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CITATION:

Nakagawa, Yoshiaki ...[et al]. Effects of brassinolide on the growing of rice plants. Journal of Pesticide Science 2021, 46(3): 274-277

ISSUE DATE:

2021-08-20

URL:

http://hdl.handle.net/2433/277266

J. Pestic. Sci. **46**(3), 274–277 (2021)
DOI: 10.1584/jpestics.D21-024

JOURNAL OF PESTICIDE SCIENCE

Brief Report

Effects of brassinolide on the growing of rice plants

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(Received March 31, 2021; Accepted April 30, 2021)

Brassinosteroids are plant steroid hormones that are essential for plant growth. When germinated rice seeds were treated with brassinolide (BL), stems were elongated and root spiral formation was observed at 5 nM of BL. Such root spiral formation was not induced by other plant hormones such as auxin and gibberellin. Since weak non-steroidal brassinolide-like compound (NSBR1) also induced spiral formation, this root spiral induction can be used as the index in the search for BL-like compounds.

Keywords: brassinolide, brassinosteroid, rice seeds, *Oryza sativa*, NSBR1.

Introduction

About a half century ago, Michel *et al.* of the USDA reported that *Zea mays* pollen contained ingredients expressing plant growth–promoting activity. The title of the paper is "Brassins—a new family of plant hormones from rape pollen."[1] The structure of the brassin was first characterized in 1979 by X-ray analysis and named *brassinolide* (BL; Fig. 1).[2] A few years later, Yokota *et al.* found castasterone (CS; Fig. 1), from chestnut insect gall, which contains a six-membered cyclohexanone ring instead of a seven-membered lactone ring of BL.[3] These compounds have sterol skeletons constructed from four fused rings (A/B/C/D) and hydroxyalkyl chains at the C17 position that were collectively called *brassinosteroids* (BRs).[4]

Before the characterization of BL by the USDA group, Marumo's group at Nagoya University also had been trying to isolate and characterize new auxin-like substances that are called *Distylium* factors. They used the rice lamina inclination assay (RLIA), which was originally designed to detect synthetic auxins,[8] to measure the activity of BRs.[5–7] Wada *et al.* also reported a simple bioassay for brassinosteroids using a wheat leaf–unrolling test.[9]

With the discovery of BR-deficient mutants,[10] BRs were recognized as the sixth plant hormone. *Arabidopsis* mutants *det2* and *cpd* are cabbage-like dwarfs in the light, and these phenotypes were not recovered to wild type by classical hormones such as auxins and gibberellin but were only rescued by BRs. Asami *et al.* discovered a BR biosynthesis inhibitor, brassinazole (Brz), which induced dwarfing in *Arabidopsis*.[11,12] To that point, more than 70 BRs had been identified in nature, chemically synthesized, and the activity evaluated using rice, *Arabidopsis*, cress, beans, *etc.* In these plants, RLIA is a good method because

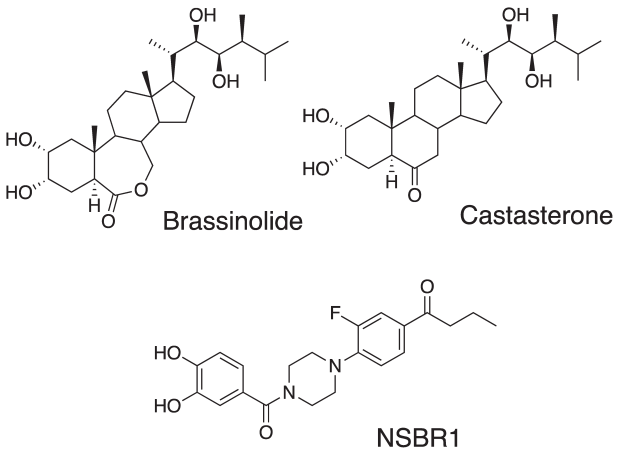

Fig. 1. Structures of brassinosteroids and an agonist (NSBR1).

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Published online June 11, 2021

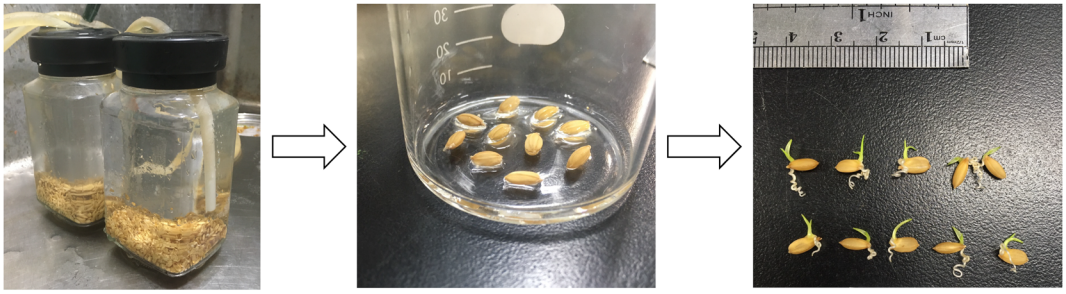

Fig. 2. Germination of rice seeds and observation of the root spiral formation. (A) Seeds were soaked in running tap water for a few days. (B) Ten germinated seeds with buds were transferred to a 50 mL beaker, and 2 mL of water was added. (C) Seeds, shoots, and roots were grown at 25°C for 2 days in the light.

the activity can be determined quantitatively. Using RLIA, we measured the hormonal activity quantitatively and discussed the structure activity relationship.[13–15] With further study, we discovered the novel non-steroidal brassinolide-like compound NSBR1 (Fig. 1)[16] using *in silico* screening; in this study, however, we used the bioassays of *Arabidopsis*, since RLIA was too time consuming and inappropriate for assaying a number of candidate compounds.

Even BRs show interesting activity and are expected to be utilized in agriculture, as the bioassay methods that have been used to date are time consuming and not reproducible. Therefore, a simple and user-friendly bioassay method is desired. Here we developed a new and simple BL-specific bioassay method using rice seeds.

Materials and methods

Rice seeds were obtained from *Oryza sativa* Koshihikari cultivated in Shiga Prefecture. Seeds were soaked in running tap water for a few days. Ten germinated seeds with buds (less than 2 mm) were put on the bottoms of 50 mL beakers, and then 2 mL of water containing test compounds was added. Ten microliters of the stock solution in ethanol or DMSO of the test compound was added to 2 mL of water in a glass test tube and mixed by repetitive pipetting. DMSO can be used to prepare the stock solution, although DMSO solution is not suitable for RLIA. Seeds were grown at 25°C for 2 days in the light, and the growth of rice plants was observed. This method is shown in Fig. 2.

Results and discussion

Rice plants were grown in water containing BL at various concentrations after germination. As shown in Fig. 3, the hypocotyl length became longer with BL treatment at higher concentrations 48 hr after germination. Hypocotyl elongation was stimulated in the concentration-dependent manner shown in Fig. 3. This phenomenon was observed reproducibly; however, measuring the hypocotyl length was cumbersome and not appropriate for the quantitative assay. We could not see any significant difference among treatments with different concentrations on the next day (64 hr after treatment), as shown in Fig. 3.

The morphology of rice plant seedlings was, however, significantly changed by BL treatment at higher concentrations. The stems became evidently robust, with stronger green color at 5×10^{-9} M BL. In particular, a big difference was observed in the root, in which the spiral was induced, as shown in Fig. 4.

In order to evaluate the effect on the root quantitatively, the seeds were treated with various concentrations of BL. As shown in Fig. 5, spiral induction was observed even at 5×10^{-9} M; as the concentration was increased, the spiral formation became more

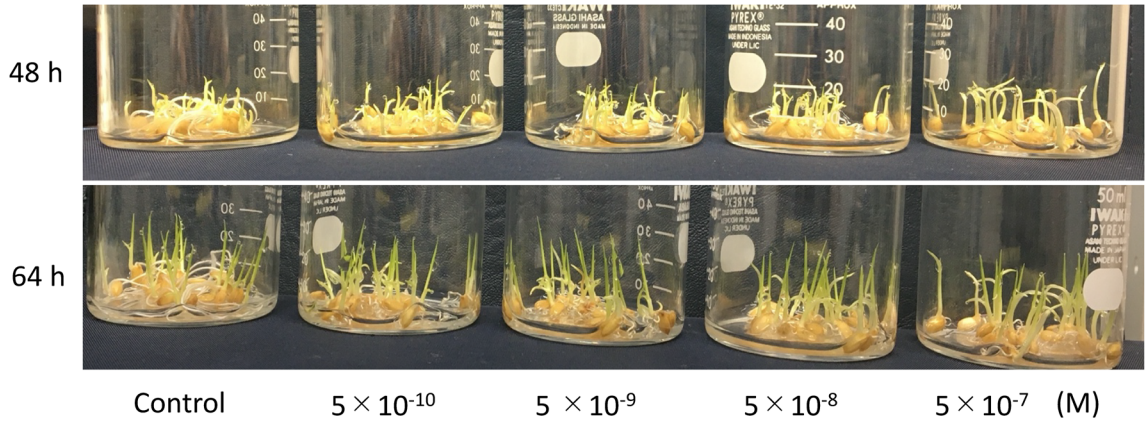

Fig. 3. Growth of rice plant in water containing BL at various concentrations.

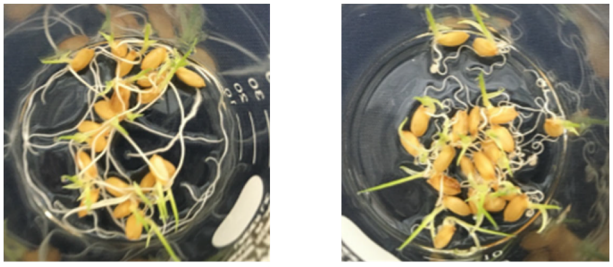

Fig. 4. Observation of roots 64 hr after the germination of seeds treated with (A) solvent and (B) BL at 5×10^{-9} M.

remarkable. NSBR1, the non-steroidal brassinolide-like compound[16] discovered in our laboratory, also induced the spiral in two of ten seeds treated at 5×10^{-5} M (Fig. 5-B). We also examined the effect of an antagonist, the 3,4-difluoro-substituted analog of NSBR1, which was discovered in the assay against *Arabidopsis*.[16] As shown in Fig. 5-C, the spiral formation induced by BL at 5×10^{-8} M was released by treating with an antagonist at 1.7×10^{-4} M. The release of spiral induction in the roots can be used to bioassay antagonists of BRs.

We also examined the effect of gibberellin on the growth of roots. No spiral was induced in the root at 5×10^{-6} M of GA_3. At a higher concentration (5×10^{-5} M) of GA_3, root growth was significantly inhibited. Likewise, auxins did not induce the spiral, even at the higher concentration (5×10^{-5} M). Since this spiral induction is specific to BRs and the potency can be determined quantitatively using minimum effective concentrations, a study of the structure–activity relationship for spiral induction by various BRs[13–15] is in progress.

Acknowledgements

We thank Mr. Sadao Hirai for kindly supplying the rice seeds.

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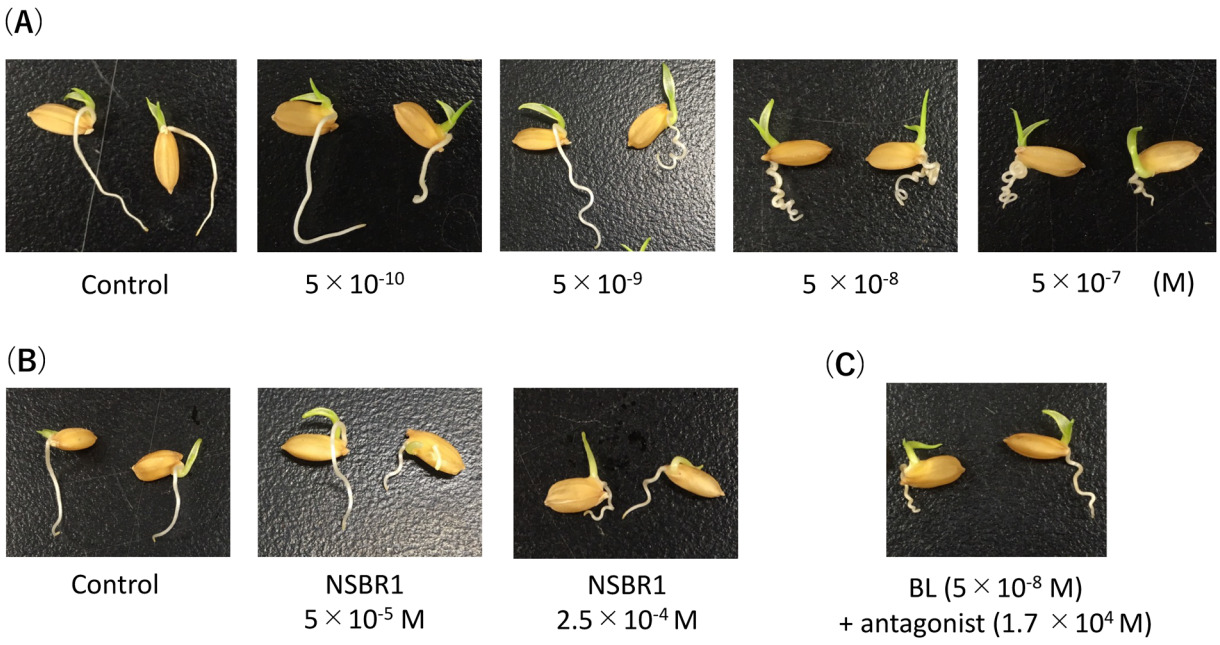

Fig. 5. Spiral induction in rice seeds. (A) BL at various concentrations. (B) NSBR1 at 5×10^{-5} M and 2.5×10^{-4} M. (C) BL antagonist at 1.7×10^{-4} M.

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