

ROS mediate brassinosteroids-induced plant stress responses

Xiao-Jian Xia,¹ Zhixiang Chen³ and Jing-Quan Yu^{1,2,*}

¹Department of Horticulture; Huajiachi Campus; Zhejiang University; Hangzhou, P.R. China; ²Key Laboratory of Horticultural Plants Growth, Development and Biotechnology; Agricultural Ministry of China; Hangzhou, P.R. China; ³Department of Botany & Plant Pathology; Purdue University; West Lafayette, IN USA

Brasinosteroids (BRs) play important roles in the complex network of plant signal transduction that regulates plant growth and development. Field and greenhouse trials have shown that exogenous BRs can also improve plant tolerance to abiotic and biotic stress. We have recently shown that application of exogenous BR enhances while inhibition of endogenous BR biosynthesis compromises the tolerance to photo-oxidative and cold stresses and resistance to cucumber mosaic virus in cucumber plants. These results suggest a possible role of endogenous BRs in plant stress responses as well. We have further shown that BR-induced stress tolerance is associated with increased accumulation of reactive oxygen species (ROS), which, in turn, is important for BR-induced stress tolerance. BR-induced ROS accumulation is sensitive to inhibitor of plasma membrane-bound NADPH oxidases. ROS mediate BR-induced stress tolerance, most likely by regulating genes involved in plant stress response pathways. Given their established roles as second messengers, ROS may also participate in other BR-regulated biological processes including plant growth, development and photosynthesis.

Since first discovered in *Brassica napus* pollen,¹ brassinosteroids (BRs) have been extensively tested for biological activities in plant growth and development. BRs affect cell elongation, division, differentiation, reproductive and vascular development, membrane polarization and proton pumping.² Despite strong evidence for BRs as potent plant growth regulators, there was no widespread recognition of

BRs as plant hormones until the identification and analysis of BR biosynthetic mutants. BR biosynthetic mutants such as *constitutive photomorphogenesis and dwarfism (cpd)* and *deetiolated2 (det2)* from *Arabidopsis thaliana* were first identified based on their altered photomorphogenesis.^{3,4} BR biosynthetic mutants were also identified in other crops.⁵ These mutants provide conclusive evidence for the important biological functions of BRs in plant growth and development.

BR biosynthetic or insensitive mutants have proven to be valuable tools for unraveling the BR signal transduction pathways in plants. Li et al. reported the first BR-insensitive *Arabidopsis* mutant *bri1*, which help lead to the subsequent identification of the BR receptor.⁶ Several other BR signaling components have been later identified and characterized. BR binding to BRI1 induces association of BRI1 with BAK1, which leads to phosphorylation of BSK1 and binding to the BSU1 phosphatase. BSU1 inactivates the GSK3-like kinase BIN2 and transduces the BR signal into the nucleus. BIN2 negatively regulates downstream signaling by phosphorylating two transcription factors BZR1 and BES1 and inhibiting their binding to BR responsive promoters.^{7,8}

In agriculture, active BR analogs can be synthesized in sufficient quantities and evaluated in greenhouse and field trials. Extensive testing in the field and greenhouse has showed that exogenous BRs have the ability to substantially increase yield in a variety of plant species.⁹ A remarkable feature of BRs is their activity to increase plant tolerance/resistance to a wide spectrum of stresses, such as low and

Key words: brassinosteroids, brassinazole, cucumber, NADPH oxidase, reactive oxygen species, stress tolerance

Submitted: 12/18/09

Accepted: 12/18/09

Previously published online:

www.landesbioscience.com/journals/psb/article/10989

*Correspondence to: Jing Quan Yu;
Email: jquyu@zju.edu.cn

Addendum to: Xia XJ, Wang YJ, Zhou YH, Tao Y, Mao WH, Shi K, et al. Reactive oxygen species are involved in brassinosteroids-induced stress tolerance in *Cucumis sativus*. *Plant Physiol* 2009; 150:801–14; PMID: 19386805; DOI: 10.1104/pp.109.138230.

high temperatures, drought, salinity and pathogens.^{10,11} In some cases, the protective effect of BRs against fungal diseases was even stronger than chemical fungicides. Additionally, BRs can be used to reduce the accumulation of heavy metals and pesticides residues and thus increase the food safety.^{12,13} The widespread beneficial effects of BRs make them environmental-friendly natural substances potentially suitable for crop protection and yield promotion in agriculture.

Although application of exogenous BRs has been reported to improve plant resistance to many environmental stresses, there are controversies about the functions of endogenous BRs in plant stress responses. Jager et al. showed that endogenous BR content in pea did not increase under drought stress and defects in BR biosynthesis or signaling had no influences on the initiation of stress response and tolerance under drought stress.¹⁴ The authors argued that endogenous BR levels are not normally responsive to water stress. In contrast, it has been shown that *Arabidopsis* BR biosynthetic and signaling mutants, *det2* and *bin2*, are more sensitive to salt stress than wild type.¹⁵ Consistent with a role of BRs in stress tolerance, the semi-dwarf *uzu* barley mutant, which is impaired in BR perception, is less tolerant to stress than non-*uzu* barley.¹⁶

We used brassinazole, a specific inhibitor of the DWF4 protein in the BR biosynthetic pathway, to reduce BR levels in cucumber plants.¹⁷ Using this chemical genetics strategy, we have shown that inhibition of BR biosynthesis reduces the tolerance of cucumber plants to cold and photooxidative stresses and cucumber mosaic virus infection, whereas feeding with bioactive BRs increases the stress tolerance. Thus, BR-induced stress tolerance is correlated with the BRs levels in cucumber plants. By analyzing the gas exchange characteristics of BR-deficient tomato mutant, we have found that stomatal conductance is reduced in the mutant. Together with the dwarf plants and thick leaves, this may lead to decreased water demand in BR mutant. This might explain the failure to observe reduced tolerance to water stress in the pea BR mutant.¹⁴

There could be several reasons for the failure to observe change in BR levels

during plant responses to biotic and abiotic stress. First, at present, the endogenous BR is below the detection level by most methods and only the intermediates of the BR biosynthetic pathway have been quantified in several reported studies.^{11,14} Failure to observe changed BR levels could be due to difficulty for sensitive detection of a small or modest changes in the levels of BRs or its biosynthetic intermediates. Second, during plant stress responses, BR levels may not change but the activity of certain components involved in BR signaling may be altered. Third, BR-mediated signaling may crosstalk with thereby modulate the concentrations or plant sensitivity to other hormones during stress conditions. Consistent with this modulator function of BRs, exogenous BRs had minor effect on endogenous ABA levels in plants maintained at normal temperatures, but increased ABA biosynthesis in plants under heat stresses.¹⁸ In sorghum, BRs also enhanced the protective effects of ABA under drought stress,¹⁹ probably by enhancing plant sensitivity to ABA. BRs may also modulate the accumulation or signaling of other plant hormones involved in stress responses. For example, microarray experiments in *Arabidopsis* have shown that BR stimulates the JA pathway through activation of OPR3, an enzyme involved in JA biosynthesis.²⁰ Intriguingly, it has been observed that BRs act upstream of the JA signaling pathway in modulating defense against herbivory in tomato.²¹

Another important and broadly significant finding from our study is that BR stimulates ROS production and this BR-induced ROS is important for subsequent establishment of stress tolerance. ROS is a key signaling molecule in plant development and environment adaptation. ROS induces gene expression and stress response via changes in cellular redox states. For example, during development of systemic acquired resistance, there is an initial ROS accumulation that perturbs the cellular redox state and, in turn, activates the NPR1 pathway to upregulate the *PRI* gene.²² Szekeres et al. reported that BR deficiency resulted in downregulation of *PRI*, whereas overexpression of a BR biosynthetic gene increased *PRI* gene expression.³ Thus, it is possible that BRs regulate *PRI* gene expression through

alteration of the cellular redox state resulting from increased ROS accumulation. Consistent with this possibility, we have observed that cucumber leaves treated with BRs inhibitor have reduced ROS level, whereas exogenous BRs induced moderate ROS accumulation mostly in the apoplast. There is evidence showing that the effect of BRs is dependent on plant developmental stage and application method. We have discovered that the interval between BR treatment and stress challenge is critical for the magnitude of induced stress tolerance. Therefore, the variation of the efficiency of BR in enhancing plant stress tolerance may be related to changes of ROS homeostasis.

ROS have regulatory roles in a wide range of important plant biological processes including stress tolerance. Foreman et al. have indicated that ROS play important roles in plant growth and development.²³ The NADPH oxidase encoded by the *AtrbohC* gene is required for root elongation in *Arabidopsis*. Studies using inhibitors of NADPH oxidase have suggested that NADPH oxidase-derived ROS control cell expansion in leaves.²⁴ Recently, we have observed that a narrow range of BR concentration leads to a moderate increase in ROS concentration, which in turn triggers a more reduced cellular redox state that is essential for BR-induced CO₂ assimilation (unpublished data). It is, therefore, likely that BR-induced ROS may also play a critical roles in BRs-regulated plant growth and development.

In conclusion, our study has revealed an important role of BRs in plant stress response. We have also revealed a critical role of ROS in BRs-induced stress tolerance. The hypothesis we have proposed here should provide novel insights into BRs action mode. Further analysis using a combinatorial approach, mutant analysis and genomics, is required to determine the source of ROS, the upstream or downstream regulator of BRs-mediated stress tolerance and the involvement of the BRI1-dependent pathway. Understanding the mode of action of BRs in inducing plant stress tolerance will help development and improvement of BR-based chemicals that can be used as agrochemicals to increase crop productivity.

References

1. Grove MD, Spencer GF, Rohwedder WK, Mandava N, Worley JF, Warthen JD, et al. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* 1979; 281:216-7.
2. Clouse SD, Sasse JM. Brassinosteroids: essential regulators of plant growth and development. *Annu Rev Plant Physiol Plant Mol Biol* 1998; 49:427-51.
3. Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, et al. Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. *Cell* 1996; 85:171-82.
4. Li JM, Nagpal P, Vitart V, McMorris TC, Chory J. A role for brassinosteroids in light-dependent development of *Arabidopsis*. *Science* 1996; 272:398-401.
5. Bishop GJ. Brassinosteroid mutants of crops. *J Plant Growth Regul* 2003; 22:325-35.
6. Li JM, Chory J. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* 1997; 90:929-38.
7. Gendron JM, Wang ZY. Multiple mechanisms modulate brassinosteroid signaling. *Curr Opin Plant Biol* 2007; 10:436-41.
8. Kim TW, Guan SH, Sun Y, Deng ZP, Tang WQ, Shang JX, et al. Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. *Nat Cell Biol* 2009; 11:1233-54.
9. Khripach V, Zhabinskii V, De Groot A. Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI century. *Ann Bot* 2000; 86:441-7.
10. Krishna P. Brassinosteroid-mediated stress responses. *J Plant Growth Regul* 2003; 22:289-97.
11. Nakashita H, Yasuda M, Nitta T, Asami T, Fujioka S, Arai Y, et al. Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant J* 2003; 33:887-98.
12. Bajguz A, Hayat S. Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol Biochem* 2009; 47:1-8.
13. Xia XJ, Zhang Y, Wu JX, Wang JT, Zhou YH, Shi K, Yu YL, Yu JQ. Brassinosteroids promote metabolism of pesticides in cucumber. *J Agric Food Chem* 2009; 57:8406-13.
14. Jager CE, Symons GM, Ross JJ, Reid JB. Do brassinosteroids mediate the water stress response? *Physiol Plant* 2008; 133:417-25.
15. Zeng H, Tang Q, Hua X. *Arabidopsis* brassinosteroid mutants *det2-1* and *bin2-1* display altered salt tolerance. *J Plant Growth Regul* (published online; DOI 10.1007/s00344-009-9111-x).
16. Divi UK, Krishna P. Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. *New Biotech* 2009; 26:131-6.
17. Xia XJ, Wang YJ, Zhou YH, Tao Y, Mao WH, Shi K, et al. Reactive oxygen species are involved in brassinosteroids-induced stress tolerance in *Cucumis sativus*. *Plant Physiol* 2009; 150:801-14.
18. Kurepin LV, Qaderi MM, Back TG, Reid DM, Pharis RP. A rapid effect of applied brassinolide on abscisic acid concentrations in *Brassica napus* leaf tissue subjected to short-term heat stress. *Plant Growth Regul* 2008; 55:165-7.
19. Xu HL, Shida A, Futatsuya F, Kumura A. Effects of epibrassinolide and abscisic acid on sorghum plants growing under soil water deficit I. Effects on growth and survival. *Jpn J Crop Sci* 1994; 4:671-5.
20. Müssig C, Fischer S, Altmann T. Brassinosteroid-regulated gene expression. *Plant Physiol* 2002; 129:1241-51.
21. Campos ML, de Almeida M, Rossi ML, Martinelli AP, Litholdo CG, Figueira A, et al. Brassinosteroids interact negatively with jasmonates in the formation of anti-herbivory traits in tomato. *J Exp Bot* 2009; 60:4346-60.
22. Mou Z, Fan W, Dong X. Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell* 2003; 113:935-44.
23. Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, et al. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 2003; 422:442-6.
24. Rodriguez AA, Grunberg KA, Taleisnik EL. Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension. *Plant Physiol* 2002; 129:1627-32.

©2010 Landes Bioscience.
Do not distribute.