Phytochemistry 104 (2014) 21–29

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Blue light-promoted rice leaf bending and unrolling are due to up-regulated brassinosteroid biosynthesis genes accompanied by accumulation of castasterone

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ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 13 April 2014
Available online 23 May 2014

Keywords:
Oryza sativa
Poaceae
Rice lamina bending and unrolling
Blue light
Brassinosteroid biosynthesis
Cytochrome P450

ABSTRACT

In this study the relationship between blue light- and brassinosteroid-enhanced leaf lamina bending and unrolling in rice was investigated. Twenty-four hours (h) irradiation with white or blue light increased endogenous brassinosteroid levels, especially those of typhasterol and castasterone, in aerial tissues of rice seedlings. There was an accompanying up-regulation of transcript levels of *CYP85A1/OsDWARF*, encoding an enzyme catalyzing C-6 oxidation, after 6 h under either white or blue light. These effects were not observed in seedlings placed under far-red or red light regimes. It was concluded that blue light up-regulates the levels of several cytochrome P450 enzymes including CYP85A1, thereby promoting the synthesis of castasterone, a biologically active brassinosteroid in rice. Based on these findings, it is considered that blue light-mediated rice leaf bending and unrolling are consequences of the enhanced biosynthesis of endogenous castasterone. In contrast to aerial tissues, brassinosteroid synthesis in roots appeared to be negatively regulated by white, blue and red light but positively controlled by far-red light.

1. Introduction

Brassinosteroids (BRs) are steroidal plant hormones that control many aspects of plant growth and development including cell expansion and division, vascular bundle differentiation, as well as responses to biotic, and abiotic stresses (Clouse and Sasse, 1998; Khripach et al., 2000; Bishop and Yokota, 2001; Bishop and Koncz, 2002).

The biosynthesis of BRs in Arabidopsis has been investigated intensively (Fujioka and Yokota, 2003). Recently, Ohnishi et al. (2009, 2012) reinvestigated the topic by means of recombinant BR biosynthetic enzyme experiments and postulated a novel pathway, the major part of which is illustrated in Fig. 1. The pathway starts with 22-hydroxylation of campesterol (1) catalyzed by CYP90B1, but appears not to proceed via campestanol (1) which, based on precursor administration experiments, had been considered to be the immediate precursor of BRs. This new pathway may also operate in rice which has BR biosynthesis genes homologous to those of Arabidopsis (Fig. 1). Such genes include: *CYP90B2/OsDWARF4* and *CYP724B1/D11* which encode C-22 hydroxylases (Sakamoto et al., 2006), *CYP90D2/D2* and *CYP90D3* encoding C-23 hydroxylases (Sakamoto et al., 2012), *CYP85A1/OsDWARF* encoding C-6 oxidase (Hong et al., 2002; Mori et al., 2002) and *CYP90A3/OsCPD1* and *CYP90A4/OsCPD2* encoding C-3 oxidases (Sakamoto and Matsuoka, 2006; Ohnishi et al., 2012), and *OsDET2* encoding 5α-reductase (Hirano et al., 2008).

The first discovered BR-deficient Arabidopsis mutants, *cpd* and *det2*, lack skotomorphogenesis, suggesting that BRs act as negative regulators of photomorphogenesis (Nemhauser and Chory, 2004). As a consequence, roles of BRs in photomorphogenesis have been studied extensively (Clouse, 2011). Negative roles of phytochromes in BR-mediated plant response have been proposed (Jeong et al., 2007). Gene expression analysis by Sun et al. (2010) revealed extensive crosstalk between BR and other hormonal and light signaling pathways at multiple levels.

In contrast, relatively few studies have investigated the role of light on the BR biosynthesis and the concurrent plant responses. It has, however, been shown that light enhances BR synthesis in pea seedlings (Symons et al., 2002; Symons and Reid, 2003), and,

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http://dx.doi.org/10.1016/j.phytochem.2014.04.017
0031-9422/

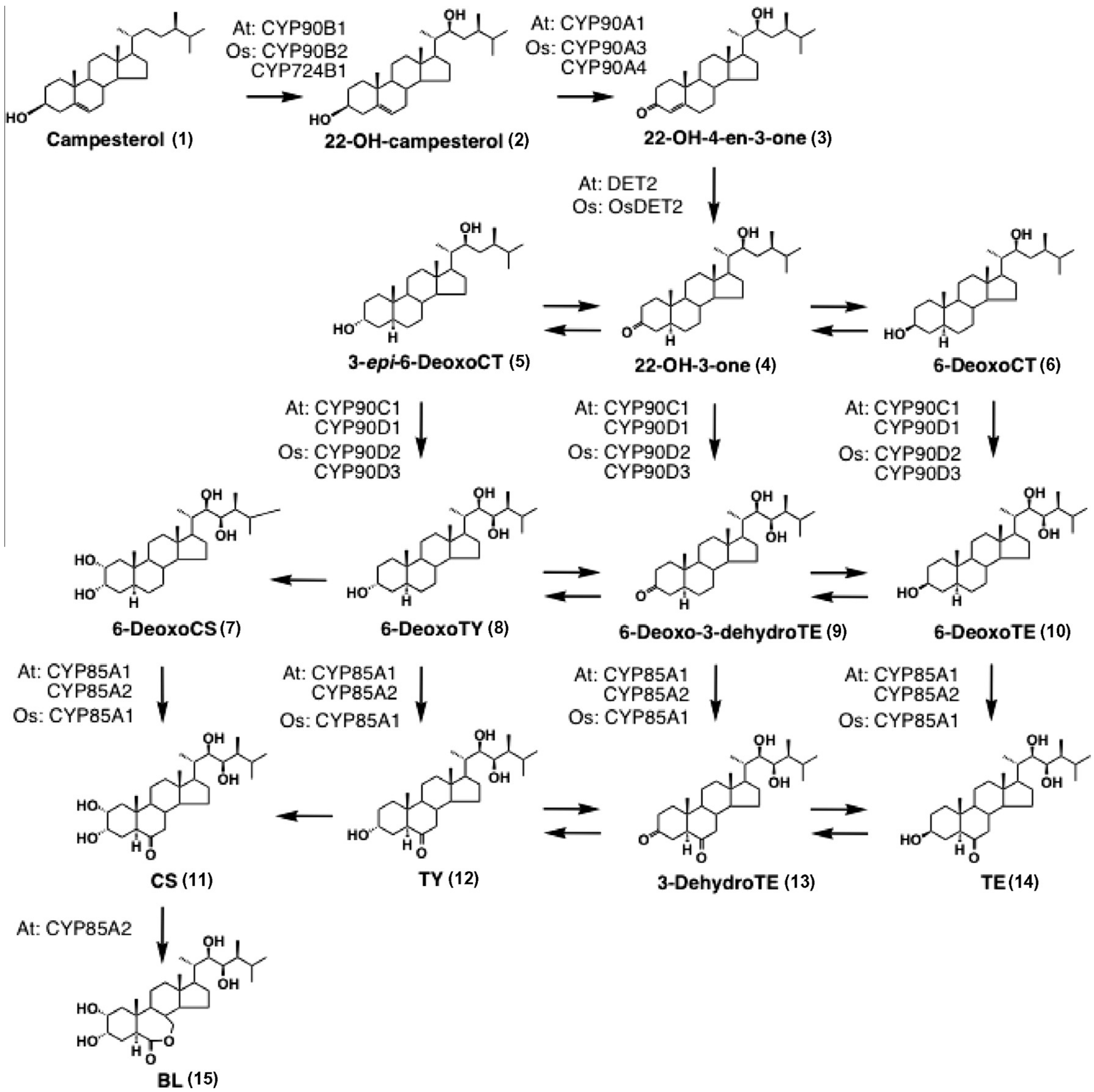

Fig. 1. Major biosynthetic pathway of BRs and associated enzymes in Arabidopsis and rice.

furthermore, diurnal regulation of Arabidopsis BR-biosynthetic genes *CPD* and *CYP85A2* as well as light-dependent synthesis of brassinolide (15) (BL) has been demonstrated (Bancoş et al., 2006).

Earlier investigations established that blue light promotes rice lamina bending (Inada, 1969) as well as lamina unrolling of intact seedlings (Katsura and Inada, 1979; Sasakawa and Yamamoto, 1980) and detached leaves (Niimi et al., 1981). Subsequently, it was shown that BRs promote rice lamina bending (Wada et al., 1981, 1984; Yamamuro et al., 2000; Sakamoto et al., 2006). Lamina unrolling has also been found to be accelerated by BRs in wheat (Wada et al., 1985a,b) and barley (Honda et al., 2003). Thus, it is likely that blue light-mediated rice lamina bending and unrolling are mediated via endogenous BRs as proposed by Wada et al. (1985a,b). Castasterone (11) (CS), but not BL (15), occurs in rice and hence CS (11) may be an endogenous regulator controlling rice lamina inclination (Kim et al., 2008) and unrolling. The current study investigated this hypothesis by examining the levels of endogenous BRs and transcripts of BR biosynthesis genes in rice seedlings grown under white, blue, red and far-red light. The data obtained demonstrate that blue light increases the synthesis of CS (11) through up-regulation of genes encoding P450 enzymes and this, in turn, induces the lamina bending and unrolling responses.

2. Results and discussion

2.1. Rice lamina bending and unrolling are accelerated by blue light and BR

Seven-day-old etiolated rice seedlings were irradiated for 24 h with either white, blue, red or far-red light and, prior to measuring leaf bending and unrolling, were grown in darkness for 24 h. This established that both white and blue light accelerated not only lamina bending (Fig. 2A) but also lamina unrolling (Fig. 2B) as compared with dark controls. However, red light was much less effective and far-red light had further smaller effects. It is, therefore, likely that blue light is the effective component of white light in eliciting rice lamina bending and unrolling. Fig. 3A and B illustrate typical effects of blue light on rice lamina bending and unrolling. These findings well supported the earlier findings of Inada (1969) and Sasakawa and Yamamoto (1980) that blue light is the most effective in inducing lamina bending and unrolling of intact rice seedlings.

To date, the effect of BR on the rice lamina bending has been extensively studied, while the effect of BR on rice lamina unrolling has been restricted to a preliminary report by Wada et al. (1985a).