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Methyl jasmonate inhibits lamina joint inclination by repressing brassinosteroid biosynthesis and signaling in rice

Lijun Gan[a,*], Hong Wu[a], Dapeng Wu[b], Zhanfang Zhang[a], Zhengfei Guo[a], Na Yang[a], Kai Xia[a], Xie Zhou[a], Keimei Oh[c], Makoto Matsuoka[d], Denny Ng[e], Changhua Zhu[a,*]

[a] College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China
[b] Key Laboratory of Separation Science for Analytical Chemistry, Chinese Academy of Sciences, Dalian Institute of Chemical Physics, Dalian 116023, China
[c] Department of Biotechnology, Faculty of Bioresource Sciences, Akita Prefectural University, 241-438, Shimoshinjo Nakano, Akita 010-0195, Japan
[d] Bioscience and Biotechnology Center, Nagoya University, Chikusa, Nagoya 464-8601, Japan
[e] CH BIOTECH R&D CO., LTD. No.121, Xian'an Rd., Xianxi Township, Changhua County 50741, Taiwan, ROC

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ABSTRACT

Lamina joint inclination or leaf angle (the angle between the leaf blade and vertical culm) is a major trait of rice plant architecture. The plant hormone brassinosteroid (BR) is the main regulator of this trait, while other plant hormones, including ethylene, gibberellin, and auxin, also influence leaf angle. In this study, we found that methyl jasmonate (MeJA) also participates in regulating lamina joint inclination. MeJA decreased lamina joint inclination and inhibited the BR-induced increase in lamina joint inclination. Furthermore, addition of a BR synthesis inhibitor increased the extent of change in lamina joint inclination in response to treatment with a low concentration of MeJA (0.05 or 0.5 mg L^{-1}), but it did not alter the lamina joint inclination of plants treated with a high concentration of MeJA (5 mg L^{-1}). Further studies showed that MeJA treatment significantly repressed the expression of BR biosynthesis-related genes and decreased endogenous BRs levels. In addition, the lamina joint inclination in the OsBRI1 mutant d61-1 was less sensitive to MeJA compared with its wild type counterpart, and lithium chloride-induced inactivation of GSK3-like kinase, a negative regulator of BR signaling, partly rescued the MeJA-induced reduction in lamina joint inclination. Further studies showed that MeJA treatment reduced the mRNA levels of BR signaling and target genes. These results indicate that MeJA-inhibition of lamina joint inclination may depend on BR biosynthesis and the BR signaling pathway.

1. Introduction

Rice (*Oryza sativa*) is one of the most important cereal crops in the world and is used to feed nearly half of the global population. Rice plant architecture is a key agronomic trait that has a strong impact on grain yield. Leaf angle, or lamina joint inclination, is a major trait of rice plant architecture, because it is directly related to photosynthetic efficiency and grain filling [1–4]. Breeding of rice varieties with an ideal plant architecture is an efficient strategy for improving rice yield [5]. New rice cultivars with more erect leaves caused by reduced lamina joint inclination enhance light utilization, allow higher density planting, and may have higher grain yields [1,2].

Most characterized rice mutants with altered leaf angle have alterations in the biosynthesis or signaling of brassinosteroids (BRs), a class of plant steroid hormones. Reduced leaf angle caused by failure of the abaxial cells of the lamina joint to elongate has been observed in BR-deficient mutants such as *dwarf4-1* [1], *BR-deficient dwarf 1 (brd1)* [6], *brd2* [7], and *d2* [8], in BR signaling mutants such as loss-of-function BR receptor mutant *OsBRI1* [9], and in transgenic plants that suppress *OsBZR1* [10]. In contrast, enhanced bending of the lamina joint has been observed in transgenic plants overexpressing the BR biosynthetic gene sterol C-22 hydroxylase [11] and in positive regulators of BR signaling such as *BRASSINOSTEROID UPREGULATED 1* (*BU1*) [12] and *OsGRAS19*, a new member of the GRAS family [13]. Furthermore, rice seedlings treated with exogenous BRs exhibited enlarged leaf angles in a dose-dependent manner. Thus, increased leaf angle is a typical effect of increased BR in rice [14,15]. The rice lamina joint inclination assay is an extremely sensitive assay for detecting BR activity, based on measuring the angles between the lamina and the second

* Corresponding authors. Fax: +86 25 8439 5869.
E-mail addresses: ganlj@njau.edu.cn (L. Gan), zch@njau.edu.cn (C. Zhu).

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leaf sheath after BR treatment. It has been used extensively to isolate naturally occurring BRs and to detect the biological activity of chemically synthesized BRs [16,17].

The BR signaling pathway in rice is similar to that in Arabidopsis. One key component of the BR signaling pathway in rice is OsBRI1 (BRASSINOSTEROID INSENSITIVE1), a plasma membrane leucine-rich repeat (LRR)-receptor kinase [9] that binds BR to activate the BR signaling pathway. Another is OsGSK2, a key negative regulator of BR signaling that acts downstream of OsBRI1. It is an ortholog of BIN2 in Arabidopsis and encodes a GSK3-like kinase [18]. A third component is OsBZR1 (BRASSINAZOLE-RESISTANT 1), a transcription factor that positively regulates BR signaling to activate the expression of BR-responsive genes; it is phosphorylated by GSK3-like kinases [19,20].

Other plant hormones are also involved in regulating leaf angle in rice plants. Ethylene was reported to participate in BR-induced rice lamina inclination [15]. Reduced expression of *SPINDLY*, a negative regulator of the gibberellic acid (GA) signaling pathway, resulted in an increase in leaf angle [21]. Auxin and BR act synergistically to increase leaf angle and a C-22-hydroxylated BR was involved in auxin-induced lamina joint bending [15,22]. The gain-of-function mutants *leaf inclination 1* (l*c1-D)* and *tld1-D* encode OsGH3, an indoe-3-acetic acid (IAA) amido synthetase, which can conjugate excess IAA into various amino acids, thereby reducing the amount of free auxin [3,23]. Interestingly the reduction in free auxin results in increased leaf angles, indicating that auxin homeostasis plays a crucial role in regulating leaf inclination. Recently, it was reported that auxin response factor OsARF19 controls leaf angle through positive regulation of *OsGH3-5* and *OsBRI1* [24]. Strigolactones (SLs) are also reported to inhibit lamina joint inclination at the seedling stage [25].

Jasmonates (JAs) are newly identified plant hormones involved in plant responses to biotic and abiotic stresses [26–28]. In addition to defense, JAs are also important regulators of several facets of plant growth and development, including induction of trichome formation, induction of tuber formation, regulation of reproductive organ development, inhibition of seed germination, and inhibition of root growth [27,29]. In this study, we characterized the interaction of JA and BR in the regulation of lamina joint inclination. We found that methyl jasmonate (MeJA) treatment could reduce leaf angle and inhibit BR-induced increases in lamina joint inclination. This effect of MeJA might be dependent on BR biosynthesis and the BR signaling pathway.

2. Methods

2.1. Plant materials

Rice (*O. sativa*) japonica cultivar Nipponbare was used in this study. BR-insensitive mutant *d61-1* [9] and its wild type counterpart Taichung 65 (T65) were used to assess the interaction of BR and MeJA.

2.2. Lamina joint inclination assay

The lamina joint assay using excised leaf segments was performed as described previously [14]. Rice seeds were sterilized, soaked and imbibed for 24 h at 30 °C. Synchronous seeds were selected and grown in the dark for 8 days at 30 °C. Uniform seedlings were then sampled by excising segments comprising 1 cm of the second leaf blade, the lamina joint and 1 cm of the leaf sheath under dim light conditions. These segments were floated on distilled water for 24 h and then incubated in 2.5 mM maleic acid potassium solution containing various treatments for 48 h at 30 °C in the dark. The angle between the lamina and sheath was measured by analyzing digital images using Motic Images Plus 2.0 software (China Group Co., Ltd.).

Brassinolide (BL; Sigma), MeJA; Wako Pure Chemical Industries and 2RS,4RS-1-{2-(4-chlorophenyl)-4-[2-(2-chloro)-ethyl]-1,3-dioxolan-2-ylmethyl}-1*H*-1,2,4-triazole (YCZ) were dissolved in 100% ethanol and then diluted to the required concentrations with sterile distilled water. The final concentration of ethanol did not exceed 0.1%. Mock treatment control and test samples contained equal concentrations of ethanol. In experiments evaluating the interaction of BL and MeJA, BL (5 $\mu g\,L^{-1}$) was combined with different concentrations of MeJA (0, 0.05, 0.5, and 5 $mg\,L^{-1}$). In experiments evaluating the interaction of YCZ and MeJA, YCZ (0.1 $mg\,L^{-1}$) was combined with different concentrations of MeJA (0, 0.05, 0.5, and 5 $mg\,L^{-1}$). In experiments evaluating the interaction of BL and MeJA in *d61-1* and its wild type, 0.5 $mg\,L^{-1}$ MeJA was combined with 5 $\mu g\,L^{-1}$ BL. In experiments evaluating the effect of MeJA and lithium chloride (LiCl) on lamina joint inclination, LiCl and KCl were dissolved in distilled water and the concentration of MeJA adjusted to 5 $mg\,L^{-1}$.

The *in vivo* lamina joint assay by the micro-drop method was performed as described previously [17]. Rice seeds were sterilized, imbibed and grown in a phytotron, Four-day-old seedlings were used to perform the lamina joint inclination assay. A 1-μl drop of MeJA (500 $mg\,L^{-1}$) or BL (100 $mg\,L^{-1}$), dissolved in 95% ethanol was applied by micro-syringe to the lamina joint of the second leaf of seedlings. The angles between the leaf lamina of the second leaf blade and sheath were measured 2 days after treatment by analyzing the digital images using Motic Images Plus 2.0 software (China Group Co., Ltd.). Twenty plants were used for each treatment. This assay was carried out three times.

2.3. Quantification of endogenous brassinosteroids

Endogenous brassinosteroids were analyzed by the in-line matrix solid-phase dispersion-tandem solid phase extraction coupled with high performance liquid chromatography-tandem mass spectrometry system. Extraction purification and measurement of BRs were performed according to the method described by Wang et al. [30].

2.4. RNA extraction and quantitative RT-PCR

Leaf segments treated with 0.5 $mg\,L^{-1}$ MeJA with or without 5 $\mu g\,L^{-1}$ BL for 6 h were used to analyze gene expression. Total RNA was extracted using TRIzol reagent (Invitrogen). First-strand cDNA was synthesized from 2 μg DNase-treated RNA using the PrimeScript II first-strand cDNA synthesis kit (TaKaRa Bio) in a total volume of 20 μl according to the manufacturer's instructions. Synthesized cDNAs were used as templates for quantitative real-time PCR (RT-qPCR). RT-qPCR was performed using the ABI PRISM 7500 Fast Real-Time PCR system (Applied Biosystems, USA) using SYBR Premix Ex Taq^{TM} (TaKaRa Bio Inc., China), according to the manufacturer's instructions. RT-qPCR was repeated at least three times using separately harvested samples. The *ACTIN* gene was used as an internal control. *OsMYC2*, *OsAOC* and *OsLOX2* were used as reporter genes of MeJA treatment[27].The primers for *OsARF19* were 5′- TGCCAACAATAGCCCATTTACC-3′ and 5′- GCCACTGCGAGTTTTTCCATCT-3′. Primer pairs for *D2, D11*, *OsDARWF* and *OsBRI1* [21] and those for *OsXTH1* [31], *OsXTR1* [31], *BU1* [12], *ACTIN* [31], *OsBZR1* [32], *OsARF23* [24], *OsAOC* and, *OsMYC2* [33] and *OsLOX2*[34] have been described previously.

2.5. Data analysis

The results were expressed as means ± SE of at least three independent experiments with at least three replicates ($n = 20$). Data