Brassica rapa Sec14-like protein gene BrPATL4 determines the genetic architecture of seed size and shape

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Abstract Seed size traits are controlled by multiple genes in crops and determine grain yield, quality and appearance. However, the molecular mechanisms controlling the size of plant seeds remain unclear. We performed functional analysis of BrPATL4 encoding Sec14-like protein to determine the genetic architecture of seed size, shape and their association analyses. We used 60 T3 transgenic rice lines to evaluate seed length, seed width and seed height as seed size traits, and the ratios of these values as seed shape traits. Pleiotropic effects on general architecture included small seed size, erect panicles, decreased grain weight, reduced plant height and increased sterility, which are common to other mutants deficient in gibberellic acid (GA) biosynthesis. To test whether BrPATL4 overexpression is deleterious for GA signal transduction, we compared the relative expression of GA related gene and the growth rate of second leaf sheath supplied with exogenous GA3. Overexpression of BrPATL4 did not affect GA biosynthesis or signaling pathway, with the same response shown under GA treatment compared to the wild type. However, the causal genes for the small seed phenotype (D1, SRS1, and SRS5) and the erection of panicles showed significantly decreased levels in mRNA accumulation compared to the wild type. These results suggest that the overexpression of BrPATL4 can control seed size through the suppression of those genes related to seed size regulation. Although the molecular function of BrPATL4 is not clear for small seed and erect panicles of BrPALT4 overexpression line, this study provides some clues about the genetic engineering of rice seed architecture.

Keywords Brassica rapa, BrPATL4, Sec14-like protein, small seed, erect panicle

Introduction In crop plants, high yield is an important agronomic trait as well as biotic/abiotic stress resistance and grain quality. To date, many studies were done to improve the agronomic traits using various approaches (reviewed by Takeda and Matsuoka 2008). Traditional plant breeding is very effective to make an improved crop variety without any engineered modification in the genome. Additionally, the new elite cultivars originated from the classical breeding method for introducing selected trait can be directly released to the market without further investigation on the possible effects in humans and the environment. However, because of the recessive nature of most genes, the fixation of desired traits of most of breeding lines derived from the crosses between parental lines requires a long period of time. Recently, the introduction of foreign genes originated from intra-species into the interested organisms which are agronomically important crops have been made to improve morphological characteristics and this approach can introduce new agronomic traits which was never been done before. Also, it is possible to select faster with lower cost because the introduction of foreign gene generally uses strong constitutive expression promoter or enhancers which makes the dominant gain-of-function mutant. The main purpose of
crop plants is to supply food, thus, the improvement of crop yield is widely used as a goal of researchers. There are many factors determining crop productivity such as grain yield, cultivation techniques, and environmental condition. Of these, the grain yield is mainly determined by number of panicles, number of grains per panicle, and grain weight. Takeda and Matsuoka (2008; Xing and Zhang 2010). Among them, number of panicles and number of grains per panicle are dominated by the differentiation of lateral organs during floral transition such as tillers and branches except grain weight which is determined by grain size. The increase of grain weight is proportional to length, width, and thickness of grain (Xing and Zhang 2010). To date, there have been several reports about causal genes regulating the seed elongation. Gibberellin (GA) insensitive mutant, Dwarf1 (d1) has mutation in the α-subunit of the heterotrimeric G protein affecting GA signaling, leading to the reduction of cell length in longitudinal direction in seeds (Ashikari et al. 1999; Ueguchi-Tanaka et al. 2000). DWARF11 (D11) encodes a novel cytochrome P450 (CYP724B1) which is involved in brassinosteroid (BR) and shows reduced seed length (Tanabe et al. 2005). dense and erect panicle 2/SMALL AND ROUND SEED 1 (DEP2/SRS1) encoding a plant specific protein without any known functional domain showed wider and shorter grain than the wild type (Abe et al. 2010; Li et al. 2010). SRS3 and SRS5 encode a kinesin 13 protein (Kitagawa et al. 2010) and an α-tubulin in rice, respectively (Segami et al. 2012). In contrast, the constitutive overexpression of OsmiR397 induces the increase of grain size through the posttranscriptional regulation of OsLAC, while most of causal genes related in seed elongation led to the reduction of seed length (Zhang et al. 2013).

In this study, we isolated a single mutant, Br322 bearing erect panicles and small seeds from the screening of B. rapa Full-length cDNA Over-expresser (FOX) gene hunting library (Abdula et al. 2013). Br322 encodes a B. rapa PATLLIN 4 (BrPATL4) and showed pleiotropic phenotypes including small and round seed, erect panicles, and dwarfism. Although, it has been reported that PATLs have functions during plant cytokinesis or viral protein movement (Peterman et al. 2004; Peiro et al. 2014), there is no report yet about its regulation of seed size. This study will provide some clues about the genetic engineering of rice seed architecture.

Materials and Methods

Plant Materials and Growth Condition

Rice variety Gopum was used as a wild type in the generation of transgenic rice. Regenerated plants were transplanted in soil (50% compost and 50% earth soil) under greenhouse condition and acclimatized for two weeks. Young leaf samples were collected for genomic DNA analysis with BrPATL4-specific primers. Subsequently, plants confirmed to contain the gene insert were harvested, and the T1 seeds were used in the next planting season in the field. Transgenic rice plants together with the wild type were sown and grown up to T3 generation (Sun et al. 2011). At vegetative stage, the young leaf tissue of five plants in each line was collected for DNA extraction and analysis. All transgenic plants used in the experiments were from T3 generation.

Isolation of gene, vector construction and plant transformation

From the large FOX rice lines we previously developed and screened for abiotic stresses tolerant line (Abdula et al. 2013). In this study, BrPATL4 was selected showing small seed, erect panicle, and dwarfism. The BrPATL4 full-length complementary DNA (cDNA) was ligated into the pSB11 vector (Komari et al. 1996). The coding sequence including untranslated regions (UTR) of both 5' and 3' of BrPATL4 gene was constructed under the control of ubiquitin-1 promoter and NOS terminator for constitutive expression (Fig. 5A) and transformed into rice by Agrobacterium-mediated method with modification (Lee et al. 2011).

Southern blot analysis of transgenic plants

Southern blot analysis was conducted to determine the copy number of transgene. HPT probe was synthesized using PCR DIG Probe Synthesis Kit (Roche Molecular Biochemicals, USA) according to the manufacturer’s instructions. The forward/reverse primers used for the probe synthesis were 5'-GGATT CAGCCATCGGTCCAGA-3'. Aliquots of DNA (10 µg) were digested overnight individually with EcoRI, BamHI, HindIII, and SacI at 37°C, fractioned by 1% agarose gel electrophoresis and transferred to a positively charged nylon membrane (Amersham Biosciences, USA). DIG-labeled probe was then added (2 µL of probe/mL of buffer) and hybridization was carried out overnight at 42°C. The DIG-labeled DNA was detected based on manufacturer’s manual (DIG Nucleic Acid Detection Kit; Roche Molecular Biochemicals, USA).

qRT-PCR analysis

Total RNA was extracted from the leaf tissues of wild type and transgenic plant by using RNaseasy Plant Mini Kit (QIAGEN, USA). The relative purity and concentration of RNA was estimated using NanoDrop-1000 spectrophotometer (NanoDrop
Table 1 Phenotypic traits of wild type and *Br322* mutant

| Trait                      | Wild type   | *Br322*    |
|----------------------------|-------------|------------|
| Seed length (mm)           | 4.83±0.19   | 2.85±0.12**|
| Seed width (mm)            | 3.88±0.15   | 3.00±0.19**|
| 1000 grain weight (g)      | 24.25±0.31  | 19.74±0.09**|
| Plant height (cm)          | 118.12±46.7 | 95.46±61.2**|
| Culm length (cm)           | 79.74±90.2  | 64.28±15.9**|
| Panicle length (cm)        | 21.16±13.3  | 15.58±4.3**|
| Leaf length (cm)           | 69.5±4.7    | 50.9±4.7**  |
| Leaf angle (cm)            | 5.0±1.2     | 3.2±0.4*    |
| Ligule length (cm)         | 1.36±0.05   | 0.62±0.08** |

Data are average of 10 plants (±SD). Gopum used as the wild type. Asterisk indicates significant different by LSD at 5% (*) and 1% (**) relative to Gopum.

Technologies, Inc. USA), and stored at -80°C. The first-strand cDNAs were synthesized using Oligo (dT)20 primer and SuperScript™ III Reverse Transcriptase (Invitrogen, USA). The specific sequences of primer pairs used in quantitative real-time PCR (qRT-PCR) were described in Supplementary Table 1.

**GA induction in shoot elongation**

To estimate the effect of GA in second leaf sheath elongation, ten rice seeds were sterilized and incubated at 30°C for 1 day and then washed 4 times with sterilized water. Seeds were allowed to imbibe water for one more day and plated on agar containing various concentrations of GA3 under continuous light at 30°C. After six days, the lengths of the second leaf sheaths were measured.

**Evaluation of agronomic traits**

At maturity, ten plants for each transgenic line and wild type were evaluated for 1000-grain weight, culm length, panicle length, leaf length, leaf angle, and ligule length. Evaluation was similar to that described in Cho et al. (2007).

**Statistical analysis**

Data requiring statistical analysis were computed using the Statistix version 8. Significant P-value was further analyzed using the two-sided Dunnett’s multiple comparisons or the least significant difference (LSD) with the wild type as control.

**Results**

**Characterization of a *BrPATL4* gene**

The *PATELLIN* gene expressed in transgenic rice plant, *Br322*, encodes a cDNA (LOC103840314) consisting of 1,695 bp with 67 bp of 5’ UTR, 1,488 bp of coding region and 140 bp of 3’ UTR. The open reading frame encodes a polypeptide of 495 amino acids with a calculated mass of 55.7 kDa. Analysis with InterProScan (http://www.ebi.ac.uk/InterProScan/) showed that the *BrPATL4* has 16 phospholipid binding pockets and two salt bridges in Sec14 domain for binding with phospholipid that either bind or transfer phosphatidylinositol (PtdIns), respectively (Fig. 5A). These two motifs were well conserved in both *AtPATL4* in *A. thaliana* and *BrPATL4* in *B. rapa* (Fig. 5C). Also, Sec14 lipid binding domain has three essential domains, Glu207, Lys239, and Gly266 for transferring PtdIns. These domains are well conserved in all six *AtPATL* s (*AtPATL1* to *AtPATL6*) and *BrPATL4* (Fig. 5B). This result suggests that *BrPATL4* could have PtdIns binding activity like *AtPATL4* that was previously reported (Peterman et al. 2004).

Phylogenetic tree analysis using the deduced amino acid of this gene showed that it is closely related to Patellin like proteins in *B. napus* and *B. oleracea* (Fig. 5C).

**BrPATL4 gene expression and generation of transgenic plants**

To investigate the expression pattern of *BrPATL4* in different tissues, qRT-PCR analyses were carried out on different tissues of *Brassica rapa*. The mRNA transcript of *Brassica rapa* was detected in seven tissues (leaf, stem, root, sepal, petal, stamen, and pistil), with the highest levels seen in the pistil, but not detected or barely detected in sepal, petal, and stamen (Fig. 2). The recombinant vector carrying *BrPATL4* was constructed under the control of *Ubiquitin-1* promoter and NOS terminator and transformed into rice using *Agrobacterium*-mediated transformation method. A total of six regenerated plants were analyzed by PCR. Confirmed plants were used to determine the expression pattern of *BrPATL4* gene in transgenic rice and wild type. mRNA transcript analysis of *Ubi-1::BrPATL4* plants showed an enhanced expression of the *BrPATL4* gene...
Fig. 1 Confirmation of transgene in Br322. (A) Schematic diagram of binary vector containing BrPATL4. Abbreviations are: P35S, CaMV 35S promoter; pUbi-1, maize Ubiquitin-1 promoter; Tg7 and Tnos, polyadenylation signals from gene 7 and nopaline synthase (nos) gene; LB, left border; RB, right border. (B) Southern blot analysis. HPT gene was used as the probe and selected enzyme for genomic DNA digestion were indicated in the upper region of blot. (C) Relative expression of BrPATL4 in wild type and Br322 by qRT-PCR. OsACT was used as internal control.

compared to that of the wild type (Fig. 1C).

Character of Br322 mutant

In this study, we report a gain-of-function rice mutant, Br322 that affects plant morphology especially in seed, panicle, and plant height. The BrPATL4 gene introduced in a rice mutant, Br322 was originally isolated from Brassica rapa by a FOX gene hunting library screening described as dwarf mutant with a defect in seed morphology (Fig. 3) (Abdula et al. 2013). The overall phenotypes of Br322 are dwarf tillers, short dark green leaves, compact panicle, and small round grains (Fig. 3). To have a better understanding of the anomalous phenotypes in Br322, the evaluation of agronomic traits was performed at the fully developed stage in both wild type and Br322 mutant (Table 1).

The overall lengths of all the organs in Br322 mutant were shorter than those of wild type which were also used for generation of Fox hunting library (Fig. 3). The plant height has reduced by 80.8% in Br322 mutant compared to the wild type. The culm length of wild type is longer than the culm length of Br322. Panicle and leaf blade length have also reduced by 73.6% and 73.2% in Br322 compared to those of wild type, respectively (Table 1). When the grains of wild type and Br322 were compared, the length and width of grains were significantly shorter than those of wild type (Fig. 3 and Table 1). The grain length and width in Br322 were 59% and 77.3% of those of wild type, respectively (Table 1). Grain weight has also reduced in Br322 mutant which was consistent with the reduction of length and grain width. 1,000-grain weight of wild type is higher than Br322.

Aside from the reduction of overall size, the leaf and panicle of Br322 showed erect phenotypes, even after the grains were fully matured (data not shown). The average leaf angle of Br322 was lower than that of the wild type (Fig. 3). There were no changes of morphologies in leaf except for reduction of size. We tried to find the reason for dense and erect panicle phenotypes of Br322. No distinct differences in
Fig. 3 Morphological phenotype of the Br322 mutant. (A) The gross morphology of wild type (left) and Br322 (right) during the filling stage. Gopum, which is a background variety of Br322, was used as the wild type plant. (B) Comparison of young florets of wild type (left) and Br322 (right 3 plants). (C) Grain size of wild type (upper) and Br322 (lower) (D) Panicle size and architecture of wild type (left) and Br322 (E) Comparison of the panicle branches of wild type (left) and Br322. All plants were grown under field condition until ripening stage.

the length of pedicels and the number of spikelet between wild type and Br322 were observed. Also, the grain numbers per panicle were not different in both wild type (121.8±10) and Br322 (124.3±9.8) (Fig. 4). However, grain sterility was higher in Br322 than in wild type (Fig. 4). These results suggest that the dense and upright phenotypes of leaf and panicle in Br322 were caused by the reduced length of panicle, while they have almost the same number of grains per panicle (Table 1 and Fig. 4).

Copy numbers and expression of the transgene

From the sequencing and expression studies of transgene used for the generation of Br322 line, we found that Br322 is a gain of function mutant of PATELLIN (PATL) of Brassica rapa (Fig. 1) which contains the conserved Sec14 lipid binding domain and Golgi dynamics (GOLD) domain as described previously (Allen-Baume et al. 2002; Bankaitis et al. 1990; Bankaitis et al. 1989; Peterman et al. 2004). The schematic representation of construct bearing BrPATL4 was shown in Figure 1A. Br322 transgenic lines showed double bands in all examined enzymes and this result showed that Br322 has two copies of T-DNA (Fig. 1). Because Br322 mutant was constructed using constitutive overexpression promoter, we analyzed the expression level of BrPATL4 using RT-PCR. Total RNA was extracted from seedling stage of wild type and Br322 and then amplified using BrPATL4 specific primer set (Supplementary Table 1). Because BrPATL4 is not a rice gene, no band was detected in wild type. However, strong expression of BrPATL4 was observed in Br322 line suggesting that the pleotropic phenotypes of Br322 were caused by the overexpression of Br322 (Fig. 1).

GA response of Br322, overexpression line of BrPATL4

The pleiotropic phenotypes of Br322 are similarly shown with the mutants such as Dwarf 1 (d1), SMALL AND ROUNG SEED 1 (SRS1), and SMALL AND ROUNG SEED 5 (SRS5) (Ashikari et al. 1999; Segami et al. 2012; Ueguchi-Tanaka et al. 2000). Of these, d1, which has a mutation in the α-subunit of the heterotrimeric G protein (Ueguchi-Tanaka et al. 2000) has the most similar phenotype with Br322 especially in seed morphology and dwarfism. It has been reported that d1 mutant was classified as GA-insensitive group leading a defect in gibberellin signal transduction (Ashikari et al. 1999). To determine whether pleiotropic phenotypes including broad, dark green leaves, compact panicles and short, round grains were caused by the defect of GA signal transduction, the length of second leaf sheath was estimated to find the reason on the pleiotropic phenotypes of Br322. As shown in Figure 6, the length of second sheath was not fully recovered in response to GA3 treatment compared to wild type, the second leaf sheath of wild type and Br322 have longer length according to the dose of GA3 and showed similar response to exogenous GA3 treatment. These results suggest that Br322 still responds to GA3 and the pleotropic phenotypes of Br322 were not caused
Fig. 5 Deduced amino acid sequence and phylogenetic analysis of BrPATL4. (A) Deduced amino acid sequence of BrPATL4. The dotted box indicates Sec14 domain (D219 to G376). Asterisks and arrows indicate phospholipid binding pockets and salt bridges, respectively. (B) Comparison of essential motifs of Sec14 domain. The three essential motif of Sec14 domain were marked with asterisk. The gray boxed amino acids indicate the phospholipid binding pocket of Sec14 domain. (C) Phylogenetic analysis of BrPATL4.

Fig. 6 Elongation of the second leaf sheath in response to GA treatment in wild type and Br322. The rice seeds were incubated on agar containing various concentrations of GA3 (10^-12 to 10^-4 M) under continuous light at 30°C. The lengths of the second leaf sheaths were measured.

Relative expression of genes related to the small and round type seeds

To get a better understanding of the abnormal phenotypes of Br322, the relative expression of D1, SRS1, and SRS5 which are causal genes related in seed morphology were examined (Ashikari et al. 1999; Segami et al. 2012; Ueguchi-Tanaka et al. 2000). D1 (RGA1) encodes a heterotrimeric G protein α-subunit which regulates cell number (Ashikari et al. 1999; Izawa et al. 2010). SRS1 encodes a novel protein with no known functional domain which regulates cell length (Abe et al. 2010), and SRS5 encodes α-tubulin and regulates seed cell elongation in rice (Segami et al. 2012). As most overall phenotypes of these mutants are similar to those of Br322, we investigated the transcriptional changes of these genes to find the reason of morphological defects in Br322. D1, SRS1, and SRS5 in Br322 showed lower level of mRNA accumulation in rice seed than in the wild type (Fig. 7). D1 showed lower level of expression of about 30 folds compared to wild type and SRS1 and SRS5 showed 17 and 10 folds decreased levels compared with the wild type respectively. Although, the molecular mechanism of transcriptional regulation of these genes through the overexpression of BrPATL4 in rice still remains to be elucidated, the pleiotropic phenotypes of Br322 such as small and round seed and dwarfism were caused by the downregulation of these genes (Fig. 7).
Br322, as three of the most important factors in grain yield (Takeda et al. 2013). Most of these genes are involved in reduction of grain size except overexpression of OsnIR397 which positively induces the grain size through the posttranscriptional regulation of OsLAC (Zhang et al. 2013). When compared to previously reported mutants with seed size defects (Segami et al. 2012), Br322 showed most severe defects in seed morphology. In Br322, the grain length and width were 59% and 77.3% of those of wild type, respectively (Table 1). It was proposed that seed elongation in rice was mainly dominated by cell division and cell elongation which are regulated by GA and brassinosteroid (BR), respectively (Segami et al. 2012). To further investigate whether the pleiotropic phenotypes are caused by overexpression of BrPATL4, we analyzed the GA response and dark condition grown phenotypes of Br322. To confirm the GA response, the length of second leaf sheath was estimated by exogenous treatment of GA3 in various concentrations. Results showed the same growth patterns in response to GA3, while the length of second leaf sheath was not fully recovered by treatment of GA3 (Fig. 6). This result suggests that Br322 is not a mutant related in, at least, GA signal transduction. Also, to find the possibility that Br322 is involved in BR signaling pathway, dark grown phenotypes were estimated (Supplementary Fig. 2). In dark condition, Br322 did not show any differences at mesocotyl and internode elongation compared to those of wild type (Supplementary Fig. 2). Taken together, these results suggest that Br322 might be independent of GA and BR signaling pathway.

In this study, we showed the possibility that the ectopic expression of BrPATL4 could suppress the accumulation of genes, D1, SRS3, and SRS5, which are involved in seed elongation including regulation of cell number or cell length (Ashikari et al. 1999; Segami et al. 2012; Ueguchi-Tanaka et al. 2000). When the transcript levels of these genes in the seeds of wild type and Br322 were compared, each gene showed significantly reduced levels in Br322 (Fig. 7). These results explain the severe phenotype of Br322 including shorter length of seed and other pleiotropic phenotypes. However, a mechanism behind such downregulation of these genes via ectopic expression of Br322 remains to be investigated.

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References

Abdula SE, Lee HJ, Lee MG, Jung YJ, Kang KK, Kou IS, Lee SB, Yang WH, Cho YG (2013) Development and Identification of Transgenic Rice Lines with Abiotic Stress Tolerance by using a Full-length Overexpressor Gene Hunting System. Plant Breeding. Biotech. 1:33-38

Abdula SE, Lee HJ, Ryu HJ, Kang KK, Kou IS, Sorrells ME, Cho YG (2014a) Overexpression of BrCIPK1 Gene Enhances Abiotic Stress Tolerance by Increasing Proline Biosynthesis in Rice. Plant Mol Biol Rep 34:501-511

Abdula SE, Lee HJ, Kim JG, Kin MC, Jung YJ, Cho YC, Kou IS, Kang KK, Cho YG (2016b) BrUGI transgenic rice showed improved growth performance with enhanced drought tolerance. Breeding Science 66:226-233

Abe Y, Mieda K, Ando T, Kono I, Yano M, Kitano H, Iwasaki Y (2010) The SMALL AND ROUND SEED1 (SRS1/DEP2) gene is involved in the regulation of seed size in rice. Genes Genet. Syst. 85:327-339

Allen-Baume V, Segui B, Cockcroft S (2002) Current thoughts on the phosphatidylinositol transfer protein family. FEBS Lett. 531:74-80

Ashikari M, Wu J, Yano M, Sasaki T, Yoshimura A (1999) Rice gibberellin-insensitive dwarf mutant gene Dwarf 1 encodes the alpha-subunit of GTP-binding protein. Proc. Natl. Acad. Sci. USA 96:10284-10289

Bankaitis VA, Aitken JR, Cleves AE, Dowhan W (1990) An essential role for a phospholipid transfer protein in yeast Golgi function. Nature 347:561-562

Bankaitis VA, Malehorn DE, Emr SD, Greene R (1989) The Saccharomyces cerevisiae SEC14 gene encodes a cytosolic factor that is required for transport of secretory proteins from the yeast Golgi complex. J. Cell Biol. 108:1271-1281

Cho YG, Kang HK, Lee JS, Lee YT, Lim SJ, Gauch H, Eun MY, McCouch SR (2007) Identification of quantitative trait loci in rice for yield, yield components, and agronomic traits across years and locations. Crop Sci. 47:2403-2417

Fujisawa Y, Kato T, Ohki S, Ishikawa A, Kitano H, Sasaki T, Ashai T, Iwasaki Y (1999) Suppression of the heterotrimeric G protein causes abnormal morphology, including dwarfism, in rice. Plant Physiol. 122:720-730

Izawa Y, Minami M, Ohki S, Iwasaki Y (2010) Expression profile of the alpha subunit of the heterotrimeric G protein in rice. Plant Signal. Behav. 5:845-847

Jacobsen JV, Zwar JA, Chandler PM (1985) Gibberellic-acid-responsive protoplasts from mature aleureone of Himalaya barley. Planta 163:430-438

Kitagawa K, Kurinami S, Oki K, Abe Y, Ando T, Kono I, Yano M, Kitano H, Iwasaki Y (2010) A novel kinesin 13 protein regulating rice seed length. Plant Cell Physiol. 51:1315-1329

Li F, Liu W, Tang J, Chen J, Tong H, Hu B, Li C, Fang J, Chen M, Chu C (2010) Rice DENSE AND ERECT PANICLE 2 is essential for determining panicle outgrowth and elongation. Cell Res. 20:838-849

Peiro A, Izquierdo-Garcia AC, Sanchez-Navarro JA, Pallas V, Mulet JM, Aparicio F (2014) Patellins 3 and 6, two members of the Plant Patellin family, interact with the movement protein of Alfalfa mosaic virus and interfere with viral movement. Mol. Plant Pathol. 15:881-891

Phillips SE, Vincent P, Rizzieri KE, Schaaf G, Bankaitis VA, Gaucher EA (2006) The diverse biological functions of phosphatidylinositol transfer proteins in eukaryotes. Crit. Rev. Biochem. Mol. Biol. 41:21-49

Segami S, Kono I, Ando T, Yano M, Kitano H, Miura K, Iwasaki Y (2012) Small and round seed 5 gene encodes alpha-tubulin regulating seed cell elongation in rice. Rice 5:4

Sun MM, Abdula SE, Lee HJ, Cho YC, Han LZ, Koh HJ, Cho YG (2011) Molecular aspect of good eating quality formation in Japonica rice. PLoS One 6:e18385. doi:10.1371/journal.pone.0018385

Takeda S, Matsuoka M (2008) Genetic approaches to crop improvement: responding to environmental and population changes. Nat. Rev. Genet. 9:444-457

Tanabe S, Ashikari M, Fujioka S, Takatsuto S, Yoshida S, Yano M, Yoshimura A, Kitano H, Matsuoka M, Fujisawa Y, Kato H, Iwasaki Y (2005) A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, dwarf11, with reduced seed length. Plant Cell 17:776-790

Ueguchi-Tanaka M, Fujisawa Y, Kobayashi M, Ashikari M, Iwasaki Y, Kitano H, Matsuoka M (2000) Rice dwarf mutant d1 improves rice yield by increasing grain size and promoting panicle branching. Annu. Rev. Plant Biol. 61:421-442

Zhang YC, Yu Y, Wang CY, Li ZY, Liu Q, Xu J, Liao JY, Wang XJ, Qu LH, Chen F, Xin P, Yan C, Chu J, Li HQ, Chen YQ (2013) Overexpression of microRNA OsmiR397 improves rice yield by increasing grain size and promoting panicle branching. Nat. Biotechnol. 31:848-852