, Kikuchi S, Kishimoto N, Yazaki J, Ando T, Yano M, Aoyama T, Sasaki T, Satoh H, Shimada H (2010) A novel factor FLOURY ENDOSPERM2 is involved in regulation of rice grain size and starch quality. Plant Cell 22:3280–3294
Sosso D, Mbelo S, Vernoud V, Gendrot G, Dedieu A, Chambrier P, Dauzat M, Heurtévin L, Guyon V, Takenaka M, Rogowsky PM (2012) PPR2263, a DYW-subgroup pentatricopeptide repeat protein, is required for mitochondrial nad5 and cob transcript editing, mitochondrial biogenesis, and maize growth. Plant Cell 24:676–691
Takenaka M (2010) MEF9, an E-subclass pentatricopeptide repeat protein, is required for an RNA editing event in the nad7 transcript in mitochondria of Arabidopsis. Plant Physiol 152:939–947
Tasaki E, Hattori M, Sugita M (2010) The moss pentatricopeptide repeat protein with a DYW domain is responsible for RNA editing of mitochondrial ccmFc transcript. Plant J 62:560–570
Vanlerberghe GC, Ordog SH (2002) Alternative oxidase: integrating carbon metabolism and electron transport in plant respiration. AIPH 12:173–191
Wang Y, Ren YL, Zhou KN, Liu LL, Wang JL, Xu Y, Zhang H, Zhang L, Feng ZM, Wang LW, Ma WW, Yunlong Wang YL, Guo XP, Hang X, Lei C, Cheng ZJ, Wan JM (2017) WHITE STRIPE LEAF4 encodes a novel P-type PPR protein required for chloroplast biogenesis during early LEAF development. Front Plant Sci 8:1116
Wu N, Matand K, Wu HU, Li BN, Li B, Majsterek I, Stoutermire B, Wu YF (2012) Natural rules for Arabidopsis thaliana pre-mRNA splicing site selection. Cent Eur J Biol 7(4):620–625
Xiu ZH, Sun F, Shen Y, Zhang XY, Jiang RC, Bonnard G, Zhang JH, Tan BC (2016) EMPTY PERICARP16 is required for mitochondrial nad2 intron 4 cis-splicing, complex I assembly and seed development in maize. Plant J 85:507–519
You XM, Zhang WW, Hu JL, Jing RN, Cai Y, Feng ZM, Kong F, Zhang J, Yan HG, Chen WW, Chen XG, Ma J, Tang XJ, Wang P, Zhu SS, Liu LL, Jiang L, Wan JM (2019) FLOURY ENDOSPERM15 encodes a glyoxalase I involved in compound granule formation and starch synthesis in rice endosperm. Plant Cell Rep 38:345–359
Zhang L, Ren YL, Lu BY, Yang CY, Feng ZM, Liu Z, Chen J, Ma WW, Wang Y, Yu XY, Wang YL, Zhang WW, Wang YH, Liu SJ, Wu FQ, Zhang X, Guo XP, Bao YQ, Jiang L, Wan JM (2016) FLOURY ENDOSPERM7 encodes a regulator of starch synthesis and amyloplast development essential for peripheral endosperm development in rice. J Exp Bot 67(3):633–664
Zhang YF, Suzuki M, Sun F, Tan BC (2017) The mitochondrion-targeted PENTATRICOPEPTIDE REPEAT78 protein is required for nad5 mature mRNA stability and seed development in maize. Mol Plant 10:1321–1333
Zhong MS, Liu X, Liu F, Ren YL, Wang YL, Zhu JP, Teng X, Duan EC, Wang F, Zhang H, Wu MM, Hao YY, Zhu XP, Jing RN, Guo XP, Jiang L, Wang YH, Wan JM (2019) FLOURY ENDOSPERM12 encoding alanine aminotransferase 1 regulates carbon and nitrogen metabolism in rice. J Plant Biol 62:61–73

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:
▶ Convenient online submission
▶ Rigorous peer review
▶ Open access: articles freely available online
▶ High visibility within the field
▶ Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com