The CaMV-35S promoter is sensitive to shortened photoperiod in transgenic tobacco

Abstract The CaMV-35S promoter is one of the most widely used promoters in transgenic plant research because it exhibits a high level of transcriptional activity in a variety of plant tissues. Here, the CaMV-35S promoter fused to the GUS gene was used as a model for constitutive expression in transgenic Nicotiana tabacum (cv ‘Xanthi’) leaves. The transgenic plants were placed under a shortened photoperiod to determine if GUS expression changed; measurements were made using fluorometry and ribonuclease protection assays. When the plants were moved from a 16:8-h photoperiod to a 8:16-h photoperiod, an increase in both specific GUS activity and gus RNA levels was observed, and these levels decreased upon returning to the 16:8-h photoperiod. These results indicate that photoperiod plays an important role in the regulation of the CaMV-35S promoter. Studies involving a comparison of this promoter to others should be limited to constant photoperiod conditions.

Key words CaMV · 35S promoter · Photoperiod

Abbreviations CaMV: Cauliflower Mosaic Virus · GUS: β-Glucuronidase

Introduction

The CaMV-35S promoter is useful in studies of promoter regulation as a model for constitutive expression (Odell et al. 1988). This promoter exhibits a high level of transcriptional activity in a variety of plant tissues (Williamson et al. 1989) and confers a high level of expression within most cells when transferred into plants (Benfey and Chua 1990).

The effects of changing photoperiod on the 35S promoter have not been reported, but light conditions are known to influence viral infection of plants (Qiu et al. 1997). An early report showed that a reduction in light intensity increased the virus content of systemically infected leaves (Bawden and Roberts 1949). Infection of cruciferous plant species by the Cauliflower Mosaic Virus is most pronounced in the Fall and Winter. By responding to the changes in light intensity and photoperiod, the virus is able to infect the plant systemically or influence the movement of the virus throughout the plant (J. Schoelz, personal communication).

In the study reported here, transgenic plants containing the CaMV-35S promoter fused to the GUS gene were used as a control for constitutive expression. These transgenic plants were subjected to treatments of shortened photoperiod, low temperature, and shortened photoperiod combined with low temperature. Leaf tissues were then assayed fluorometrically and RNA was isolated for analysis. These results are significant because this is the first report of an increase in activity of the CaMV-35S promoter under shortened photoperiod.

Materials and methods

Plant transformation

The plasmid pBI121 contains the CaMV-35S-gus construct (Sigma Chemical Co, St. Louis, Mo.). The plasmid pSS81,
containing the cor15a-gus fusion, was a gift from M. Thomashow (Baker et al. 1994). Agrobacterium- transformation of Nicotiana tabacum cv ‘Xanthi’ was carried out as previously described (Horsch et al. 1989) using strain LBA4404. Polymerase chain reaction (PCR) was used to screen transgenic plant lines containing the gus gene (Schnurr 1997). The primary transgenic plant lines that contained the gus gene as determined by PCR were self-pollinated, and the seeds were germinated and selected on 150 mg/l kanamycin. Plants were transplanted to 4-inch pots containing soil-less mix and grown in growth chambers maintained at 22 ± 2 °C under fluorescent light in growth chambers emitting 4000 lux under a 16:8-h (light:dark) photoperiod. Four independent transgenic plant lines were selected (designated 35S-2, 35S-5, 35S-8, and 35S-11) on the basis of high levels of GUS activity using the histochemical GUS assay (Jefferson 1987). Three representative plants from each line were used in the treatments described below, except the data generated in Fig. 2, where 20 plants from one line were used.

Photoperiod, cold and cold + photoperiod treatments

For the photoperiod treatment, the plants were grown under a 16:8-h (light:dark) hour photoperiod at 22 °C, and the third fully expanded leaf from the apical meristem was removed and stored at −80 °C. The photoperiod was changed to 8:16 h for 3 full day/night cycles, and one leaf above the first sample was removed and stored at −80 °C. The photoperiod was then returned to 16:8 h, and after 3 full day/night cycles the first fully expanded leaf from the meristem was removed and stored at −80 °C. This sampling technique was used because mature leaves express higher levels of GUS than younger leaves (Jefferson et al. 1987). Therefore, the leaves were sampled by using the more mature leaves before the treatment, so that any increases in GUS expression could not be attributed to leaf age. Under normal growth conditions, GUS activity is consistently higher in the more mature leaves (Schnurr 1997).

For the cold treatment, the plants were grown under a 16:8-h photoperiod at 22 °C. The third fully expanded leaf from the apical meristem was removed and stored at −80 °C. The temperature in the growth chamber was lowered to 4 °C over a 30-min period, and the plants remained at 4 °C for 3 full days during which point the second fully expanded leaf was sampled. After the temperature was returned to 22 °C and maintained for 3 days, the first fully expanded leaf from the meristem was sampled.

The cold + photoperiod treatment consisted of the plants growing under a 16:8-h photoperiod at 22 °C at which point the third fully expanded leaf was removed. The second fully expanded leaf was removed after the plants were subjected to a 8:16-h photoperiod at 4 °C for 3 full day/night cycles. The first fully expanded leaf was sampled after the plants were returned to the 16:8-h photoperiod at 22 °C for 3 full cycles.

Fluorometric assay

The fluorometric assay for specific GUS enzyme activity was performed as described previously (Jefferson et al. 1987). Approximately 150 mg of leaf tissue was ground in 0.4 ml extraction buffer, and 10 μl of the extract was added to 2 ml assay buffer. For each plant line, three 200-μl aliquots were removed at 0 and 60 min and placed in 1.8 ml stop buffer. The samples were read on a Mini TKO-100 DNA fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.). The specific activity was normalized to nmol/min per milligram protein by using a modified Bradford protein assay to determine the protein concentrations of each sample (Read and Northcote 1981).

Ribonuclease protection assays

Total leaf RNA was extracted from frozen plant tissues using the RNeasy Plant Total RNA kit from Qiagen according to manufacturer’s protocol (Chatsworth, Calif.). The 187-nucleotide GUS probe was synthesized with the T7 MAXiScript in vitro transcription kit from Ambion (Austin, Tex) using α-32P-CTP (NEN Life Science Products, Boston, Mass.). Each ribonuclease protection assay was performed according to the protocol in the RPAII kit (Ambion) using 5 ug of RNA with 1×104-cpm labeled probe. RPA blots were quantified using Ultra-Lum software (Paramount, Calif.), version 1.58.

Results and discussion

Although the CaMV-35S promoter is often used as a control for constitutive transgene expression, it has been suggested that 35S promoter activity is not constitutive (Jefferson 1987; Nagata 1987; Williamson 1989).

There is documentation on the effects of light quality and intensity on the expression of genes directed by the CaMV-35S promoter (Frohnmeyer 1994; Bovy 1995). The response of the 35S promoter to changing photoperiod has not been studied using sensitive techniques such as the fluorometric and ribonuclease protection assays. For instance, transgenic aspen containing 35S-gus exhibited GUS activity in the stem that did not vary during the cycle of growth-dormancy-reactivation, which included a photoperiod change from 18:6 h to 8:16 h (Nilsson et al. 1996). However, this conclusion was based on the qualitative results of histochemical staining, a technique less sensitive than the fluorometry assay (Beney and Chua 1989).

In this study, transgenic tobacco plants were grown under a shortened photoperiod to determine if these conditions cause a change in GUS expression, with the latter measured using a fluorometric assay (Jefferson et al. 1987). Transgenic tobacco plants were grown under a 16:8-h photoperiod, then a 8:16-h photoperiod for 3 day/night cycles and returned to the 16:8-h photoperiod for 3 full cycles. Fluorometric analyses of leaf extracts made from samples taken before, during, and after the treatment showed a reproducible increase in GUS expression in three independent transgenic 35S-gus tobacco lines (see Table 1). The GUS activity of the 35S-gus lines, 35S-2, 35S-5, and 35S-11, was up-regulated during the 8:16-h photoperiod treatment, by 21% 19%, and 96%, respectively. The levels of GUS activity consistently declined when the plants were returned to a 16:8-h photoperiod, by 38%, 23% and 21%, respectively. Analysis of RNA levels using ribonuclease protection assays showed similar results. GUS RNA transcripts were highest in 35S-gus leaf tissues sampled on the first and second days after the plants were grown under a 8:16-h photoperiod, and declined when the plants were returned to the normal 16:8-h photoperiod (Fig. 1). Both the fluorometry and RNA analyses suggest that transcription and translation of GUS in these transgenic plants increased upon exposure to a shortened photoperiod, and after returning the plants to a 16:8-h photoperiod, GUS levels decreased. The cor15a-gus plants did not show an increase in specific GUS activity or gus transcript levels (Table 1; Schnurr 1997).