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BZS1, a B-box Protein, Promotes Photomorphogenesis Downstream of Both Brassinosteroid and Light Signaling Pathways

Xi-Ying Fan[a,b,c,2], Yu Sun[b,2], Dong-Mei Cao[a,d,2], Ming-Yi Bai[e], Xiao-Min Luo[a], Hong-Juan Yang[a], Chuang-Qi Wei[b], Sheng-Wei Zhu[a], Ying Sun[b], Kang Chong[a] and **Zhi-Yong Wang[e,1]**

a Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
b Institute of Molecular Cell Biology, College of Life Science, Hebei Normal University, Shijiazhuang, Hebei 050016, China
c Graduate School of the Chinese Academy of Sciences, Beijing 100049, China
d Institute of Horticulture, Shanxi Academy of Agriculture Sciences, Shanxi 030031, China
e Department of Plant Biology, Carnegie Institution for Science, Stanford, CA 94305, USA

ABSTRACT Photomorphogenesis is controlled by multiple signaling pathways, including the light and brassinosteroid (BR) pathways. BR signaling activates the BZR1 transcription factor, which is required for suppressing photomorphogenesis in the dark. We identified a suppressor of the BR hypersensitive mutant *bzr1–1D* and named it *bzr1–1D* suppressor1-*D*ominant (*bzs1–D*). The *bzs1–D* mutation was caused by overexpression of a B-box zinc finger protein BZS1, which is transcriptionally repressed by BZR1. Overexpression of *BZS1* causes de-etiolation in the dark, short hypocotyls in the light, reduced sensitivity to BR treatment, and repression of many BR-activated genes. Knockdown of *BZS1* by co-suppression partly suppressed the short hypocotyl phenotypes of BR-deficient or insensitive mutants. These results support that BZS1 is a negative regulator of BR response. BZS1 overexpressors are hypersensitive to different wavelengths of light and loss of function of BZS1 reduces plant sensitivity to light and partly suppresses the *constitutive photomorphogenesis 1* (*cop1*) mutant in the dark, suggesting a positive role in light response. BZS1 protein accumulates at an increased level after light treatment of dark-grown *BZS1–OX* plants and in the *cop1* mutants, and BZS1 interacts with COP1 *in vitro*, suggesting that light regulates BZS1 through COP1-mediated ubiquitination and proteasomal degradation. These results demonstrate that BZS1 mediates the crosstalk between BR and light pathways.

Key words: photomorphogenesis; light signaling; Brassinosteroid; *BZS1*; *Arabidopsis*.

INTRODUCTION

Wild-type *Arabidopsis* seedlings grown in the dark have long hypocotyls and closed cotyledons with undifferentiated chloroplast, a phenomenon termed etiolation or skotomorphogenesis. When exposed to light, seedlings undergo de-etiolation (also called photomorphogenesis) and thus display short hypocotyls, open cotyledons, and chloroplast differentiation (Wei and Deng, 1996). This light-induced developmental switch is controlled by the photoreceptor-mediated signaling transduction pathways, which act in part by inactivating the COP1 E3 ubiquitin ligase and stabilizing positive transcription factors. In addition to light signaling, brassinosteroid (BR) is another key signal that controls the switch between skotomorphogenesis and photomorphogenesis, as BR-deficient and insensitive mutants show de-etiolation phenotypes in the dark (Chory et al., 1991; Li et al., 1996; Szekeres et al., 1996; Song et al., 2009). Light and BR act antagonistically in photomorphogenesis. However, the molecular mechanisms of this antagonism are not fully understood.

BR signals are perceived by BRI1 receptor kinase and transduced through a well-defined signal transduction pathway to activate members of the BZR family transcription factors (Kim et al., 2009; Kim and Wang, 2010; Clouse, 2011). BZR1 and BZR2/BES1 transcription factors play central roles in BR regulation of plant growth and gene expression (Wang et al.,

[1] To whom correspondence should be addressed. E-mail zywang24@stanford.edu.

[2] These authors contributed equally to this work.

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2002; Yin et al., 2002; He et al., 2005; Yin et al., 2005). The dominant *bzr1–1D* mutation causes constitutive BZR1 activation due to enhanced dephosphorylation by PP2A (Tang et al., 2011), and *bzr1–1D* fully suppresses the photomorphogenesis phenotype of *bri1-116* and reverses the expression changes of about 80% of the genes affected in *bri1-116* (Sun et al., 2010). A large number of light-induced genes are activated in the BR-deficient or insensitive mutants in the dark (Chory et al., 1991; Li et al., 1996; Szekeres et al., 1996; Song et al., 2009), suggesting that light and BR may regulate common transcription factors. Indeed, a recent study showed that a member of the GATA family transcription factors (GATA2) is transcriptionally repressed by BZR1 but posttranslationally activated by light signaling (Luo et al., 2010). GATA2 controls a subset of genes co-regulated by light and BR to promote photomorphogenesis. Many other transcription factors mediate light-regulated gene expression (Lau and Deng, 2010), but whether any of them are also regulated by BR remains unknown.

B-box proteins are a group of transcription factors containing one or more B-box domains that are stabilized by binding to zinc ions (Klug and Schwabe, 1995). There are 32 B-box containing proteins with diverse functions in *Arabidopsis* (Khanna et al., 2009). For example, CONSTANS (CO) is an important regulator of photoperiodic flowering (Putterill et al., 1995; Onouchi et al., 2000). The CO-like genes *COL1* and *COL2* are involved in regulating the circadian clock (Ledger et al., 2001). *STH2*, *STH3*, and *COL3* are positive regulators of light responses (Datta et al., 2006, 2007, 2008), whereas *STO* and *STH1* are negative regulators of photomorphogenesis (Khanna et al., 2006; Indorf et al., 2007).

In this study, we identified an activation-tagged *bzr1–1D* suppressor (*bzs1–D*), which showed dwarfism and suppressed *bzr1–1D*'s phenotypes of stem kink and insensitivity to BR biosynthesis inhibitor BRZ. The phenotypes of *bzs1–D* are caused by overexpression of a B-box protein we named BZS1 (also named BBX20), which is repressed at the transcription level by BR. Overexpression and loss-of-function experiments demonstrate that BZS1 plays a negative role in BR responses but positively regulates light responses. BZS1 protein level is increased upon light treatment of dark-grown seedlings and in the *cop1* mutant. BZS1 interacts with COP1 *in vitro*, suggesting that light may regulate BZS1 accumulation through COP1. These data demonstrate that BZS1 acts downstream of both BR and light pathways. This study supports a mechanism of BR-light crosstalk through transcriptional repression and posttranslational activation of photomorphogenesis-promoting transcription factors.

RESULTS

Isolation and Characterization of the *bzr1–1D* Suppressor Mutant

The activation tagging strategy has been successfully used in identification of novel BR signaling components, including BRS1, BAK1, BRL1, BSU1, BEN1, ATBS1, and TCP1 (Li et al., 2001, 2002; Mora-Garcia et al., 2004; Zhou et al., 2004; Yuan et al., 2007; Wang et al., 2009; Guo et al., 2010; Kang et al., 2010). To better understand the molecular mechanism of BZR1 function and identify additional components of the BR pathway, we previously carried out a large-scale activation tagging screen in *bzr1–1D* background (Cao et al., 2008). One of the mutant lines partially suppressed phenotypes of *bzr1–1D*; therefore, we named it *bzr1–1D suppressor1-Dominant* (*bzs1–D*). The *bzr1–1D;bzs1–D* mutant showed stronger BR-deficient phenotypes, such as dwarfism and extended lifecycle, compared to *bzr1–1D* itself (Figure 1A). The *bzr1–1D;bzs1–D* plants showed no stem kink, which is one of the most obvious phenotypes of light-grown *bzr1–1D* (Figure 1B), and a reduced degree of silique kink compared to *bzr1–1D* (Figure 1C).

The *bzr1–1D* mutant is insensitive to brassinazole (BRZ), a BR biosynthesis inhibitor (Wang et al., 2002). BRZ inhibits hypocotyl elongation of wild-type in the dark but not of *bzr1–1D* (Figure 1D and 1E). While *bzr1–1D;bzs1–D* plants had similar hypocotyl length compared to wild-type and *bzr1–1D* when grown on medium without BRZ, their hypocotyls were shorter than *bzr1–1D* on the medium containing

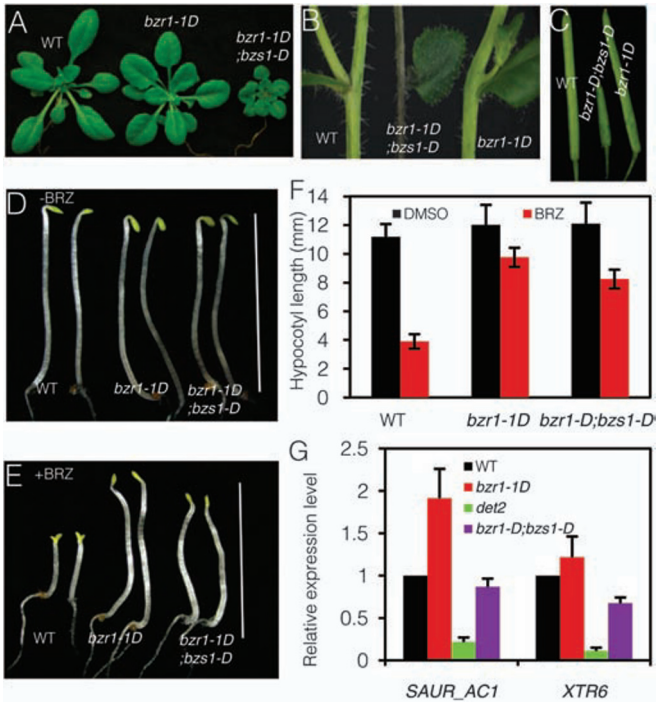

Figure 1. The *bzs1–D* Mutation Partly Suppresses *bzr1–1D*'s Phenotypes.

(A) Phenotypes of soil-grown 3-week-old plants.
(B) The stem kink phenotypes.
(C) The silique kink phenotype.
(D, E) Selective dark-grown seedlings on regular half-strength MS medium (D) or half-strength MS medium containing 2 μM BRZ (E).
(F) The average hypocotyl length of seedlings grown on medium with or without 2 μM BRZ. Error bars indicate SD (n = 40).
(G) Quantitative RT–PCR analysis of the expression levels of *SAUR_AC1* and *XTR6*. *UBC30* was used as internal control.