Note

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Light-Controlled Expression of β-Glucuronidase Gene Driven by *palg2b* Promoter from *Populus kitakamiensis* in *Nicotiana plumbaginifolia*

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Abstract

We investigated the induction of β-glucuronidase (GUS) activity in transgenic *Nicotiana plumbaginifolia* harboring *palg2b* promoter-*uidA* chimeric gene after light irradiation. GUS activity was increased distinctly in leaves of transgenic plants by white light irradiation and was slightly increased in transgenic green calli by blue light irradiation. The present results indicate that the *g2b* promoter might be useful to control the expression of a foreign gene in transgenic plants.

Development of commercially useful products by the production of primary and secondary metabolites in transgenic plant cells requires regulation of the expression of the foreign genes. Light, which causes dramatic changes in many processes involving primary or secondary metabolism, is an attractive factor for regulating expression of the genes encoding the enzymes which synthesize industrially important metabolites (Kurata *et al.*, 1997; Zhong *et al.*, 1995). Light-responsive promoters of ribulose bisphosphate carboxylase (*rbcS*), chlorophyll a/b binding protein (*cab*), chalcone synthase (CHS) and phenylalanine ammonia-lyase (PAL) genes have been studied extensively (Feinbaum *et al.*, 1988; Sugita *et al.*, 1987, Tobin *et al.*, 1985; Wehmeyer *et al.*, 1990; Logemann *et al.*, 1995). However, only a few examples using light-regulated promoters to produce foreign gene products have been reported. The *rbcS3B*, *cab* and *CHS* promoters were expressed in tobacco cell cultures (An 1987; Uozumi *et al.*, 1994; Kurata *et al.*, 1998), but there has been little investigation of the PAL promoter for foreign gene expression.

We investigated the GUS expression driven by *palg2b* promoter which was isolated from hybrid aspen *Populus kitakamiensis* (Osakabe *et al.*, 1995 a) to evaluate whether the promoter is able to be used in further applications for production of useful metabolites. We confirmed that *g2b* promoter-driven GUS expression was induced by irradiation of white light in transgenic plants, and found that *g2b* promoter might be useful to control the expression of a foreign gene in transgenic plants.

Nicotiana plumbaginifolia was transformed by infection with *Agrobacterium tumefaciens* LBA4404 harboring plasmid g2bGUS-1780 as previously described (Moriwaki *et al.*, 1999). (The plasmid was kindly provided by Dr. Kawai, Tokyo University of Agriculture and Technology.) Transformed plantlets were cultured in glass containers at 27℃ under continuous light (22 μE s^{-1} m^{-2}). Transgenic calli were induced by culturing leaves of transgenic plantlets on Murashige & Skoog's (MS) medium (Murashige and Skoog, 1962) containing 0.2 ppm kinetin, 2 ppm NAA, and 3% (w/w) sucrose. Non-green calli were cultured in darkness at 27℃, and green calli were cultured under continuous light (22 μE s^{-1} m^{-2}) at 27℃.

For measuring GUS activity of transformed plantlets, 2- to 5- cm long leaves from 2- to 3-month-old plantlets were used. The influence of light on the activation of *g2b* promoter was examined using leaves cut and placed on solid MS medium, and kept in complete darkness for 5 days, because there was a report that mRNA of light-responsive genes was not observed after dark adaptation (Yamamoto *et al.*, 1997). Then the leaves were irradiated with white light (22 μE s^{-1} m^{-2}). Two fluorescent light tubes (20W) were used as the light source in all experiments. After light irradiation, leaves were incubated in complete darkness for about 18 h, because maximum level of GUS activity was observed for 12-24 h after induction in an other report (Moriwaki *et al.*, 1999). GUS activity was measured

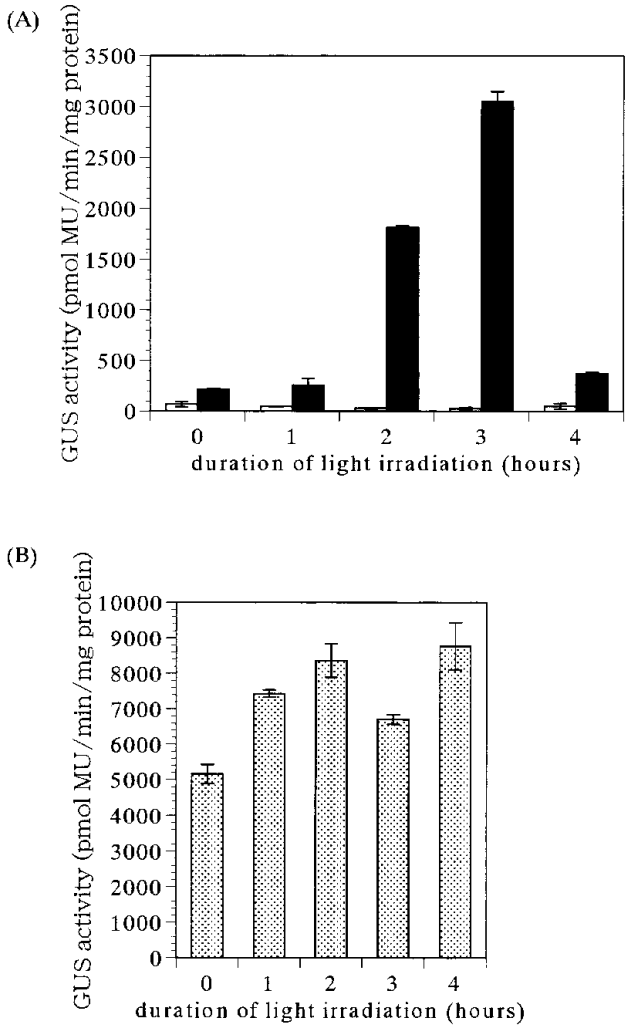

Fig. 1. Induction of GUS activity by white light irradiation.
GUS activity of plantlets harboring g2bGUS (A) and CaMV 35S GUS (B) are indicated. Open bars in (A) indicate the activity of wild type plantlets, and closed bars indicate that of transformants. Error bars represent the SE of five independent experiments.

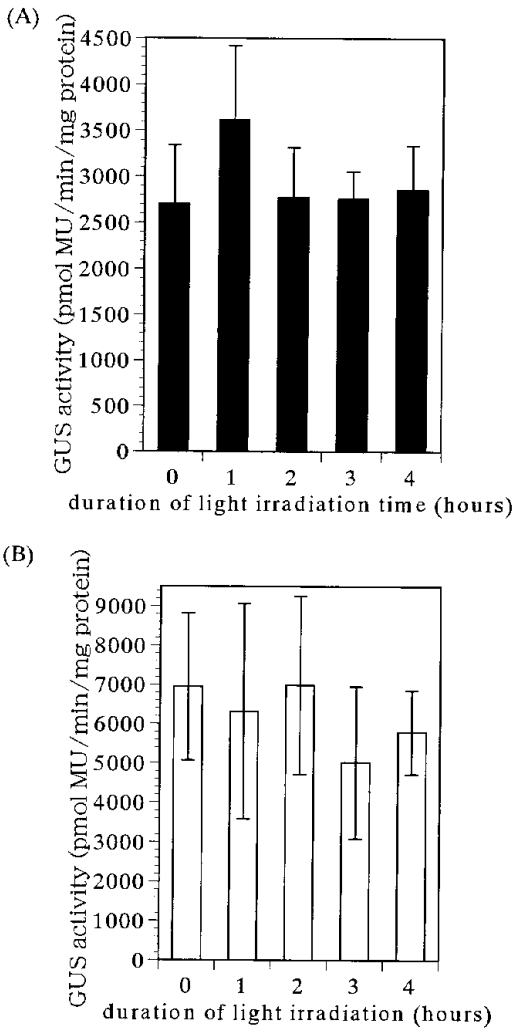

Fig. 2. GUS activity in transgenic calli.
The activity of green calli and that of non-green calli are indicated in (A) and (B), respectively. Error bars represent SE of five independent experiments.

as described previously (Moriwaki *et al.*, 1999; Jefferson *et al.*, 1987).

To investigate the influence of the duration of white light irradiation on GUS activity, we irradiated the leaves for 1, 2, 3 and 4 h. The highest GUS activity was observed in the leaves irradiated for 3 h, and it was about 14.5 times higher than the activity in mock-treated leaves (**Fig. 1A**). However, when the leaves were irradiated for 4 h, GUS activity was only about 1.8 times higher than that of mock-treated leaves. In transformed plantlets harboring the CaMV 35S promoter-*uidA* chimeric gene, GUS activity was increased with the irradiation period (**Fig. 1B**). Hence, the lower GUS activity after a 4 h irradiation than after a 3 h irradiation is attributed to the *g2b* promoter.

GUS activity was measured in transgenic calli treated in the same way as the leaves. In green calli, GUS activity seemed to increase after irradiation with white light, while an apparent increase was not observed in non-green calli (**Fig. 2**).

There were great differences in GUS activity among mock-treated samples of leaves, green calli, and non-green calli (**Fig. 1, 2**). GUS activity in leaves was about 1/35 of that of non-green calli, and that in green calli was about 1/3 of that of non-green calli. The difference observed here is accountable, because in the dedifferentiated state, high expression of the genes which respond to stress is observed (Fritzemeier *et al.*, 1987). The *g2b* promoter is considered to be under the control of light irradiation with the differentiation progress.

We also investigated the influence of light with various wave lengths on the induction of GUS activity of green calli. Red (λmax=650nm) light was generated by covering fluorescent tubes by red cellophane film so as not to affect conditions other

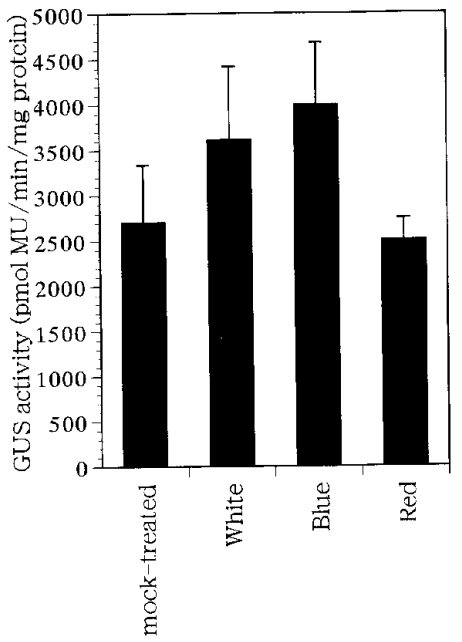

Fig. 3. GUS activity in green calli irradiated with light at various wave lengths.
Error bars represent SE of five independent experiments.

than wave length. Blue light (λmax=390nm) was provided in the same way using blue cellophane film. Light irradiation was performed for 1 h, and light source was adopted according to the report of Kurata *et al.* (1998). The level of GUS activity induced by blue light was similar to that induced by white light (**Fig. 3**). On the other hand, the activity after exposure to red light was similar to that in the mock-treated calli (**Fig. 3**). The PAL gene has been reported to be induced by exposure to 290 nm in carrot cell suspension cultures (Takeda *et al.*, 1994), and by UV irradiation in *P. kitakamiensis* (Osakabe *et al.*, 1995 b). In another report, GUS expression driven by the CHS promoter was induced only by blue light (Kurata *et al.*, 1998). In agreement with these observations GUS activity was induced by blue light and not by red light in this study. Blue light will be as useful in controlling foreign gene expression as white light will be.

The present results indicate that the expression of foreign genes will be controlled by the g2b promoter in transgenic plants, and that the g2b promoter is going to be under the control of light irradiation with differentiation progress. In transgenic plants, the g2b promoter might be useful to control the expression of a foreign gene that would be harmful when overexpressed.

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