Effect of Synthetic Cytokinin Application on Pod Setting of Individual Florets within Raceme in Soybean

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Abstract: The application of synthetic cytokinin (6-benzylaminopurine, BA) to racemes of soybean genotype IX93-100 at 7 days after anthesis (DAA) enhanced pod-set percentage of the florets at the 5th position and above (numbered from the base on racis). The endogenous cytokinin (trans-zeatin riboside) content of individual florets was measured at the 1, 3, 5, 7th position every 3 days after anthesis. Cytokinin was detected only from the florets at 9 DAA, and the content was higher in the more proximal florets while it became negligible in the 7th floret. These results suggest that an increase in the amount of cytokinin in individual florets might enhance the pod setting of the florets positioned at the middle or distal part within the raceme.

Key words: Cytokinin application, Endogenous cytokinin, Pod setting, Soybean.
0.5 g N per pot as LP-70, a coated fertilizer that releases 80% of its total N in 70 days at 25°C in water (Chisso Asahi Fertilizer Co. Ltd., Tokyo). The other fertilizers applied were 2 g P₂O₅, 2 g K₂O and 5 g slaked lime per pot. The soil type used was a fine-textured Terrace Yellow soil, clayey (Classification Committee of Cultivated Soils, 1996). The seeds were inoculated with a strain (J1065) of *Bradyrhizobium japonicum* (Tokachi-noukyouren, Obihiro, Japan). The plants were grown in a greenhouse until the application of BA, and thereafter grown outdoors. The plants were irrigated adequately.

In 2007, endogenous cytokinin contents of individual florets were analyzed. Seeds were sown in the experimental field of Tohoku University on 7 June at a spacing of 100×10 cm. Soil type was the same as that used in the 2005 experiment. Nitrogen fertilizer was applied at a rate of 2 g m⁻² N as LP-70. The other fertilizers applied were 10 g m⁻² P₂O₅ and 10 g m⁻² K₂O. The seeds were inoculated with a strain (J1065) of *Bradyrhizobium japonicum* (Tokachi-Nokyoren, Obihiro). Weeds were removed by hand and insects were controlled using insecticides.

### 2. Examination of the effect of BA application

For preparation of BA solutions, a certain amount of BA was dissolved in 1 N KOH and diluted to 1 mM (BA) with 16 mM K-phosphate buffer (pH 6.4) containing 0.05% (v/v) Tween 80. The solution was dropped, using a syringe (0.05 mL per raceme) on racemes at the 7, 8 and 9th nodes (numbered from the most proximal node) on the main stem. BA was applied at 7 days after anthesis (DAA) of the most proximal florets within individual racemes. The DAA of individual floret was estimated according to the morphological change of control plants labeled with the date of anthesis. The BA concentration applied and the growth stage at the time of application were found to be most effective in enhancing pod setting of this genotype in our previous study (Nonokawa et al., 2007). Control plants received the solution without BA. The flowering, pod initiation and abscission of the treated racemes were monitored everyday until maturity. The pod-set percentage was calculated as follows; (number of pods)/(number of flowers) × 100.

### 3. Analysis of endogenous cytokinin content

For analysis of endogenous cytokinin contents of florets, several grams of racemes in fresh weight were collected from several plants at three-day intervals from anthesis (0, 3, 6, 9, 12 DAA). Sampled racemes were immediately divided into individual florets (1, 3, 5, 7th and above on rachis) and weighed, then frozen in liquid N and stored at −80°C until analysis.

Cytokinin contents of the florets were analyzed in the same way as described in our previous study (Nonokawa et al., 2007). Briefly, samples were extracted with cold methanol (80%, v/v), loaded onto a Bond Elute C18 column, then the content of cytokinin (trans-zeatin riboside (t-ZR) equivalent) in the samples was quantified using enzyme immunoassay (EIA) with Phytodetek plant growth regulator immunoassay detection kit t-ZR (Agdia Inc., ID, USA). The EIA procedures were based on the directions provided with the kit. Since t-ZR kit crossreacts with t-Z, the cytokinin contents shown in the figure are the sum of the content of t-ZR and t-Z.

**Results and Discussion**

BA application to soybean florets has been shown to have a positive effect on pod setting (Crosby et al., 1981; Carlson et al., 1987; Dyer et al., 1987; Peterson et al., 1990; Mosjibis et al., 1993; Reese et al., 1995; Nagel et al., 2001), but the effect on individual florets remained uncertain. In the present study, we attempted to clarify the effects on pod setting in terms of individual florets. The BA application to florets at 7 DAA enhanced the pod setting in the florets on the middle to distal parts of the rachis (Fig. 1). BA application did not affect the number of bloomed florets (data not shown).

Our previous study revealed that the endogenous cytokinin content of whole racemes peaked at 9 DAA (Nonokawa et al., 2007), but the variation in the content of individual florets within the raceme and its daily change remained unclear. In the present study, we analyzed the daily change in the content of individual florets. The cytokinin content was detected only from the florets at 9 DAA, the content being higher in the 1st and 3rd florets, lower in the 5th floret, and negligible in the 7th
floret (Fig. 2). The difference in the content between the 3rd floret and 5th floret was substantial. These results suggest that the florets at the 3rd and above within the raceme contain endogenous cytokinin below the level necessary for pod setting, and that the application of exogenous cytokinin to those florets can enhance the pod setting of the florets.

The effect of BA application on pod setting in field-grown plants is unstable as compared with that in pot-grown plants (Nonokawa et al., 2007). Therefore, the use of synthetic cytokinins as a chemical enhancer of pod setting in the field may be impractical. Genetic improvement of the synthesis of cytokinins in the root system, via conventional breeding or molecular methods, may strengthen the pod-setting capacity of soybean cultivars. Clarification of the physical and chemical properties of the rhizosphere which optimizes the synthesis of endogenous cytokinins in roots should help enhance pod setting through agronomical methods (Kokubun, 2011).

References

Abernethy, R.H. et al. 1977. Can. J. Plant Sci. 57: 713-716.
Antos, M. and Wiebold, W.J. 1984. Agron. J. 76: 715-719.
Brevendan, R.E. et al. 1978. Agron. J. 70: 81-84.
Brun, W.A. and Betts, K.J. 1984. Plant Physiol. 75: 187-191.
Carson, D.R. et al. 1987. Plant Physiol. 84: 233-239.
Classification Committee of Cultivated Soils. 1996. Classification of Cultivated Soils in Japan, the 3rd approximation, National Institute of Agro-Environmental Sciences, Tsukuba, Japan.
Crosby, K.E. et al. 1981. Plant Physiol. 68: 985-988.
Dyer, D.J. et al. 1987. Plant Physiol. 84: 240-243.
Heindl, J.C. et al. 1982. Plant Physiol. 70: 1619-1625.
Heitholt, J.J. et al. 1986a. Crop Sci. 26: 589-595.
Heitholt, J.J. et al. 1986b. Crop Sci. 26: 999-1004.
Huff, A. and Dybing, C.D. 1980. J. Exp. Bot. 31: 751-762.
Jiang, H. and Egli, D.B. 1993. Agron. J. 85: 221-225.
Kato, I. 1964. Tokai-Kinki Natl. Agr. Exp. Sta. Bull. 11: 1-52*.
Kokubun, M. and Honda, I. 2000. Plant Prod. Sci. 3: 354-359.
Kokubun, M. 2011. In T.B. Ng ed., Soybean−Biochemistry, Chemistry and Physiology. InTech. 541-554.
Kurosaki, H. et al. 2003. Plant Prod. Sci. 6: 17-23.
Mosjidis, C.O. et al. 1993. Ann. Bot. 71: 193-199.
Nagel, L. et al. 2001. Ann. Bot. 88: 27-31.
Nonokawa, K. et al. 2007. Plant Prod. Sci. 10: 199-206.
Peterson, C.M. et al. 1990. Bot. Gaz. 151: 322-330.
Reese, R.N. et al. 1995. J. Exp. Bot. 46: 957-964.
Saitoh, K. et al. 1999. Jpn. J. Crop Sci. 68: 396-400*.
Spollen, W.J. et al. 1986a. Agron. J. 78: 280-283.
Spollen, W.J. et al. 1986b. Crop Sci. 26: 1216-1219.
Wiebold, W.J. et al. 1981. Agron. J. 73: 43-46.
Yarrow, G.L. et al. 1988. Plant Physiol. 86: 71-75.
Yashima Y. et al. 2005. Plant Prod. Sci. 8: 139-144.

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