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Comparison of Effect of Brassinosteroid and Gibberellin Biosynthesis Inhibitors on Growth of Rice Seedlings

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Abstract: Brassinosteroid (BR) and gibberellin (GA) are two predominant plant hormones that regulate plant cell elongation. Mutants disrupt the biosynthesis of these hormones and display different degrees of dwarf phenotypes in rice. Although the role of each plant hormone in promoting the longitudinal growth of plants has been extensively studied using genetic methods, their relationship is still poorly understood. In this study, we used two specific inhibitors targeting BR and GA biosynthesis to investigate the roles of BR and GA in growth of rice seedlings. Yucaizol, a specific inhibitor of BR biosynthesis, and Trinexapac-ethyl, a commercially available inhibitor of GA biosynthesis, were used. The effect of Yucaizol on rice seedlings indicated that Yucaizol significantly retarded stem elongation. The IC_{50} value was found to be approximately 0.8 μmol/L. Yucaizol also induced small leaf angle phenocopy in rice seedlings, similarly to BR-deficient rice, while Trinexapac-ethyl did not. When Yucaizol combined with Trinexapac-ethyl was applied to the rice plants, the mixture of these two inhibitors retarded stem elongation of rice at lower doses. Our results suggest that the use of a BR biosynthesis inhibitor combined with a GA biosynthesis inhibitor may be useful in the development of new technologies for controlling rice plant height.

Key words: brassinosteroid; gibberellin; plant hormone; plant growth regulator; rice

Rice (*Oryza sativa* L.) is one of the predominant dietary energy sources for a large part of humans in the word, especially in Asia. Great efforts have been made to develop new methods for improving the production and quality of rice. Rice architecture is crucial for grain yield, and is determined by plant height, leaf angle, tillering number, and panicle morphology. Plant hormones are important in the regulation of plant growth and development. Brassinosteroid (BR) and gibberellin (GA) are two important plant hormones involving in controlling plant height by regulating cell elongation (Clouse and Sasse, 1998; Richards et al, 2001). In particular, BR plays important roles in the development of rice architecture (Wang and Li, 2005). To date, researches indicate that manipulation of BR biosynthesis or signaling that is used to modify rice architecture can be a feasible approach for improving rice yield. One of the remarkable technologies used in the Green Revolution was the introduction of dwarfing genes into wheat, thereby increasing plant yields and providing a stronger and shorter stem that prevents the plant from falling over (Sasaki et al, 2002; Hedden, 2003). Currently, there are tremendous needs to control plant height in modern agriculture.

The functions of BR and GA in plant growth and development have been studied in considerable detail. GA binds with a soluble receptor, GIBBERELLIN-INSENSITIVE DWARF1 (GID1), and also interacts with SLENDER1. The GA-signaling repressor encodes the DELLA family protein (Dill et al, 2001). The stable triple complex (GA-GID1-DELLA) is then recognized by the SCFGID2 (for Skp1-Cullin-F-box) E3 ubiquitin ligase complex, leading to the ubiquitination and, consequently, degradation of the DELLA repressor protein, thereby reversing the repression of the GA response in rice (Sasaki et al, 2003; Gomi et al, 2004; Ueguchi-Tanaka et al, 2007). Most of our knowledge about BR signaling is obtained from studies in *Arabidopsis thaliana*. BR signal transduction is thought to function by BR binding to the extracellular domain of the leucine-rich-repeat receptor-like kinase (LRR-RLK) BRI1 (brassinosteroid insensitive 1) (Clouse, 2011) and BAK1, a coreceptor of BRI1 (Li et al, 2002), which convey the BR signal to downstream components including BIN2 (BR-insensitive

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2) (He et al, 2002) and BSU1 (BRI1 suppressor 1) (Kim et al, 2011). Subsequent phosphorylation of BZR1 and BZR2/BES1 inhibits their activity through multiple mechanisms (Wang et al, 2002). BR-induced dephosphorylation activates BZR1 and BZR2/BES1 proteins that directly regulate the transcription of BR-responsive genes.

Although BR and GA signal transduction pathways have been studied, the mechanism of their involvement in the regulation of rice plant height remains unclear (Tong et al, 2014). GA biosynthesis mutants display dwarf phenotypes in rice, while BR biosynthesis mutant rice plants also exhibit dwarf phenotypes with erect leaves (Sakamoto et al, 2006). The architectural styles of BR-deficient mutant rice plants are beneficial for dense planting because of the increased light capture required for photosynthesis and nitrogen storage for grain filling (Sakamoto et al, 2006).

The objective of this study is to use specific inhibitors of BR and GA biosynthesis to explore the relationship of BR and GA in the regulation of rice plant height. Yucaizol is a potent BR biosynthesis inhibitor developed in our laboratory (Oh et al, 2012, 2013, 2015; Yamada et al, 2012, 2013) that is now commercially available from Wako Pure Chemical Industries, Ltd., Tokyo, Japan (Fig. 1). Trinexapac-ethyl (TPE) is a commercially available specific inhibitor of GA biosynthesis (Rademacher, 2000) (Fig. 1). Herein, we report the effects of a BR biosynthesis inhibitor combined with a GA biosynthesis inhibitor in rice plants.

MATERIALS AND METHODS

Chemicals

The BR biosynthesis inhibitor Yucaizol is synthesized by a previously described method (Yamada et al, 2013). Stock solutions of the test compound were dissolved in DMSO at a concentration of 100 mmol/L and stocked at -30 °C. Trinexapac-ethyl was purchased from Syngenta as Primo MAXX® and dissolved in DMSO at a concentration of 100 mmol/L as a stock solution according to the manufacturer's instructions. The other reagents were of the highest grade and purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Plant culture and growth conditions

Rice seeds (*Oryza sativa* L. cv. Akitakomachi) were sterilized in a 0.86 mmol/L benomyl solution (a commercially available fungicide purchased from Sumitomo Chemical Garden Products, Tokyo, Japan) for 24 h and then washed with distilled water for five times. The seeds were cold-treated (4 °C) for 3 d and then transferred to a 30 °C incubator for 2 d to promote simultaneous germination. The germinating seeds were placed on gauze set on top of 200-mL disposable plastic cups (Sunplatec) and stimulated to germinate in distilled water for another 3 d. Subsequently, the distilled water was replaced with Hoagland's solution containing several chemicals (Hoagland and Arnon, 1950). The growth conditions were set to 27 °C in 14 h light (approximately 18 000 lux) and 25 °C in 10 h dark with 70% humidity. The plant height of 10-day-old seedlings was measured and the average plant height of eight rice seedlings was used after with or without chemical treatment.

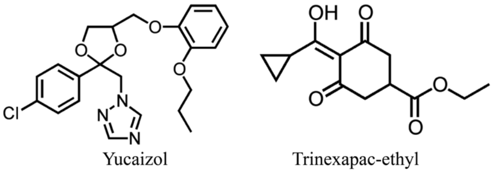

Fig. 1. Chemical structure of brassinosteroid biosynthesis inhibitor (Yucaizol) and gibberellin biosynthesis inhibitor (Trinexapac-ethyl).

Lamina joint bending assay

The angle between the lamina and the second leaf sheath in 5-day-old seedlings after treatment with or without chemical treatment was measured. The second lamina joints were used for the lamina bending assay as described by Wada et al (1981).

Statistical analysis

All measurements were carried out at least in triplicate. Data analyses (*t*-test and analysis of variance) were applied to determine the significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of Yucaizol on longitudinal growth of rice

The first BR biosynthesis inhibitor was discovered by Min et al (1999). Assessment of the target site of brassinazole revealed that it binds with CYP90B1 from *Arabidopsis* (Asami et al, 2001). To date, brassinazole has been widely used in BR research (Blackwell and Zhao, 2003). Determining the biological activity of brassinazole in rice indicated that it did not retard rice plants at a high concentration (Sekimata et al, 2001). To determine the biological activity of Yucaizol on the retardation of rice seedlings, we treated rice seedlings with different concentrations of Yucaizol. As shown in Fig. 2, Yucaizol induced dwarfism and retardation in rice seedlings in a dose-dependent manner. The IC50 value was found approximately 0.8 μmol/L. Our results clearly indicated that Yucaizol exhibits potent inhibitory activity on the retardation of rice seedlings.

Effect of Yucaizol on bending angle of lamina joint of rice

To distinguish the dwarfism characteristics induced by Yucaizol from GA biosynthesis inhibitors, we used leaf angle as a factor because BR deficient mutant rice plants display an erect leaf phenotype with a small leaf angle (Sakamoto et al, 2006). Thus, we determined the effects of Yucaizol on lamina joint bending angle in rice, which is a sensitive and specific assay method to