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PERSPECTIVE

Phytohormones and rice crop yield: strategies and opportunities for genetic improvement

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Abstract To feed an estimated world population of 8.9 billion by 2050, strategies for increasing grain production must be developed. Several agronomically important traits for increasing yield, such as plant height, grain number, and leaf erectness, have recently been characterized in rice (*Oryza sativa* L.). These traits are regulated primarily by three phytohormones: gibberellins, cytokinins, and brassinosteroids. The control of biosynthesis and degradation of these key phytohormones is discussed in terms of its importance for normal plant growth. Genes involved in the biosynthesis and regulation of these phytohormones can be used to develop effective strategies to increase grain yield. Genetic manipulation of phytohormone-related gene expression is thus a practical strategy to generate high-yielding transgenic plants through the modification of levels and profile of endogenous phytohormones.

Keywords Brassinosteroids · Cytokinins · Gibberellins · Grain yield · Rice

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Introduction

Since the 1960s, the world's population has doubled to more than 6.5 billion people. This rapid increase, together with drastic decreases in the area of cultivated land, has raised concerns for a likely global food crisis. The development and widespread adoption of high-yielding semi-dwarf varieties of wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) led to major increases in food production. As a result of this Green Revolution large-scale famine was averted (Conway and Toenniessen 1999). However, by 2050 global population is projected to reach 8.9 billion people with much of this increase in South-East Asia. To feed 8.9 billion people, the productivity of food crops will have to increase by an additional 50%.

Rice is one of the most important staple crops because it feeds more than half of the world's population. It is particularly important for people living in Asia. Rice has been established as a model crop because it has the smallest genome of the major cereals (390 Mbp; Sasaki et al. 2005). The sequence of the entire rice genome (Sasaki et al. 2005) provides the basis for genome-based identification and functional analysis of genes for other cereals, including maize (*Zea mays* L.), wheat, and barley (*Hordeum vulgare* L.), because these cereals share a syntenic relationship with rice (Gale and Devos 1998). Many molecular rice markers have been developed and a large number

of mutants have been generated. These accomplishments have greatly facilitated the analysis of agronomically important traits of rice, including yield.

Gibberellins and semi-dwarf phenotype

The major factor that made the Green Revolution possible was the introduction of high-yielding semi-dwarf varieties of wheat and rice, in combination with the application of large amounts of nitrogen fertilizer. Nitrogen fertilization is essential to increasing grain yield, but it also promotes leaf and stem elongation, resulting in an overall increase in plant height. However, tall plants are easily damaged by wind and rain, and dramatic yield losses can occur as a result of storms. This is a particular problem in the monsoon season in Asia. In contrast, semi-dwarf cultivars of wheat and rice are more resistant to damage by wind and rain (lodging-resistant), and the reduction in plant height also improves the harvest index (the ratio of grain to grain plus straw) and enhances biomass production (Khush 1999).

It is noteworthy that two so-called Green Revolution genes—wheat *Reduced height1* (*Rht1*) and rice *semi-dwarf1* (*sd1*)—are involved in gibberellin (GA) signaling and biosynthesis, respectively (Peng et al. 1999; Sasaki et al. 2002). Characterization of the Green Revolution genes has taught us that controlling GA metabolism or sensing is the best target for producing high-yield semi-dwarf cultivars by means of traditional crop breeding. Today, however, we can adopt genetic engineering approaches to create a more desirable plant architecture. Two approaches to reduce endogenous GA content have been described: suppression of GA biosynthesis and enhancement of GA catabolism. An example of the first approach involves antisense expression of GA biosynthetic genes. Itoh et al. (2002) generated antisense transformants of the rice GA3-oxidase gene, *OsGA3ox2*. However, only a few plants showed a semi-dwarf phenotype; moreover, this phenotype was not heritable. Similar results were observed in antisense transformants of Arabidopsis (*Arabidopsis thaliana* L.) GA20-oxidase genes (Coles et al. 1999). These phenotypic instabilities may have resulted from GA homeostasis, since reduced GA content stimulates feedback up-regulation of other GA biosynthetic genes (Coles et al. 1999; Itoh et al. 2002). Increased expression of these genes led to higher levels of substrates of target enzymes in order to compensate for the reduced activity of the target enzyme by antisense expression.

The second approach is exemplified by overproduction of the GA-catabolic enzyme GA2-oxidase or ectopic expression of its gene. Overproduction of GA2-oxidase causes drastic decreases in the level of bioactive GA, leading to reduced plant height. Overexpression of the rice GA2-oxidase gene, *OsGA2ox1*, under the control of a constitutive actin promoter resulted in a severely dwarfed phenotype (Fig. 1a; Sakamoto et al. 2001). Similar results were observed in transgenic *Arabidopsis* and wheat carrying the bean GA2-oxidase gene, *PcGA2ox1* (Hedden and Phillips 2000a, b). These transformants also showed severe defects in flower and grain development, indicating that GA is essential for the development of reproductive organs. As such defects prevent breeding, it is essential to employ organ- or tissue-specific promoters when GA2-oxidase overproduction is used as a tool to produce dwarf phenotypes of agronomic significance.

One solution to this problem involves using the promoter of the rice GA3-oxidase gene (Sakamoto et al. 2003). GA3-oxidase catalyzes the final step of the GA biosynthetic pathway. Rice has two GA3-oxidase genes: *OsGA3ox1*, which is specifically expressed in reproductive organs, and *OsGA3ox2*, which is active in vegetative organs. The loss-of-function mutant of *OsGA3ox2*, *d18*, is severely impaired in shoot elongation but not in flower or grain development (Itoh et al. 2001). Thus, when *OsGA2ox1* is ectopically expressed in transgenic rice under the control of the *D18* promoter, inhibition of GA synthesis should be restricted to leaves and stems. In fact, *D18:OsGA2ox1* rice transformants exhibit only moderate dwarfing and form normal flowers and grains (Fig. 1b) in contrast to the over-expressing transformants discussed above (Fig. 1a).

Using the *OsGA3ox2/D18* promoter has the additional benefit of stabilizing inheritance of the semi-dwarf trait. GA homeostasis is achieved by

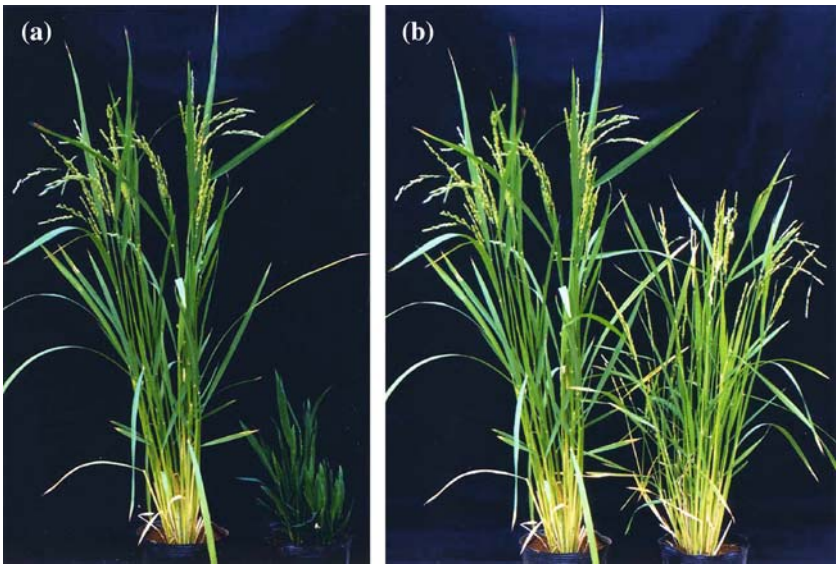

Fig. 1 Phenotypes of transgenic rice plants expressing the GA catabolic enzyme gene, *OsGA2ox1*. (**a**) Left, wild-type 'Nipponbare' rice. Right, typical phenotype of transgenic rice overexpressing *OsGA2ox1* under the control of the constitutive rice actin promoter. Most transformants show a severely dwarfed phenotype and do not bear any grains. (**b**) Left, wild-type 'Nipponbare' rice. Right, typical phenotype of transgenic rice ectopically expressing *OsGA2ox1* under the control of the *D18* promoter. Most transformants show a semi-dwarf phenotype without any effects on reproductive development

means of negative feedback regulation of biosynthetic enzyme genes and positive feed-forward regulation of catabolic enzyme genes. This homeostatic regulation may cause unexpected phenotypes when one attempts to alter GA levels by genetic manipulation. However, when the *OsGA3ox2/D18* promoter is used to control expression of GA2-oxidase, the resulting reduced levels of GA will up-regulate expression of *OsGA3ox2* and that of the introduced *OsGA2ox1*. Consequently, one can overcome the homeostatic regulation of GA. Although the complexity of GA biosynthesis and signaling allows for a number of targets for manipulation. The negative regulator of GA signaling, such as *Arabidopsis* GAI, wheat Rht, and rice SLR are good candidates as suggested (Peng et al. 1999; Fu et al. 2001; Ikeda et al. 2001). Even though different approaches will probably be necessary for different species, the strategy outlined above enables the introduction of a single dominant dwarfing gene that generates semi-dwarf plants avoiding conventional, long-term breeding programs.

Cytokinins and grain number

Grain number is an important trait that contributes directly to yield. During the past decade, many attempts have been made to characterize quantitative trait loci (QTLs) for grain number. Recently, a QTL that increases grain number in rice, *Gn1a*, has been identified (Ashikari et al. 2005). To detect this QTL, researchers studied an *indica* rice variety, 'Habataki', and a *japonica* variety, 'Koshihikari', because these two varieties exhibit large variations in the target trait. On average, 'Habataki' and 'Koshihikari' plants bear 300 and 160 grains per panicle, respectively (Fig. 2). Ashikari et al. detected five QTLs for increasing grain number, and the most effective QTL, *Gn1* on the short arm of chromosome 1, explained 44% of the difference in grain number between 'Habataki' and 'Koshihikari'. *Gn1* consists of two loci, *Gn1a* and *Gn1b*, *Gn1a* was found to encode a cytokinin (CK) oxidase/dehydrogenase (CKX) gene, *OsCKX2*. CK is best known as a phytohormone that promotes

Fig. 2 Variations in the number of rice grains. 'Habataki' (right) and 'Koshihikari' (left) plants bear 300 and 160 grains per panicle, on average, respectively. Photographs provided by Dr. M. Ashikari (Nagoya University)

cell division (Mok 1994), and CKX preferentially and irreversibly degrades CKs (Mok and Mok 2001). Because at least 11 putative *CKX* genes are present in the rice genome, the *OsCKX* genes could be functionally differentiated based on their spatial and temporal expression patterns. Indeed, *OsCKX2* expression was observed mainly in inflorescence meristems and young flowers, and transcript accumulation was less abundant in 'Habataki' than in 'Koshihikari'. The expression of *OsCKX2* in inflorescence meristems might regulate the CK level and thus control the number of flowers. Transgenic rice plants generated with antisense *OsCKX2* that had reduced levels of endogenous *OsCKX2* expression developed higher numbers of grains (Ashikari et al. 2005). In contrast, overexpression of *AtCKX3*, the *Arabidopsis* ortholog of rice *OsCKX2*, in transgenic *Arabidopsis* plants reduced the number of flowers because of a decreased rate of primordia formation in the flower meristem (Werner et al. 2003). These findings demonstrate the feasibility of genetic improvement of crop yields by modulation of CK catabolism and levels of bioactive CK.

Brassinosteroids and plant stature

Since the harvest index (ratio of grain to grain plus straw) for rice is approaching a ceiling, future increases in yield potential will have to involve an increase in crop biomass; that is, there will have to be more net photosynthesis (Mann 1999). It is well known that phenotypes with erect leaves can capture more light for photosynthesis and can be planted more densely, with a higher leaf area index (one-sided leaf area per unit of land area), both of which contribute to increased yields (Sinclair and Sheehy 1999). In rice, the contribution of the lower leaves to photosynthesis is significant even through their photosynthetic capacity is considerably less than that of the upper leaves (Horton 2000). Erect leaves allow greater penetration of light to the lower leaves, thereby optimizing canopy photosynthesis (Sinclair and Sheehy 1999).

The erect-leaf phenotype is the most common among all brassinosteroid (BR)-related rice mutants, suggesting that this is the most sensitive of the BR-related phenotypes. The erect-leaf phenotype caused by BR-related mutations has not been utilized in traditional rice breeding, probably because most BR-related mutants have small grains or decreased fertility, both of which are unfavorable for crop breeding (Hong et al. 2003, 2005; Tanabe et al. 2005). However, the weakest recently identified mutant, *osdwarf4-1*, exhibits erect leaves without abnormal flower or grain morphology (Fig. 3a–c; Sakamoto et al. 2006). This phenotype is a loss-of-function mutant of *OsDWARF4*, which encodes a BR biosynthetic cytochrome P450. In rice, two different cytochrome P450s, CYP90B2/OsDWARF4 and CYP724B1/D11, redundantly catalyze the C-22 hydroxylation step in BR biosynthesis. It is noteworthy that C-22 hydroxylation is catalyzed by a single enzyme, CYP90B1/DWARF4 (the ortholog of OsDWARF4) in *Arabidopsis*, but this step is dominantly catalyzed by CYP724B1/D11 in rice according to the phenotypic severity in rice loss-of-function mutants (Sakamoto et al. 2006). The mutant phenotypes indicate that *CYP724B1/D11* is an important gene in controlling the synthesis of bioactive BR for shoot elongation and normal reproductive development, and that *OsDWARF4* is an important gene controlling the synthesis of bioactive BR for leaf inclination but not for reproductive development. This is the reason *osdwarf4-1* shows slight dwarfism, and erect leaves without abnormal leaf, flower, or grain morphology, and is thus practical for cultivation.

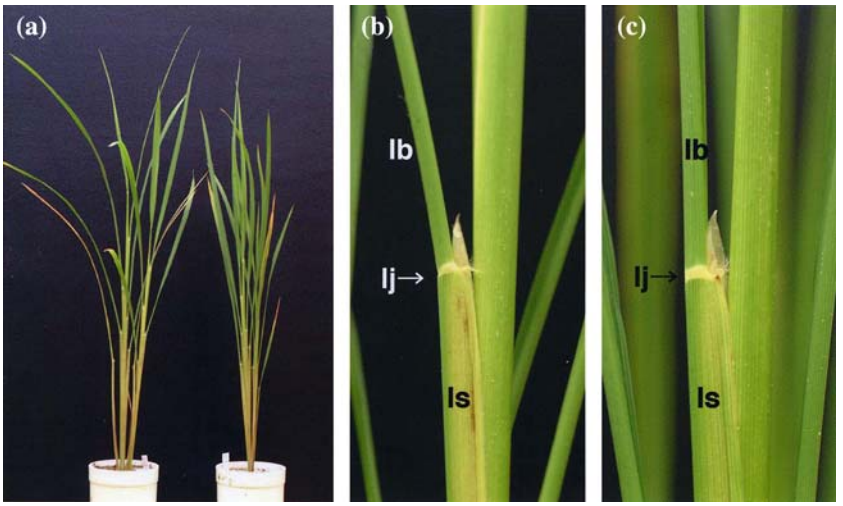

Fig. 3 A brassinosteroid (BR)-deficient mutant shows erect leaves. (**a**) Comparison of gross morphology between wild-type 'Nipponbare' (left) and a BR-deficient *osdwarf4-1* mutant (right); (**b**) and (**c**) erect-leaf phenotype of *osdwarf4-1*. The angle between the leaf blade and leaf sheath of *osdwarf4-1* (**c**) is less than that in the wild-type (**b**). lb, leaf blade; lj, lamina joint; ls, leaf sheath

Under dense planting conditions, the presence of erect leaves greatly increased the above-ground biomass and grain yield of *osdwarf4-1* rice, even without extra fertilizer. These results provide a strategy for genetic improvement of crop production by modulation of BR biosynthesis. We have recently identified a rice homolog of *Arabidopsis BAS1* that encodes a BR catabolic enzyme, CYP734A1 (Neff et al. 1999). Although overexpression of these rice homologs in transgenic rice induced a severely dwarfed phenotype with defects in leaf, flower, and grain development, ectopic expression under the control of the *OsDWARF4* promoter induced a moderately dwarfed phenotype with very erect leaves (T. Sakamoto et al. unpublished results). We have also generated transgenic rice that partially suppresses expression of the BR receptor gene *OsBRI1*. These transformants also exhibited a moderately dwarfed phenotype with very erect leaves and thus have the potential to increase grain yield (Morinaka et al. in press). Although further studies are needed to confirm that these transgenic plants will have a higher grain yield at high planting densities in paddy fields, our recent results suggest the feasibility of generating plants with erect leaves without defects in reproductive development by modifying the metabolism and sensing of BR. This will make it possible to introduce a single dominant transgene that would generate plants with erect leaves to increase grain yield at high planting density without the need for conventional, long-term breeding programs and without the negative environmental effects caused by excessive use of fertilizers.

Conclusions

The results reported here indicate the value of further research on the genetic control of phytohormones and the incorporation of appropriate mutant alleles in important rice varieties to improve productivity. In particular, the development of genotypes that exhibit erect-leaf phenotypes because of mutations in the genes related to BR production and sensing shows promise.

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