J. Plant Biol. 40 (4) : 240~244 (1997)

Optimization of a Rice Lamina Inclination Assay for Detection of Brassinosteroids: I. Effect of Phytohormones on the Inclination Activity

Han, Kwang-Seok, Kwang-Wook Ko, Sang-June Nam, Seung-Hyun Park and Seong-Ki Kim*

Department of Biology, Chung-Ang University, Seoul 156-756, Korea

Effect of ABA, GA_3, zeatin (Zea) and IAA on the inclination activity was examined by a rice lamina inclination assay using a Korean cultivar, Tongjin. Treatment with ABA, GA_3, Zea or IAA alone failed to increase the inclination response significantly at the concentration tested from 0.1 ppm to 10 ppm. However, treatment with 0.1 and 1 ppm ABA in the presence of brassinolide inhibited the inclination activity induced by treatment with brassinolide alone. And treatment with 0.1 and 1 ppm GA_3 or Zea in the presence of brassinolide strongly inhibited the inclination activity induced by treatment with brassinolide alone. On the other hand, the inclination activity by treatment with brassinolide alone was clearly promoted by treatment with 0.1 and 1 ppm IAA in the presence of brassinolide. Based on the synergistic effect induced by treatment with BR and IAA, we could develope an improved rice lamina inclination assay whose minimum detectable concentration of brassinolide is 0.00001 ppm /petri dish. The minimum detectable concentration in our assay was five times as low as that of the previous rice lamina inclination assay.

Keywords: a rice lamina inclination assay, plant hormones, brassinosteroids, synergistic response

A rice lamina inclination assay was originally developed for detecting indole-3-acetic acid (IAA) (Maeda, 1965). Wada *et al.* noticed that the assay was more sensitive to brassinosteroids (BR) rather than IAA (Wada *et al.*, 1981; Wada *et al.*, 1984). Since then, all naturally-occurring BR except the first BR, brassinolide have been successfully identified by the assay for guiding purification steps (Grove *et al.*, 1979; Kim, 1991). The relative biological activities of the BR have also been evaluated by the assay (Adam and Marquardt, 1986; Mandava, 1988; Yokota and Takahashi, 1986). The rice lamina inclination assay is thus considered to be a highly specific bioassay for BR.

Although a minium detectable concentration for BR in the rice lamina inclination assay is as low as 5×10^{-5} ppm (Wada *et al.*, 1984), it is not always low enough to detect BR in plant tissues. Especially, because the contents of BR in vegetatively growing tissues are as low as less than 10^{-3}-10^{-4} ng/kg fr. wt. (Yokota and Takahashi, 1986), the minium detectable amount of BR by the assay should be reduced for detecting BR in the tissues. In order to improve the sensitivity for detecting BR by the rice lamina inclination assay, we examined the effect of several phytohormones on the inclination activity induced by BR in the assay using a Korean cultivar, Tongjin. In this paper, the enhancement patterns of the hormones in the assay are discussed.

MATERIALS AND METHODS

Plant Materials

Seven rice (*Oriza sativum*) cultivars used in this study were donated by Dr. Kwang-Yun Cho in Korea Research Institute of Chemical Technology, Teajon, Korea in 1993. For increasing germination rate of the rice, the cultivars were kept at 4°C for more than one year before using in the rice lamina inclination assay.

Chemicals

Brassinolide [(2α, 3α, 22*R*, 23*R*)-tetrahydroxy-24*S*-methyl-7-oxa-5α-cholestan-6-one], castasterone [(2α,

*Corresponding author: Fax +82-2-820-5206

3α, 22*R*, 23*R*)-tetrahydroxy-24S-methyl-5-cholestan-6-one] and typhasterol [(3α, 22*R*, 23*R*)-trihydroxy-24S-methyl-5-cholestan-6-one] used as authentic standards in the assay were provided by Prof. Takao Yokota in Teikyo Universiy, Ustunomiya, Japan. Indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA) and zeatin (Zea) were purchased from Sigma Chemical Co., St. Louis, USA.

A Rice Lamina Inclination Assay

After soaking rice seeds in tap water for 48 h, the seeds were selected and cultivated on 1% agar plate in darkness at 25°C. Etiolated rice seedlings were grown for 7 days, and then uniform seedlings were selected. At approximate, 4 cm segment from shoot apex containing the second leaf lamina, the second lamina joint and sheath were excised. These segments were floated on distilled water for 24 h and uniformly bent segments were selected. Ten of the segments were incubated in a petri dish containing 20 ml distilled water and a finite amount of the test sample for 48 h under the same condition. Finally, the magnitude of the angle induced between the lamina and the sheath was measured. Every operation was conducted under red light wavelength except the final angle-measurement step.

RESULTS

Selection of a Korean Rice Cultivar for a Rice Lamina Inclination Assay

The usefulness of seven Korean rice cultivars for the rice lamina inclination assay was examined. All cultivars tested showed positive inclination activities for a standard brassinosteroids, brassinolide at the concentration tested (Fig. 1 and 2). In respect of concentration-activity relationship, however, three cultivars mentioned in Fig. 1 showed too strong (Choochung and Pongkwang) or too weak (Tongil) inclination activities at the concentration tested, giving rise to undesirable concentration-dependent results shown in Fig. 1. It thus appeared that the three cultivars were not suitable for the rice lamina inclination assay for detection of brassinosteroids. On the other hand, four cultivars mentioned in Fig. 2 revealed relatively reasonable concentration-dependent inclination activities at the same concentration ranges. Especially, a cul-

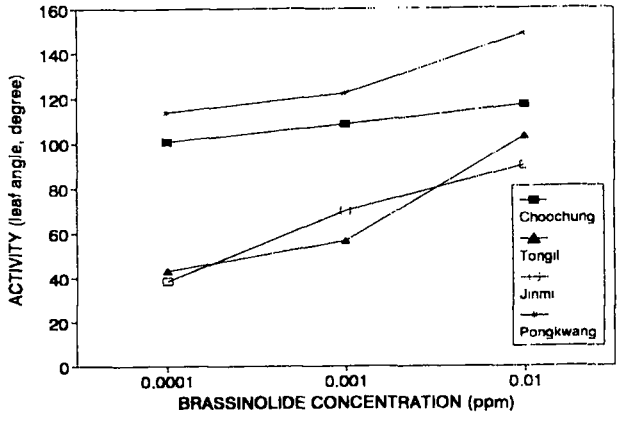

Fig. 1. The inclination activity of Korean rice cultivars in the rice lamina inclination assay I. The leaf angle of control in this assay was 30°. Each data point represents the mean of 10 replicates.

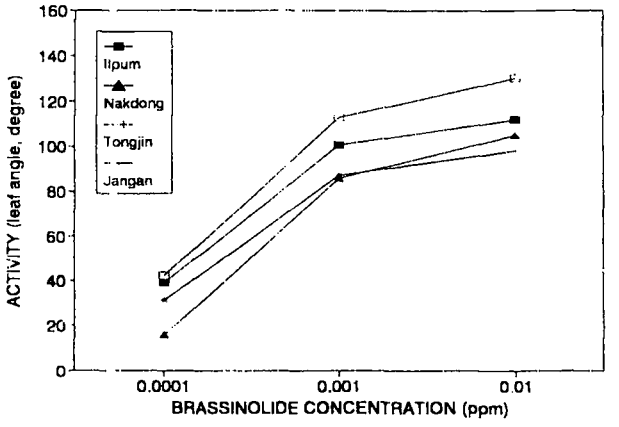

Fig. 2. The inclination activity of Korean rice cultivars in the rice lamina inclination assay II. The leaf angle of control in this assay was 300. Each data point represents the mean of 10 replicates.

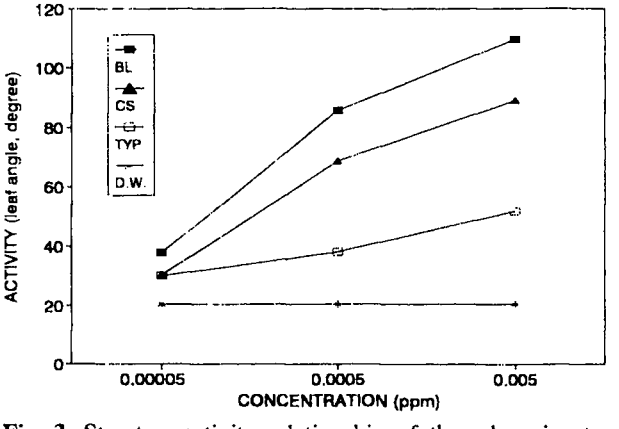

Fig. 3. Structure-activity relationship of three brassinosteroids in the rice lamina inclination assay using a rice cultivar, Tongjin. Each data point represents the mean of 10 replicates. BL: brassinolide, CS: castasterone, TYP: typhasterol and D.W.: distilled water (control).

tivar Tongjin showed a stronger inclination activity than those of other three cultivars. Among the seven Korean rice cultivars tested, therefore, a cultivar Tongjin was considered to be the most useful rice cultivar for the rice lamina inclination assay for detecting brassinosteroids.

Three authentic brassinosteroids, brassinolide, castasterone and typhasterol were evaluated by the rice lamina inclination assay using the rice cultivar Tongjin. As shown in Fig. 3, they showed different activities in the assay. Castasterone was about one-third as active as brassinolide. And typhasterol was about one-tenth as active as castasterone. These relative values for inclination activity are almost the same as those reported by a rice lamina inclination assay using a cultivar Koshihikari used as the most common rice cultivar for detecting brassinosteroids (Kim *et al.*, 1990). Thus our rice lamina inclination assay using a cultivar Tongjin is also useful for examination of brassinosteroids structure-activity relationship.

Effect of Plant Hormones on the Rice Lamina Inclination Activity

The inclination response for four kinds of plant hormones was examined in our rice lamina inclination assay using a cultivar Tongjin. As shown in Fig. 4, treatment with GA_3, ABA or Zea alone showed no inclination activity at concentration ranges from 0.1 ppm to 10 ppm. Treatment with IAA alone exhibited little inclination activity up to 1 ppm, and relatively weak activity at 10 ppm. Thus it is thought that a sole treatment of the plant hormones gives no significant inclination response at the concentration ranges in the assay.

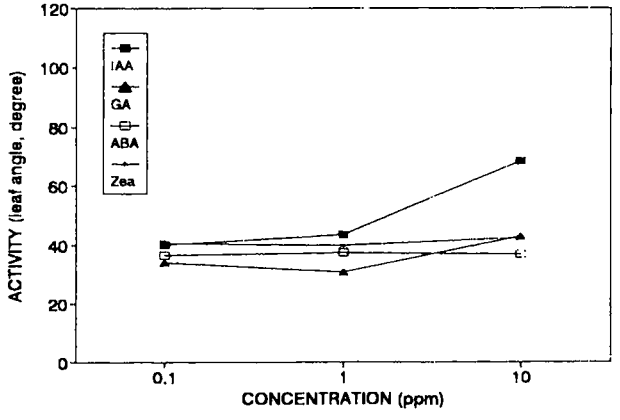

Fig. 4. Effect of phytohormones on the inclination activity measured by the rice lamina inclination assay using a rice cultivar, Tongjin. The leaf angle of control in this assay was 37°. Each data point represents the mean of 10 replicates.

The effect of the plant hormones on the inclination activity induced by treatment of brassinolide in the assay was investigated. As shown in Fig. 5, treatment with 0.1 ppm or 1 ppm ABA in the presence of brassinolide reduced the inclination activity about 2-3 and 5-7 fold less rather than treatment with brassinolide alone, respectively. Higher inhibitory responses for the inclination activity were obtained from treatment with GA_3 or Zea in the presence of brassinolide (Fig. 6 and Fig. 7). Treatment with 0.1 ppm GA_3 or Zea in the presence of brassinolide revealed about 5-10 fold less activity rather than that of treatment with brassinolide alone. And treatment with 1 ppm GA_3 or Zea in the presence of brassinolide de-

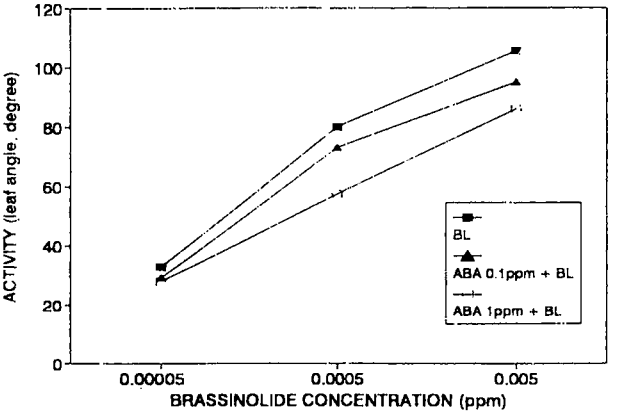

Fig. 5. Effect of ABA on the inclination activity induced by treatment with brassinolide (BL) in the rice lamina inclination assay using a rice cultivar, Tongjin. The leaf angle of control was 280. Each data point represents the mean of 10 replicates.

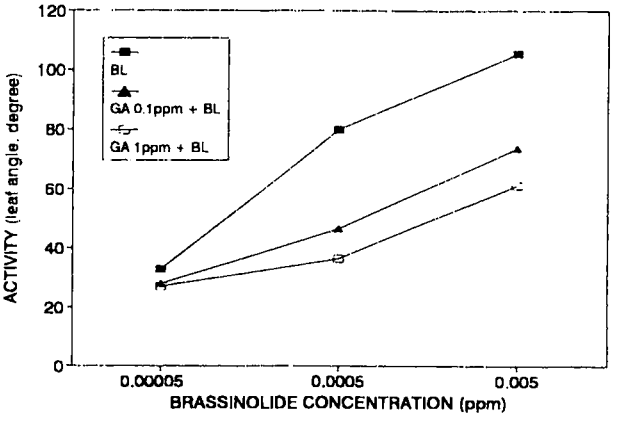

Fig. 6. Effect of GA3 on the inclination activity induced by treatment with brassinolide (BL) in the rice lamina inclination assay using a rice cultivar, Tongjin. The leaf angle of control in this assay was 28°. Each data point represents the mean of 10 replicates.

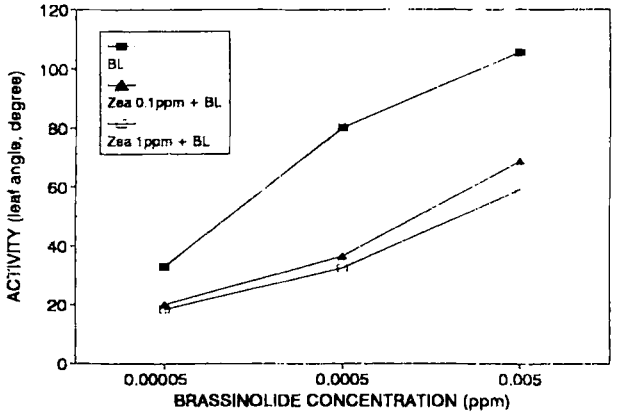

Fig. 7. Effect of Zea on the inclination activity induced by treatment with brassinolide (BL) in the rice lamina inclination assay using a rice cultivar, Tongjin. The leaf angle of control in this assay was 28°. Each data point represents the mean of 10 replicates.

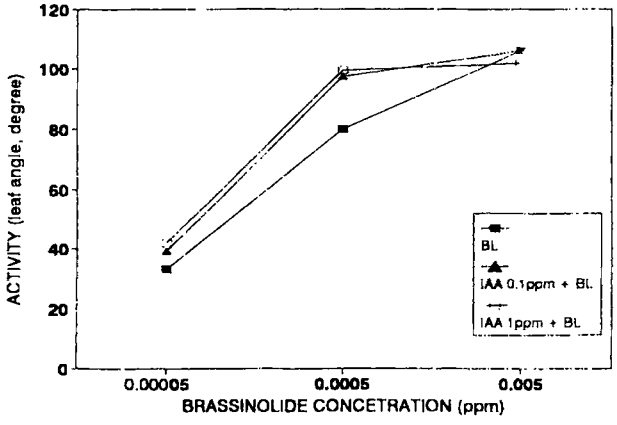

Fig. 8. Effect of IAA on the inclination activity induced by treatment with brassinolide (BL) in the rice lamina inclination assay using a rice cultivar, Tongjin. The leaf angle of control in this assay was 28°. Each data point represents the mean of 10 replicates.

creased the inclination activity as less as over 20 fold. On the other hand, treatment with IAA in the presence of brassinolide activated the inclination activity in the assay. Treatment with only 0.1 ppm IAA in the presence of brassinolide enhanced the activity about 5-7 fold higher than treatment with brassinolide alone. And treatment with 1 ppm IAA in the presence of brassinolide accelerated the synergistic response induced by 0.1 ppm IAA and brassinolide.

DISCUSSION

Interactions between BR and three plant hormones (IAA, ABA and cytokinin) have been investigated in the rice lamina inclination assay using Japanese rice cultivars (Wada *et al.*, 1984). They have reported that treatment with ABA or cytokinins in the presence of BR revealed a significant inhibitory effect on the inclination activity induced by treatment with BR alone. They have also reported that treatment with IAA in the presence of BR gave no significant change of the activity induced by treatment with BR alone. In our bioassay using a rice cultivar Tongjin, a similar inhibitory effect of treatment with ABA or cytokinin (Zea) in the presence of BR was also shown. However treatment with IAA in the presence of BR revealed a strong activating effect (a synergistic effect) on the activity induced by treatment with BR alone. Interaction between BR and gibberellins in the rice lamina inclination assay has not been established yet. Thus the strong inhibitory effect of gibberellins on the activity induced by BR is the first evidence for interaction between BR and gibberellins in the rice lamina inclination assay.

Takeno and Pharis (1982) have reported that a synergistic response between BR and IAA was shown in -drop assay using dwarf rice (Tanginbozu) seedlings for detecting gibberellins. Based on the synergistic effect between BR and IAA in the dwarf rice seedlings, then, Kim *et al.* (1990) developed a simple and improved dwarf rice lamina inclination assay (a dwarf rice u-drop assay) for detecting BR, which is also useful for detecting gibberellins co-currently. Thus it is thought that the synergistic response between BR and IAA in our rice lamina inclination assay using a Korean rice cultivar, Tongjin can be also used to develop a improved rice lamina inclination assay for detecting BR. Indeed the minimum detectable amount of BR in our rice lamina assay by treatment with IAA was less than 0.00001 ppm/petri dish (brassinolide equivalent), indicating that our bioassay is the most sensitive bioassay for detecting BR. Especially our bioassay may be very powerful for detecting BR in vegetatively growing plant tissues containing relatively low amount of BR.

ACKNOWLEDGEMENTS

This research was supported by the Chung-Ang University Research Grants in 1993. This paper was also supported in part by SPECIAL FUND for UNIVERSITY RESEARCH INSTITUTE, Korea Research Foundation.

LITERATURE CITED

Adam, G and V. Marquqrdt. 1986. Brassinosteroids. *Ph-*

ytochemistry. **25**: 1787-1799.

Grove, M.D., G.F. Spencer, W.K. Rohwedder, N. Mandava, J.F. Worley, Jr. J.D. Warthen, G.L. Steffens, J.L. Flippen-Aderson and Jr. J.C. Cook. 1979. Brassinolide, a plant growth-promoting steroid isolated *Brassica napus pollen. Nature.* **281**: 216-217.

Kim, S.K. 1991. Natural occurrences of brassinosteroids. *In* Brassinosteroids-Chemistry, Bioactivity and Application (ACS symposium series 474). H.G.C. Cutler, T. Yokota and G. Adam (eds). American Chemical Society, Washington D.C., pp. 26-35

Kim, S.K., H. Abe, C.H.A. Little and R.P. Pharis. 1990. Identification of two brassinosteroids from cambial region of Scots pine (*Pinus silverstris*) by gas chromatography-mass spectrometry, after detection using a dwarf rice lamina inclination bioassay. *Plant Physiol.* **94**: 1709-1713.

Mandava, N.B. 1988. Plant growth-promoting brassinosteroids. *Ann. Rev. Plant. Physiol. Mol. Biol.* **38**: 23-52.

Maeda, E. 1965. Rate of lamina inclination in excised rice leaves. *Physiol. Plant.* **18**: 813-927.

Takeno, K. and R.P. Pharis. 1982. Brassinosteroid-induced bending of the leaf lamina of dwarf rice seedlings: an auxin mediated phenomenon. *Plant Cell Physiol.* **23**: 1275-1281.

Wada, K., S. Marumo, H. Abe, T. Morishita., K. Nakamura, M. Uchiyama and K. Mori. 1984. A rice lamina inclination test-a micro-quantitative bioassay for brassinosteroids. *Agri. Biol. Chem.* **48**: 719-726.

Wada, K., S. Marumo, N. Ikekawa, M. Morisaki and K. Mori. 1981. Brassinolide and homobrassinolide promotion of lamina inclination of rice seedlings. *Plant Cell Physiol.* **22**: 323-325.

Yokota, T. and N. Takahashi. 1986. Chemistry, physiology and agricultural application of brassinolide and related steroids. *In* Plant growth substances 1985. Bopp M. (ed), Springer-Verlag, Berlin/Heidelberg/New York. pp. 129-138.

Received July 18, 1997
Accepted September 18, 1997