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Effects of gibberellin and strigolactone on rice tiller bud growth

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Strigolactones (SLs) regulate diverse developmental phenomena. Rice SL biosynthesis and signaling mutants have an increased number of tillers and a reduced plant height relative to wild-type (WT) rice plants. In this study, we tested the effectiveness of gibberellin (GA) on restoring more tillering phenotype and dwarfism observed in both SL biosynthesis and signaling mutants. The application of GA to these mutants rescued the tiller bud outgrowth; however, the sensitivity to GA was different between the WT and the SL biosynthesis mutant. © Pesticide Science Society of Japan
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Introduction

Shoot branching, an important agronomic trait that determines crop yield, is primarily controlled by plant hormones. Auxin and cytokinin are major hormones that control shoot branching. Strigolactone (SL), a member of a new class of phytohormones, is also known to control shoot branching.^{1,2)} The relationship between the two classical hormones (auxin and cytokinin) and SLs in the regulation of shoot branching has been actively discussed with regard to rice, Arabidopsis, and pea plants.³⁻⁵⁾

SLs are branching inhibitors biosynthesized from beta-carotene by beta-carotene isomerase (D27), two carotenoid cleavage dioxygenases (D10 and D17), and P450s (Os01g0700900 and Os01g0701400) in rice.^{6,7)} The SL signaling component *D3* encodes an F-box leucine-rich repeat (LRR)-containing pro-

tein, which functions downstream of this branching inhibitor.⁸⁾ After the discovery of SL as a branching inhibitor, a rice mutant, *d14/d88/htd2*, which encodes the SL receptor, has been reported to be defective in novel genes that transduce the SL signal.⁹⁻¹¹⁾ The *d14* and *d3* mutants are insensitive to exogenous SL application and contain higher levels of SL than wild-type (WT) plants.⁹⁻¹¹⁾ Rice mutants defective in both SL biosynthesis and signal transduction also exhibit partial dwarfism.¹²⁾

Gibberellin (GA) is a crucial plant hormone that regulates developmental processes throughout the life cycle of plants.¹³⁾ Plants defective in both GA biosynthesis and GA signal transduction show typical phenotypes such as dwarfism; small, dark green leaves; prolonged germination dormancy; retardation of root growth; suppression of flowering; reduced seed production; and male sterility.¹³⁾ Although it is reported that GA-deficient rice plants also exhibit early and increased tillering and dwarfism,^{14,15)} there have been no reports of the relationship between GA signaling and SL signaling in the tillering and dwarfism of rice plants. This report investigated the relationship between GA and SL in controlling tiller bud outgrowth and plant height.

Materials and Methods

1. Plant materials

The wild-type rice varieties (*Oryza sativa*) used in this study were Shiokari (*d3-1*, *d10-1*, *d14-1*, *d17-1*, and *d27-1*)^{9,12,16)} and Nipponbare (*d10-2*).¹⁷⁾

2. Rice hydroponic culture

Rice seeds were sterilized in 2.5% sodium hypochlorite solution containing 0.01% Tween 20 for 30 min, washed with sterile water six times, and incubated in sterile water at 25°C in the dark for 2 days. Germinated seeds were transferred into hydroponic culture media²⁾ solidified with 0.6% agar and cultured at 25°C under fluorescent white light with a 14-hr light and 10-hr dark photoperiod for 6 days. Each seedling was transferred to a brown glass vial containing 12 mL of hydroponic culture solution with or without GA₃ or GR24 (synthetic SL analog)¹⁸⁾ and grown under the same condition for 7 days. Plant height was measured from the ground to the tip of the longest leaf of the plant.

For prolonged cultivation, 2-week-old rice seedlings were transferred to a new vial containing 12 mL of hydroponic culture solution with or without GA₃ and grown under the same condition for 7 days.

Results and Discussion

The growth of second tiller buds in SL mutants, such as *d3-1*, *d10-1*, *d14-1*, *d17-1*, and *d27-1*, was observed when 2-week-old rice seedlings were grown hydroponically, but those of WT plants remained dormant.²⁾ To assess the role of GA in SL mu-

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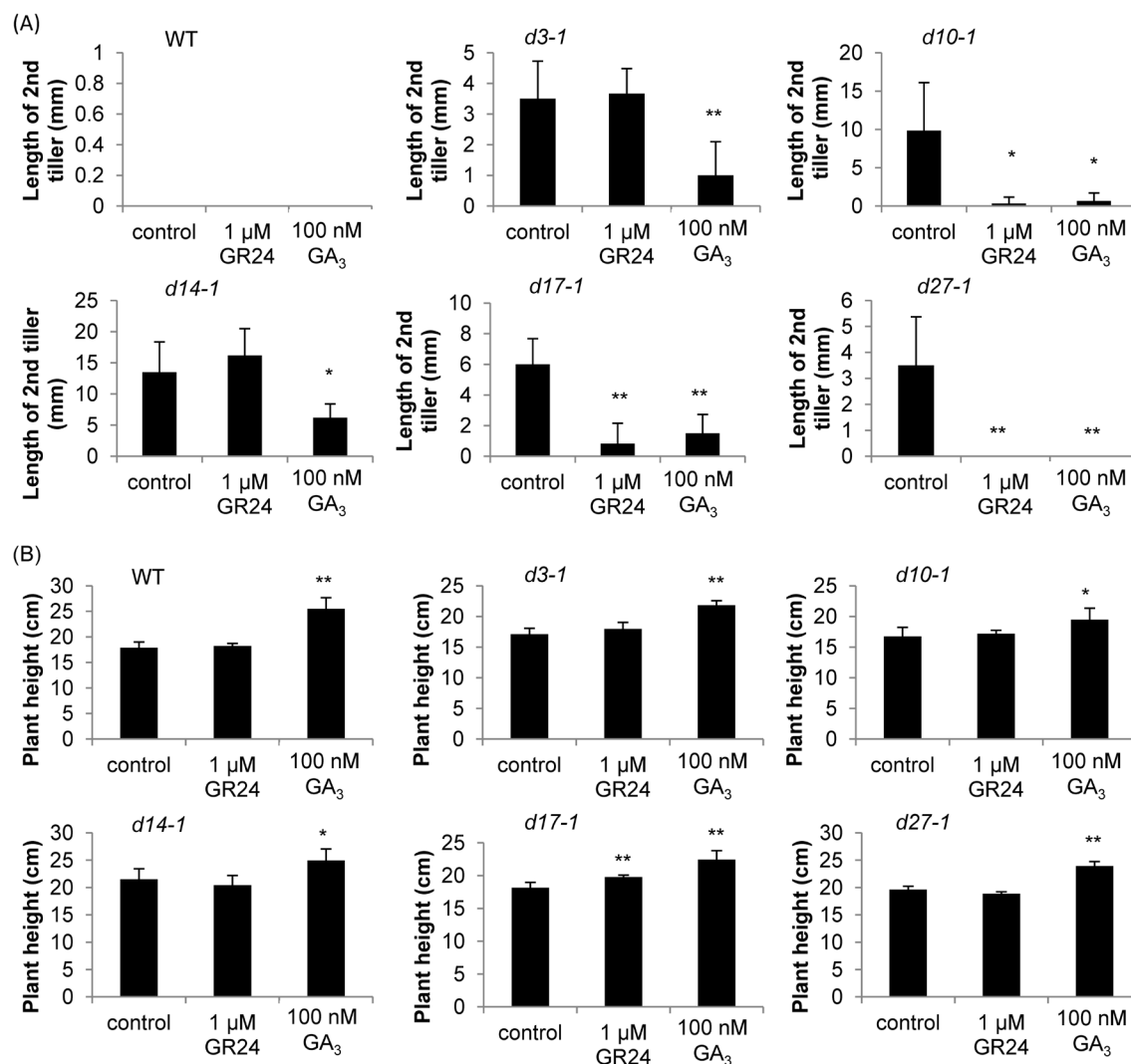


Fig. 1. Effects of SL and GA on tiller bud outgrowth and plant height in 2-week-old rice seedlings. (A) Lengths of second tillers in 2-week-old seedlings. The data are means \pm SD of six samples. (B) Plant heights of 2-week-old seedlings. The data are means \pm SD of six samples. * ($p < 0.05$) and ** ($p < 0.01$) indicate significant differences relative to controls.

tants, we first examined the effect of GA_3 on the outgrowth of second tiller buds and the plant height of mutants defective in SL biosynthesis (*d10-1*, *d17-1*, and *d27-1*) and signaling (*d3-1* and *d14-1*). Two-week-old WT rice ‘*Shiokari*’ did not show elongation of the second tiller as previously reported.²⁾ On the other hand, following application of GA_3 but not SL, the outgrowth of second tiller buds in all of the mutants was significantly suppressed, and plant height in these mutants increased. The application of GR24 suppressed tiller bud outgrowth in SL biosynthesis mutants (*d10-1*, *d17-1*, and *d27-1*) but not in SL signaling mutants (*d3-1* and *d14-1*). In addition, GR24 treatment did not produce any increase in the plant height of these mutants (Fig. 1A and B).

Next, we estimated the differences in GA sensitivity between WT ‘*Nipponbare*’ and *d10-2* rice seedlings. Two-week-old rice seedlings did not show second and third tiller bud outgrowth in the WT, but the second and third tiller buds grew out in hydro-

ponically grown 3-week-old rice seedlings of both the WT and *d10-2* mutants.²⁾ The effects of GA_3 were determined by measuring the tiller lengths and plant heights in 3-week-old seedlings. In the WT, GA_3 treatment suppressed the elongation of the second and third tiller buds and increased plant height in a concentration-dependent manner. In the *d10-2* mutant, although the elongation of the first and second tiller buds and the increase in plant height showed tendencies similar to those in the WT treated with GA_3 , its responses to GA_3 were weaker than those of the WT (Fig. 2). The outgrowth of the second tiller buds was significantly inhibited in the *d10-2* mutant by treatment with 10 nM and 50 nM GA_3 , whereas it was significantly suppressed in WT plants with only 1 nM GA_3 (Fig. 2). Plant height was also significantly increased in the *d10-2* mutant by treatment with 50 nM and 200 nM GA_3 , whereas it was significantly elongated in WT plants with 1 nM GA_3 (Fig. 2). In addition, the outgrowth of the third tiller bud was not suppressed by the GA_3 treatment.

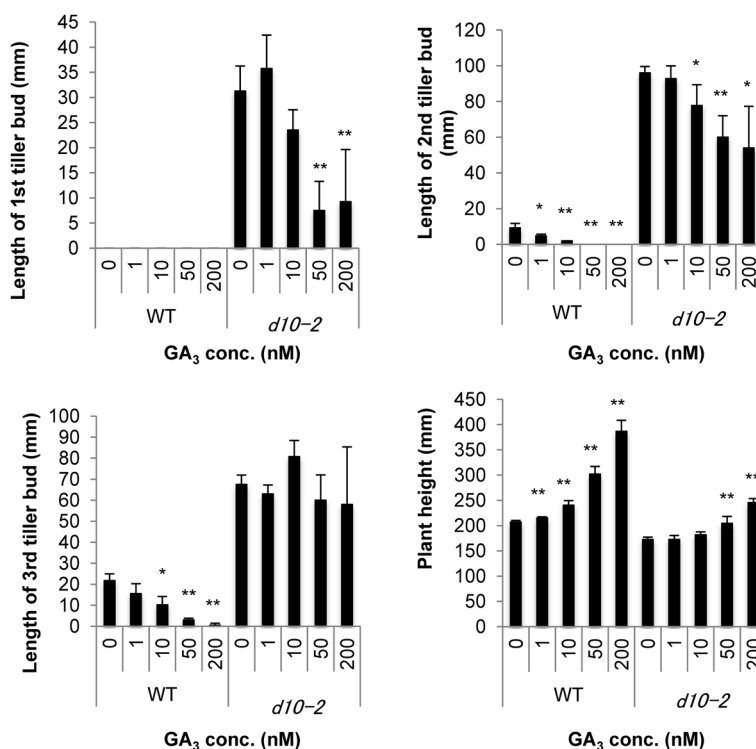


Fig. 2. Effect of GA on tiller bud growth and plant height in 3-week-old rice. First, second, and third tiller bud lengths and plant heights of 3-week-old rice. Using a *t*-test, the significant difference of * ($p < 0.05$) and ** ($p < 0.01$) as compared to 0 nM GA₃-treated plants was obtained. Data are means \pm SD ($n = 6$).

These results indicate the existence of novel cross-talk between SL and GA signaling in the outgrowth of tiller buds and elongation of plant height.

GA and SL can reduce the number of tillers or branching. In Arabidopsis, it has been reported that both SL and GA regulate auxin transport.^{19,20} It is suspected that both SL and GA may play a role in regulating tiller bud outgrowth by regulating auxin transport. In addition, Luisi *et al.* (2011) reported that the endogenous level of bioactive GA modulates the response of decapitated pea plants to SL, suggesting the possible involvement of the auxin transport in SL–GA cross-talk.²¹ However, the ways SL and GA regulate auxin transport appear to be different: SL promotes the accumulation of PIN proteins, whereas GA represses the accumulation of PIN proteins.^{19,20} This may be one of the reasons GA sensitivity in tiller bud outgrowth and the increase in plant height were different between the WT and the *d10-2* mutant.

SL regulates various morphological changes, such as branching, leaf senescence, root hair elongation, and mesocotyl elongation.^{1,2,22–25} Because auxin, cytokinin, and gibberellin also control similar morphological changes with SL, studies of SL-signaling cross-talk with auxin and cytokinin continue to expand. However, there are very few reports on the cross-talk between SL and GA. Notably, de Saint Germain *et al.* (2013) reported that SL acts independently of GA in stimulating internode elongation in pea plants.²⁶ Recently, we found two evidences of the existence of cross-talk between SL and GA signaling. One is the SL-dependent interactions of D14 with SLR1, which is one of the

components of GA signaling *in vitro* and *in vivo*.²⁷ Another is the GA-signaling-dependent regulation of the expression levels of SL biosynthesis genes.²⁸ In the present study, we found that the responses to GA in tiller bud outgrowth and plant elongation were different in the WT and *d10-2* mutants. Our findings indicate the existence of physiological cross-talk between SL and GA in not only the regulation of SL biosynthesis but also tiller bud outgrowth and plant elongation. Further investigation will reveal the important mechanism of SL–GA cross-talk that is useful for controlling the morphological changes affecting crop yield.

Acknowledgements

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References

- 1) V. Gomez-Roldan, S. Fermas, P. B. Brewer, V. Puech-Pagès, E. A. Dun, J. P. Pillot, F. Letisse, R. Matusova, S. Danoun, J. C. Portais, H. Bouwmeester, G. Bécard, C. A. Beveridge, C. Rameau and S. F. Rochange: *Nature* **455**, 189–194 (2008).
- 2) M. Umehara, A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N. Take-da-Kamiya, H. Magome, Y. Kamiya, K. Shirasu, K. Yoneyama, J. Kyo-zuka and S. Yamaguchi: *Nature* **455**, 195–200 (2008).
- 3) J. Xu, M. Zha, Y. Li, Y. Ding, L. Chen, C. Ding and S. Wang: *Plant Cell Rep.* **34**, 1647–1662 (2015).
- 4) H. Sun, J. Tao, M. Hou, S. Huang, S. Chen, Z. Liang, T. Xie, Y. Wei, X. Xie, K. Yoneyama, G. Xu and Y. Zhang: *Ann. Bot.* **115**, 1155–1162 (2015).
- 5) H. Sun, J. Tao, S. Liu, S. Huang, S. Chen, X. Xie, K. Yoneyama, Y.

- Zhang and G. Xu: *J. Exp. Bot.* **65**, 6735–6746 (2014).
- 6) A. Alder, M. Jamil, M. Marzorati, M. Bruno, M. Vermathen, P. Bigler, S. Ghisla, H. Bouwmeester, P. Beyer and S. Al-Babili: *Science* **335**, 1348–1351 (2012).
 - 7) Y. Zhang, A. D. van Dijk, A. Scaffidi, G. R. Flematti, M. Hofmann, T. Charnikhova, F. Verstappen, J. Hepworth, S. van der Krol, O. Leyser, S. M. Smith, B. Zwanenburg, S. Al-Babili, C. Ruyter-Spira and H. J. Bouwmeester: *Nat. Chem. Biol.* **10**, 1028–1033 (2014).
 - 8) P. Stirnberg, K. van De Sande and H. M. Leyser: *Development* **129**, 1131–1141 (2002).
 - 9) T. Arite, M. Umehara, S. Ishikawa, A. Hanada, M. Maekawa, S. Yamaguchi and J. Kyozuka: *Plant Cell Physiol.* **50**, 1416–1424 (2009).
 - 10) Z. Gao, Q. Qian, X. Liu, M. Yan, Q. Feng, G. Dong, J. Liu and B. Han: *Plant Mol. Biol.* **71**, 265–276 (2009).
 - 11) W. Liu, C. Wu, Y. Fu, G. Hu, H. Si, L. Zhu, W. Luan, Z. He and Z. Sun: *Planta* **230**, 649–658 (2009).
 - 12) S. Ishikawa, M. Maekawa, T. Arite, K. Onishi, I. Takamura and J. Kyozuka: *Plant Cell Physiol.* **46**, 79–86 (2005).
 - 13) S. Yamaguchi: *Annu. Rev. Plant Biol.* **59**, 225–251 (2008).
 - 14) S. F. Lo, S. Y. Yang, K. T. Chen, Y. I. Hsing, J. A. Zeevaart, L. J. Chen and S. M. Yu: *Plant Cell* **20**, 2603–2618 (2008).
 - 15) S. Ito, N. Kitahata, M. Umehara, A. Hanada, A. Kato, K. Ueno, K. Mashiguchi, J. Kyozuka, K. Yoneyama, S. Yamaguchi and T. Asami: *Plant Cell Physiol.* **51**, 1143–1150 (2010).
 - 16) H. Lin, R. Wang, Q. Qian, M. Yan, X. Meng, Z. Fu, C. Yan, B. Jiang, Z. Su, J. Li and Y. Wang: *Plant Cell* **21**, 1512–1525 (2009).
 - 17) T. Arite, H. Iwata, K. Ohshima, M. Maekawa, M. Nakajima, M. Kojima, H. Sakakibara and J. Kyozuka: *Plant J.* **51**, 1019–1029 (2007).
 - 18) E. M. Mangnus and B. Zwanenburg: *J. Agric. Food Chem.* **40**, 1066–1070 (1992).
 - 19) S. Crawford, N. Shinohara, T. Sieberer, L. Williamson, G. George, J. Hepworth, D. Müller, M. A. Domagalska and O. Leyser: *Development* **137**, 2905–2913 (2010).
 - 20) B. C. Willige, E. Isono, R. Richter, M. Zourelidou and C. Schwechheimer: *Plant Cell* **23**, 2184–2195 (2011).
 - 21) A. Luisi, R. Lorenzi and C. Sorce: *Plant Growth Regul.* **65**, 415–419 (2011).
 - 22) Y. Yamada, S. Furusawa, S. Nagasaka, K. Shimomura, S. Yamaguchi and M. Umehara: *Planta* **240**, 399–408 (2014).
 - 23) H. Koltai: *New Phytol.* **190**, 545–549 (2011).
 - 24) S. Ito, T. Nozoye, E. Sasaki, M. Imai, Y. Shiwa, M. Shibata-Hatta, T. Ishige, K. Fukui, K. Ito, H. Nakanishi, N. K. Nishizawa, S. Yajima and T. Asami: *PLoS ONE* **10**, e0119724 (2015).
 - 25) Z. Hu, H. Yan, J. Yang, S. Yamaguchi, M. Maekawa, I. Takamura, N. Tsutsumi, J. Kyozuka and M. Nakazono: *Plant Cell Physiol.* **51**, 1136–1142 (2010).
 - 26) A. de Saint Germain, Y. Ligerot, E. A. Dun, J. P. Pillot, J. J. Ross, C. A. Beveridge and C. Rameau: *Plant Physiol.* **163**, 1012–1025 (2013).
 - 27) H. Nakamura, Y. L. Xue, T. Miyakawa, F. Hou, H. M. Qin, K. Fukui, X. Shi, E. Ito, S. Ito, S. H. Park, Y. Miyauchi, A. Asano, N. Totsuka, T. Ueda, M. Tanokura and T. Asami: *Nat. Commun.* **4**, 2613 (2013).
 - 28) S. Ito, D. Yamagami, M. Umehara, A. Hanada, S. Yoshida, Y. Sasaki, S. Yajima, J. Kyozuka, M. Ueguchi-Tanaka, M. Matsuoka, K. Shirasu, S. Yamaguchi and T. Asami: *Plant Physiol.* **174**, 1250–1259 (2017).