



# Shoot-applied polyamines suppress nodule formation in soybean (*Glycine max*)

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Received 8 March 2005; accepted 13 May 2005

## KEYWORDS

Brassinolide;  
*Glycine max*;  
Nodule;  
Polyamine;  
Super-nodulating mutant

## Summary

In legumes, the number of root nodules is controlled by a mechanism called autoregulation. Recently, we found that the foliar brassinosteroid (BR), a plant growth-regulating hormone, systemically regulates the nodule number in soybean plants. In the present study we report that such down-regulation of root nodule formation by a BR may occur through a change of the polyamine contents, with the experimental evidence as follows.

The foliar contents of both spermidine (Spd) and spermine (Spm) in the super-nodulating soybean mutant, En6500, were always lower than those in its parent line, Enrei. This lower Spd and Spm content accompanied a striking accumulation of putrescine (Put) in the former plant. This finding indicates that Spd and Spm biosynthesis from their precursor Put is repressed in En6500. The foliar treatments with Spd or Spm of En6500 led to a reduction of both nodule number and root growth. On the other hand, foliar treatment with MDL74038, a specific inhibitor of Spd biosynthesis, apparently increased the root nodule number in Enrei. Foliar application of brassinolide (BL) of En6500 increased the leaf Spd level and reduced the nodule number. These results suggested that BL-induced Spd synthesis in shoots might suppress the root nodule formation.

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**Abbreviations:** BL, Brassinolide; BR, Brassinosteroid; DMSO, Dimethyl sulfoxide; HR, Hypersensitive response; PA(s), Polyamine(s); PCA, Perchloric acid; Put, Putrescine; SAMDC, S-adenosylmethionine decarboxylase; Spd, spermidine; Spm, spermine; TMV, tobacco mosaic virus

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## Introduction

Legume plants strictly control nodule formation to ameliorate the consumption of photosynthetic products, which are necessary for the growth of nodules and the maintenance of nitrogen-fixing activity. This mechanism is generally referred to as autoregulation or feedback regulation that involves the inhibition of nodule formation on young roots due to prior nodulation events (Pierce and Bauer, 1983; Kosslak and Bohlool, 1984). Recently, it has been shown that HAR1- and NTS-deficient mutants of *Lotus japonicus* and *Glycine max*, respectively, lose their ability to regulate the nodule number and thereby produce an extraordinary number of root nodules (Krusell et al., 2002; Nishimura et al., 2002; Searle et al., 2003). Grafting experiments between wild-type lines and super-nodulating mutants revealed that leaf tissues are the sites where the substances that regulate the root nodule number are produced, thus implicating shoot-to-root signaling (Caetano-Anolles et al., 1991; Sheng and Harper, 1997). The gene that is involved in this signaling encodes a putative receptor kinase with high similarities to *CLAVATA1* of *Arabidopsis* (Krusell et al., 2002; Nishimura et al., 2002; Searle et al., 2003), although the principal signal molecule(s) involved in the communication between the shoot and root remains to be determined.

Polyamines (PAs); spermidine (Spd), spermine (Spm) and their obligate precursor putrescine (Put) are small aliphatic amines that are ubiquitous in all plant cells. PAs are known to mediate hormone-induced plant responses in many physiological processes including wound responses (Perez-Amador et al., 2002; Walters et al., 2002) and internode growth (Hanzawa et al., 2000). In fact, it has been demonstrated that exogenous treatments with plant hormones changed tissue PA contents and/or caused the genes that are involved in the biosynthetic pathways of PAs in plant tissues to be expressed at high levels (Ghachtouli et al., 1996; Hanzawa et al., 2002; Perez-Amador et al., 2002; Walters et al., 2002). Previously, we found that foliar treatment with brassinolide (BL), a highly effective molecular species among plant hormone brassinosteroids (BRs), decreased the nodule number in the super-nodulating soybean mutant, En6500, depending on the dose, but not in the wild-type soybean Enrei (Terakado et al., 2005). Furthermore, we observed that the foliar application of brassinazole, an inhibitor of BR synthesis, increased the nodule number in the wild-type Enrei, suggesting the participation of leaf BRs in the regulation of root nodule formation.

BRs are able to control vascular differentiation, photo-morphogenesis, fertility, root growth and seed germination (Bishop and Yokota, 2001) and were recently reported to be able to induce the systemic resistance of higher plants to fungal pathogens and bacterial diseases (Nakashita et al., 2003). However, to our knowledge, there is no information regarding the effect of BRs on PA metabolism, and in addition, their relation to nodule formation in leguminous plants. In order to ascertain whether PAs mediate BRs-induced nodule regulation in soybean plants, we investigated the level of PAs after foliar treatment with BL, and the effect of foliar treatment with PAs and/or a PA-inhibitor of root nodule formation using the super-nodulating mutant En6500 and its parent line Enrei. The possible involvement of PAs in the BR-induced regulation of nodule formation was examined.

## Materials and methods

### Experiments with field-grown soybeans

Soybean plants (*G. max* [Merr.] L. Enrei and En6500) were grown in an experimental field of National Agricultural Research Center, Tsukuba, Japan. A nitrogen (N)-phosphorus (P)-potassium (K) fertilizer was applied at the rate of 1000 kg ha<sup>-1</sup> (3–10–10; N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O) using a commercial mixed fertilizer made in Japan. At 29 and 49 d after being sown, the plants were harvested and dissected into leaves, roots and nodules and stored at –40 °C.

### Growth-pouch cultivation of soybeans

The seeds of Enrei and En6500 were immersed in 70% (v/v) ethanol for 1 min and washed once with sterile distilled water. The seeds were then surface-sterilized with 3% (w/v) sodium hypochlorite for 5 min and washed three times with sterile distilled water and kept wet at 25 °C for 3 d in the dark in a growth cabinet. The seedlings were transplanted to plastic growth-pouches (Mega International, Minneapolis, USA) moistened with nitrogen-free medium (Akao and Kouchi, 1989) 3 d after germination. *Bradyrhizobium japonicum* USDA110, grown in a liquid yeast–mannitol medium for 7 d at 28 °C as described in the report of Terakado et al. (2003), was used as the inoculant. The bacterial suspension was diluted at the rate of 10<sup>9</sup> cells and inoculated to the plants 10 d after germination. The Bradyrhizobia-inoculated seedlings were grown under a day/night regime of 16/8 h at a constant temperature of 25 °C. These soybean

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