INTRODUCTION

Brassinosteroids (BRs) are a unique class of plant polyhydroxysteroids that are structurally related to animal and insect steroid hormones. Many studies have shown the importance of BRs in plant growth and development by demonstrating the dramatic morphological changes that occur in BR biosynthetic and perception mutants, including those of Arabidopsis, pea, and tomato.\(^1\)

In contrast to the rapid advances in BR research on dicot plants, there had been no reports dealing with the molecular biological function of BRs in monocot plants. We have isolated and characterized three independent BR-related mutants of rice. All three exhibit a common phenotype: erect leaves, shortened internodes, and constitutive photomorphogenesis in the dark. The erect leaves with shortened culms suggested that the BR-related phenotype might improve crop architecture and, thereby, increase crop yield. Indeed, recently, it was revealed that a semi-dwarf barley cultivar, uzu, contains a single nucleotide mutation in the BR receptor gene, BRI1.

Keywords: brassinosteroid, rice, dwarf, breeding.

Fig. 1. Gross morphology of rice BR-related mutants. (Bar: 20 cm).
1. DWARF PHENOTYPE OF RICE BR-RELATED MUTANTS

Figure 2a shows the gross structure of a rice plant (left) and a schematic diagram of rice culm (right). The rice leaf consists of a leaf sheath and a leaf blade with jointing at the lamina joint, or collar. Only leaves are seen on the outside of the plant before panicle formation, heading, and flowering, which occur with the rapid elongation of internodes after the transition from the vegetative to the reproductive stage. The internodes are usually numbered from top to bottom in rice; accordingly, the uppermost internode, just below the panicle, is the first internode. Based on the elongation pattern of internodes, rice dwarf mutants were previously categorized into five groups: dn, dm, d6, nl, and sh.12) In the dn-type mutants, the length of each internode is almost uniformly shortened, resulting in an elongation pattern similar to that of the wild-type plant. In contrast, the dm-type mutants are noted for the unusual non-elongation of the second internode. Similarly, the shortening of specific internodes is also observed in the sh-type and d6-type mutants, in which only the uppermost internode or the internodes below it are shortened, respectively (Fig. 2b).

Plant dwarfism is often caused by defects in the biosynthesis or perception of plant hormones such as gibberellin (GA) or BRs. At present, many rice dwarf mutants have been identified as GA-deficient or GA-insensitive mutants, and a few BRs. At present, many rice dwarf mutants have been identified as GA-deficient or GA-insensitive mutants, and a few BRs. A rice BR mutant with an intermediate phenotype, OsBRI1 antisense plants allow us to order the internode elongation pattern according to the degree of severity of the dwarfism, from mild to severe, as follows, dn, dm, d6-, nl, and sh-types, adapted from Takeda (see Ref. 12). (c) The d2-2 and d2-1 mutants show a typical dm-type phenotype, d61-2 shows d6-type phenotype and the brd1-1 mutant does not elongate at any internodes. Arrowheads indicate the positions of the nodes. (Bar: 10 cm)

Taken together, the results with BR-related mutants and OsBRI1 antisense plants allow us to order the internode elongation pattern according to the degree of severity of the dwarfism, from mild to severe, as follows, dn, dm, d6-, nl, and sh-types, adapted from Takeda (see Ref. 12). (c) The d2-2 and d2-1 mutants show a typical dm-type phenotype, d61-2 shows d6-type phenotype and the brd1-1 mutant does not elongate at any internodes. Arrowheads indicate the positions of the nodes. (Bar: 10 cm)
notype. The \( D1 \) gene has been isolated and found to encode a \( \text{Ga}\alpha \)-like protein that is involved in the GA signal transduction pathway.\(^{14}\) Thus, perhaps a specific mechanism links BR and GA signal transduction at the second internode and is responsible for the induction of elongation at this site.

Very recently, Chono et al.\(^{15}\) reported that a semi-dwarf gene in barley, \( zuu \), encodes a putative BR receptor that is highly homologous to \textit{Arabidopsis} \( A\text{bRII} \) and rice \( Os\text{BRII} \). They found that this semi-dwarf barley cultivar, called \( zuu \) barley in Japanese, is less responsive to brassinolide and has a high accumulation of BRs. Based on the syntenic relationship between the chromosome locations of the \( zuu \) gene in the barley genome (chromosome 3H) and the rice BR-insensitive mutant \( d61 \) (chromosome 1), they predicted the barley \( zuu \) gene to be an ortholog of the rice \( D61 \) (\( Os\text{BRII} \)) gene. When they compared the nucleotide sequence of the barley \( B\text{RII} \)-like gene in \( zuu \) with that of the wild-type barley gene, they found that the barley \( zuu \) gene contains a single nucleotide substitution, which results in an amino acid substitution at a conserved residue in the kinase domain.

The semi-dwarf phenotype has been used for developing high-yield varieties of rice and wheat because the semi-dwarf phenotype resists lodging under high fertilization conditions; while a high fertilizer concentration is essential to increase grain production, it also induces taller plant height. The widespread adoption of rice and wheat semi-dwarf varieties has led to major increases in crop production in Asia and middle and southern regions of the USA. This remarkable achievement has been referred to as the “green revolution”.\(^{16–18}\) Recently, the semi-dwarf genes that contributed to the green revolution have been isolated and characterized. The rice \( SD1 \) gene encodes an enzyme in the GA-biosynthetic pathway,\(^{19,20}\) and the wheat dwarf gene \textit{Reduced height 1} (\( R\text{hl}1 \)) encodes a negative regulator of GA signaling.\(^{21}\) These findings suggested that GA-related dwarfism might be a good mechanism for producing semi-dwarf cultivars in a breeding program.

In addition to GA, BRs are another important factor determining plant height, as mentioned previously. Even so, BR-related genes have not been considered as genetic resources for the production of dwarf cultivars because BR-related mutations induce not only dwarfism but also many other abnormal leaf and root morphologies (see below). However, the identification and characterization of the barley \( zuu \) gene taught us that BR-related genes can serve as available targets to produce semi-dwarf cultivars. The positive application of BR-related dwarf mutants should expand the genetic resources for the breeding of semi-dwarf cultivars with high-yields. In this context, we are now attempting to produce novel semi-dwarf rice by decreasing bioactive BR biosynthesis.

### 2. ORGAN MORPHOLOGY

Besides dwarfism, one of the most characteristic phenotypes of BR-related mutants is an erect leaf. In rice, when the leaf blade and sheath are fully elongated, cells at the adaxial side of the lamina joint start to elongate, causing the leaf blade to bend away from the vertical axis of the leaf sheath and toward the abaxial side (Fig. 3a, left). The leaf of the \( D2-2 \) mutant is erect (right). Arrowheads indicate the positions of the lamina joints. (b) Short leaf sheath. The leaf sheath of wild-type rice (left) is longer than that of the \( d61 \) (two in center) or \( brd1 \) (right) mutant. The leaf blade-to-sheath length ratio is increased in the mutants. Arrowheads indicate the positions of the lamina joints. (c) Inhibited root growth. The roots of the \( brd1-1 \) mutant (right) are shorter and the crown root is less developed than in wild-type rice (left). (d) Small seeds. The mutant (lower) has slightly shortened grains relative to wild-type grains (upper).

!![](image)

**Fig. 3.** BR-related mutants display pleiotropic abnormalities. (a) Erect leaf. The leaf blade of wild-type rice bends away from the vertical axis of the leaf sheath and toward the abaxial side (left). The leaf of the \( d2-2 \) mutant is erect (right). Arrowheads indicate the positions of the lamina joints. (b) Short leaf sheath. The leaf sheath of wild-type rice (left) is longer than that of the \( d61 \) (two in center) or \( brd1 \) (right) mutant. The leaf blade-to-sheath length ratio is increased in the mutants. Arrowheads indicate the positions of the lamina joints. (c) Inhibited root growth. The roots of the \( brd1-1 \) mutant (right) are shorter and the crown root is less developed than in wild-type rice (left). (d) Small seeds. The mutant (lower) has slightly shortened grains relative to wild-type grains (upper).
a cell elongation defect. This discerning response of lamina joint cells to BRs has been used in a sensitive bioassay known as the rice lamina inclination assay.22)

BR-related mutants also show abnormal leaf morphology in addition to a lack of lamina bending. The rice leaf is separated almost equally into a blade and a sheath at the lamina joint. However, in the mutant plants, the leaf sheath is much shorter than the blade, and the blade-to-sheath length ratio is increased with the severity of the mutant (Fig. 3b).2,5) indicating that BRs affect the development of the leaf sheath more than the blade. Defects of BR homeostasis also affect the formation and development of the roots and grains of mutant plants. Root elongation is inhibited and the frequency of the formation of crown roots is decreased in BR-deficient mutants, whereas the formation of lateral roots is relatively unaffected (Fig. 3c).5) Some weak mutants, although they can produce seeds, exhibit a further abnormal phenotype in that the grains are slightly shortened (Fig. 3d).9)

3. SHAPE AND ARRANGEMENT OF CELLS AT THE INTERNODE AND LEAF EPIDERMIS

Culm elongation and lamina bending are caused by the division of internode cells and the differentiation of specific cells on the adaxial surface of the lamina joint, respectively, followed by elongation of these cells. Hence, the dwarfing of the culm and the lack of lamina bending could be the result of defects in the differentiation of divisional cells and/or cell elongation. Similarly, abnormal leaf morphology could be a result of a disordering of the normally organized cell arrangement and/or cell elongation. To more completely understand BR-related defects, we examined the internal structures of the internodes and malformed leaves by microscopy.5) During rapid elongation, internodes differentiate in response to the formation of intercalary meristems at the internodes. In the intercalary meristems, active cell division occurs in an anticlinal manner to form longitudinal cell files, and longitudinal elongation of the arranged cell files also occurs (Fig. 4a). The anticlinal divisions and longitudinal elongation of the cells that produce the well-organized cell files (Fig. 4c) are the driving force for the rapid elongation of the rice culm. In contrast to wild-type plants, the culms of BR mutants are not separable into nodes and internodes (Fig. 4b). In the mutant culm, moreover, the organized longitudinal arrangement of cells that typifies the internode of wild-type plants is not observed (Fig. 4d), indicating that the development of the intercalary meristem is defective. These findings demonstrate that BRs are essential for the organized arrangement of cell files at the internodes.

A disorganization of the cell files also occurs on the leaf surface of mutant plants. We observed the structure of the leaf surface of wild-type and mutant plants by scanning electron microscopy (SEM) (Fig. 4e to 4h). In wild-type plants, the epidermal cells of the leaf blade and sheath were arranged in a longitudinal manner, running parallel to the vascular tissues (Fig. 4e and 4g). However, the organized arrangement of leaf epidermal cells was disrupted and an abnormal expansion in a vertical direction against the cell file was observed in the mutants (Fig. 4f and 4h). Consequently, these cells in the mutants became round, square, or otherwise distorted, whereas the wild-type cells had a uniform rectangular shape.5)

In plant cells, the cortical microtubules are arranged transversely to the cell axis and determine the orientation of cellulose microfibrils in the cell wall, thereby controlling the shape of plant cells and the direction of cell expansion. It is possible that a defect in the organized cell arrangement at the internodes and the abnormal shape of the leaf surface cells in BR-related mutants might be owing to a failure in cortical microtubule formation. BRs have been reported to promote the transverse arrangement of cortical microtubules, accelerating cell elongation.23) Therefore, a decreased level of BRs should affect not only microtubule formation but also the transverse arrangement of the microtubules. Indeed, in the internode cells of 61, we could not detect microtubules in unelongated internode cells and found that the microtubules in elongating internode cells were disorganized (Fig. 4i and 4m); whereas, the microtubules in elongating internode cells of wild-type
plants were arranged in an orderly manner, at right angles to the direction of elongation (Fig. 4k and 4m). These observations indicate that the internode cells of BR-related mutants also have a failure in microtubule formation and microtubule arrangement. It is important to distinguish between a deficiency of microtubule formation and that of microtubule arrangement when considering the biological function of BRs. In fact, the GA-deficient dwarf mutant d18, which retains normal microtubule formation but not correct microtubule arrangement, shows an inhibition of leaf-surface cell elongation, but does not exhibit aberrant cell shape or cell files (Ueguchi-Tanaka et al., unpublished data). In this context, the malformed cell shape observed in the leaf surface as mentioned above is a typical characteristic of the BR-related phenotype.

**CONCLUSION AND PROSPECTS**

As we discussed in this review, BR biosynthesis and signaling play important roles in the cell elongation and organ development of rice, as reported previously in dicot plants. Although we have gained some knowledge of the mechanism by which BR regulates growth and development, we remain some time away from being able to manipulate this mechanism. More components of the BR response pathways need to be isolated and characterized. On the other hand, the dwarfism and erect leaves of BR-related mutants make them potentially ideal cultivars for breeding. For example, erect leaves would allow rice to be planted more densely, and dwarfism would reduce the straw biomass and increase lodging resistance. It can be expected that there will be a broad perspective in molecular breeding involving plant architectural modifications via the control of BR synthesis and signaling pathways.

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**REFERENCES**