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ARTICLE

Developmentally regulated expression of the BRI1 brassinosteroid receptor in *Arabidopsis thaliana*

Lidia Hategan, János Bindics, Miklós Szekeres*

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

ABSTRACT Brassinosteroids (BRs) are important regulators of morphogenic events during plant development. The lack of active transport and well-characterized biosynthesis offer ideal conditions for studying the local and temporal effects of this hormone group. While recent studies have found clear coincidence between the sites of BR accumulation and organ differentiation, they have also provided evidence for developmental changes in hormone susceptibility. In order to investigate the role of the BR receptor BRI1 in the modulation of hormone sensitivity, we studied the time course and localization of *BRI1* gene activity in *Arabidopsis* seedlings. To this end, we generated transgenic lines carrying *BRI1* promoter-driven luciferase or *GUS* reporter genes and characterized the expression patterns of these chimeric genes. Our results showed increased *BRI1* expression in dark grown seedlings, particularly in the elongation zone of the hypocotyl, and also at the sites of organ development in green seedlings. These data indicate that, in addition to local increases in the hormone level, the abundance of the receptor can also be instrumental in eliciting the BR response. **Acta Biol Szeged 52(1):57-58 (2008)**

KEY WORDS

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The steroidal plant hormones brassinosteroids (BRs) have an important role in coordinating morphogenic events of plant development from germination to seed production. Their regulatory function is often inter-dependent with those of other phytohormones but, unlike those hormonal substances, BRs are known to act primarily at or near the site of their synthesis (Symons and Reid 2004). Expression studies of key BR biosynthetic genes revealed particularly high transcript levels during germination and the formation of reproductive organs, and found strong accumulation of bioactive BRs during these periods (Montoya et al. 2005; Symons et al. 2006). Whereas increased hormone levels proved indispensable for the onset of these morphogenic programs, other experimental data pointed out marked temporal and organ specific changes in BR sensitivity (Choe et al. 1998; Bancos et al. 2006). Since BR signaling initiates from the BRI1 BR receptor (Li and Jin 2007), elevated expression of the *BRI1* gene was expected to be an indicator of increased hormone responsiveness. Using transgenic *Arabidopsis* lines expressing *BRI1* promoter-driven glucuronidase (*GUS*) or firefly luciferase (*LUC*) transgenes, we found clear coincidence between the timing and localization of strong *BRI1* promoter activity and BR-dependent morphogenic processes.

Materials and Methods

Stable transgenic lines carrying transcriptional fusions between the *Arabidopsis BRI1* promoter (gene identifier: At4g39400) and the firefly luciferase (*LUC*) or *Escherichia coli uidA* (*GUS*) reporter genes were generated in *Arabidopsis thaliana* Col-0 background, as described in Bancos et al. (2006). Transgenic seeds were germinated and grown on agar-supported Murashige-Skoog medium (Sigma) at 22ºC, under daily 12 h white light (50-60 µmol m^{-2} s^{-1}) and 12 h dark regimes (LD), or in constant dark (DD).

Luciferase transgene activity was detected by *in vivo* imaging with a liquid nitrogen-cooled back illuminated digital CCD camera (Princeton Instruments, Trenton, NJ). Quantitative data were obtained from patches of 25 seedlings using 25 min exposures every second hour. Seedlings used for glucuronidase histochemical assays were fixed in 2% (w/v) formaldehyde solution, then subjected to overnight staining with the method of Jefferson (1987).

The reliability of the reporter-based expression data was verified by semi-quantitative RT-PCR mRNA analyses. For these assays total RNA was isolated from 0.5 g seedling material using TRI-reagent (Sigma). Sample preparation and the RT-PCR analysis were carried out as in Bancos et al. (2006).

Results and Discussion

In order to determine the temporal expression pattern of *BRI1* during germination, we measured the luminescence of developing *BRI1:LUC Arabidopsis* seedlings. Following imbibition, strong induction could be observed in LD-grown seedlings, which resulted in a well-defined maximum of transgene activity on the third day of the experiment. This

*Corresponding author. E-mail: szekeres@brc.hu

was then followed by a rapid decrease of the expression level to less then 50% of the transient maximum after the fifth day, and a slow attenuation to about 20% by day 14. The initial induction phase was very similar in DD-germinated etiolated seedlings, but here a broad maximum was observed between the third and fifth days, and only after day 10 did the expression level decrease below 50% of the maximum value. The extended period of *BRI1* promoter activity coincided with that of hypocotyl elongation in DD seedlings. CCD camera images showed intense luminescence in the hypocotyl, and *BRI1:GUS* seedlings allowed precise localization of the transgene activity to the upper, elongation zone of the hypocotyl.

During etiolation the temporal and spatial coincidence between hypocotyl elongation and elevated *BRI1* expression suggests that, in addition to accumulation of the hormone, BR-induced elongation is also dependent on the abundance of the receptor. The observed high *BRI1* activity in DD seedlings seems to resolve the apparent controversy between the enhanced BR accumulation observed in light, and the stronger growth-promoting effect of the hormone in the dark (Symons et al. 2008).

Further data of *BRI1* expression also indicated good coincidence between the loci of enhanced transgene activity and BR-dependent morphogenic events. In LD seedlings strong luciferase luminescence and GUS staining were observed in the shoot apical meristem, differentiating and expanding leaves, the root tip, and the sites of lateral root initiation. These data, as well as those of the temporal expression, imply that local enrichment of the BRI1 receptor can be instrumental in enhancing the developmental effects of BRs.

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