ANALYSIS OF PLANT GENE RESPONSE TO THE STRESS OF COPLANAR PCB USING THE TRANSGENIC *ARABIDOPSIS THALIANA*

Shigenori Sonoki, Atsushi Kobayashi, Shiori Matsumoto, Shizuka Nitta and Shin Hisamatsu

Dept. of Environmental Health Science, Azabu University, Kanagawa 229-8501, Japan

Introduction

Polychlorinated biphenyls (PCBs), one of the halogenated aromatic hydrocarbons, are industrial compounds which have been widely identified as environmental pollutants. Among the possible 209 PCB congeners, the environmental toxicity of coplanar PCBs (Co-PCBs) is getting more serious. In this study, the searching of gene(s) responding to the stress ofCo-PCBs in the genome of plant, *Arabidopsis thaliana*, was tried to investigate the efficiency of monitoring the gene expression changed by Co-PCBs exposure in the environmental risk assessment of Co-PCBs contamination. Two kinds of transformed *Arabidopsis thaliana* were used to look for the gene(s) whose expressions were controlled by Co-PCBs. These two transformed lines have been constructed either by T-DNA tagging or Ac/Ds transposon tagging to facilitate the identification of insertions of the reporter gene, a β-glucuronidase (GUS) gene, in the vicinity of enhancers in the genome[1, 2]. Seedlings were exposed to 3,3',4,4',5-pentachlorobiphenyl (PeCB), which is potentially the most toxic Co-PCBs. After exposure to PeCB, the expression of GUS gene was detected histochemically by staining the whole plant in the GUS dye solution, then the staining patterns were compared to those in the control plant. As a result, one interesting line showing the different GUS expression pattern was found.

Materials and Methods

Chemicals

3,3',4,4',5-Pentachlorobiphenyl (PeCB) was purchased from Wellington Labs., and the standard solution was prepared in toluene to a concentration of 5 mg/L. The standard solution was diluted to 0.5 ng/ml in distilled water for the exposure experiment to plants. All solvents were pesticide free reagent grade. All other chemicals used were of analytical grade.

Enhancer trapped *Arabidopsis thaliana*

Enhancer trap lines of *Arabidopsis thaliana* generated by T-DNA tagging were obtained from Arabidopsis Biological Resource Center at Ohio State University. The vector used to generate the enhancer trap lines is a derivative of the binary plant transformation vector pCGN1547[3]. The vector contains the -60CaMV minimal promoter fused to the GUS gene[4]. By itself the -60 CaMV promoter directs very low levels of transcription, and in the absence of a closely linked enhancer

element, the level of GUS activity is undetectable. After integration, enhancer elements in genomic DNA adjacent to the T-DNA insertion cause an increase in transcription from the -60 promoter resulting in an increased level of expression of the GUS gene, so the expression patterns of the GUS gene in plants were directed by the adjacent enhancer. These patterns can be detected by staining for GUS activity using the chromogenic substrate X-gluc.

Histochemical detection of GUS

Seedlings of enhancer trap lines of *Arabidopsis thaliana* grown on the rock fiber at 26□ on a 16hr light / 8hr dark cycle were exposed to PeCB (0.5 ng/ml) for two days. The seedlings were carefully pulled out of the rock fiber, then put into the staining solution for GUS expression prepared according to the procedure by A-M Stomp[5]. The staining was performed at 37□ overnight. The staining patterns for the localization of GUS activity in plants were detected under the microscope, and were compared with those of the control plants for the differences.

Results and Discussion

Generation of enhancer trap lines of *Arabidopsis thaliana* by Ac/Ds transposon tagging

To identify the gene(s), whose expressions are controlled by Co-PCBs, so many kinds of enhancer traps have to be investigated. For that purpose, Ac/Ds transposon tagging method was tried to generate the enhancer trap lines. Ds Transposon carrying the GUS gene and the minimal CaMV 35S promoter, and Ac element containing the transposase were kindly gifted by Dr. Nina V. Fedoroff. As shown in Figure 1., these two constructs, Ds-GUS transposon and Ac transposase, were introduced into *Arabidopsis thaliana* by *Agrobacterium*-mediated vacuum infiltration[6]. The progenies showing kanamycin-resistant and hygromycin-resistant for Ds-GUS transformants, and kanamycin-resistant for Ac transformants were selected, respectively. The crosses between Ds lines as pollen parents and Ac lines as egg parents followed by the selection of transformants were carried out according to Fedoroff et al.[2]. As a result, 467 R2 progenies having the phenotypes of hygromycin-resistant, chlorsulfuron-resistant and napthalene acetamide-resistant were selected. Among them, 59 lines expressed the GUS activities as shown in Table 1 and the percentage of GUS expression was 12.6%. This value seems to be considerably lower than that in enhancer trap lines, 35.1%, produced by T-DNA tagging shown in Table 2.

The effect of PeCB on the GUS expression

One line in the T-DNA tagging lines showing the different GUS expression pattern was found after exposure to PeCB. This line showed no GUS activities in one-month-old seedling, however, the distinct GUS activities appeared only in the first leaf responding to PeCB stress. But we have to pay attention to the age of plant to be used for comparison in the GUS activity, because the GUS expression patterns varied due to aging.

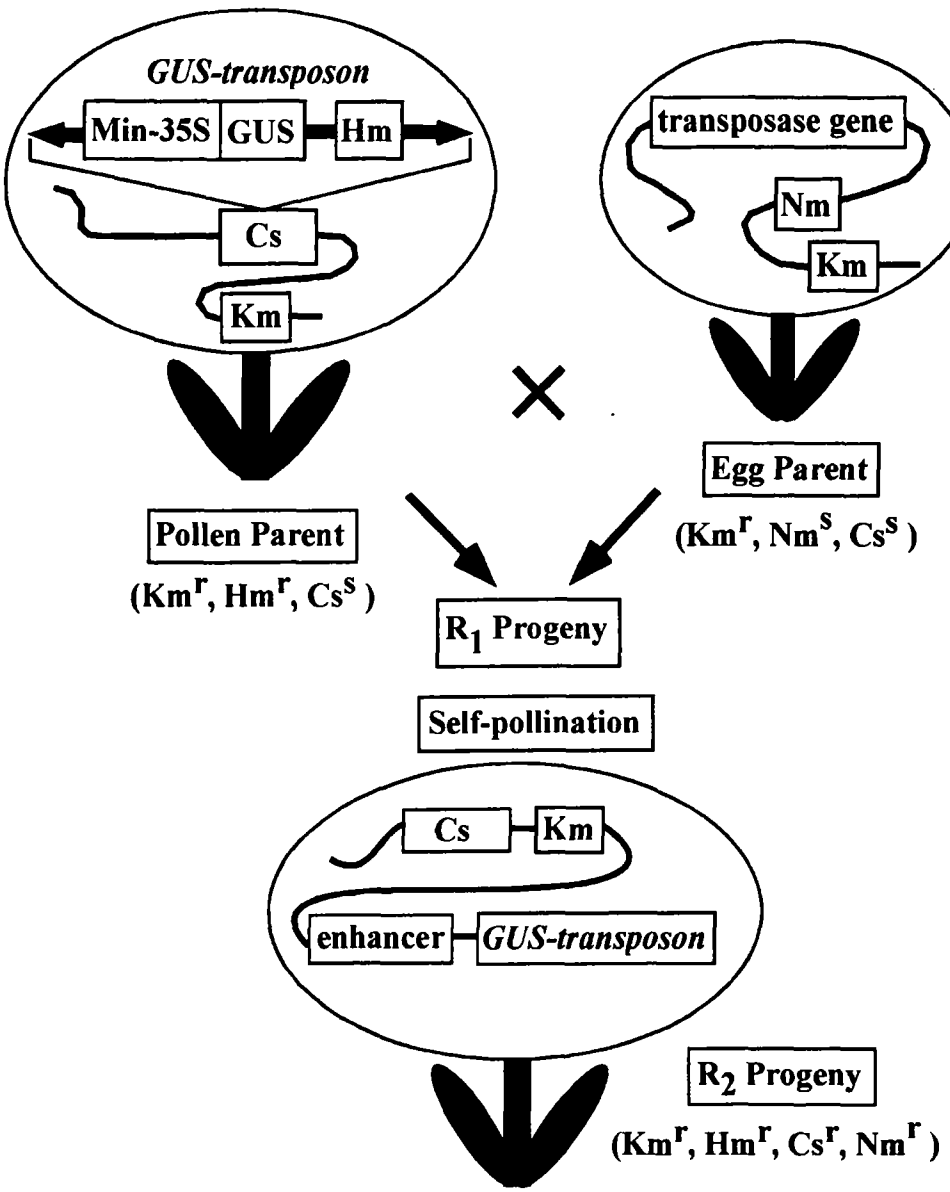

Figure 1. Generation of the enhancer trap line of *Arabidopsis thaliana* by Ac/Ds transposon tagging

Abbreviations: Km: kanamycin resistant gene; Hm: hygromycin resisitant gene; Cs: chlorsulfuron resisitant gene; Nm: naphthalene acetamide sensitive gene; Min-35S: minimal CaMV 35S promoter

Table 1. GUS staining pattern in enhancer trap lines of *Arabidopsis thaliana* generated by Ac/Ds transposon tagging

Staining Location	Number Lines
Leaf	31
Stem	12
Root	9
Vascular	4
Pistil	3
(Total)	**59**

Of 467 lines analyzed, 59 lines (12.6 % of the total) exhibit a GUS staining pattern.

Table 2. GUS staining pattern in enhancer trap lines of *Arabidopsis thaliana* generated by T-DNA tagging

Staining Location	Number Lines
Leaf	137
Stem	80
Root	72
Pistil	60
Vascular	21
Anther	6
Petal	5
Stamen	3
Pollen	2
Sepal	1
(Total)	**387**

Of 1103 lines analyzed, 387 lines (35.1 % of the total) exhibit a GUS staining pattern.

Acknowledgements

This work was partly supported by the grant of the Ministry of Education (subject # 11839028) and of High-Tech Research Center project from the Ministry of Education. We greatly thank Dr Nina V. Fedoroff of Pennsylvania State University for giving Ds-Gus and Ac transposase constructs for Ac/Ds transposon tagging. We would also like to acknowledge Dr FumiakiAkahori and Dr Kenji Nakaaki for their helpful comments.

References

1. Koncz C., Martini N., Mayerhofer R., Koncz-Kalman Z., Korber H., Redei G.P. and Schell J. (1989) *Proc. Natl. Acad. Sci. U S A* 86(21), 8467-8471
2. Fedoroff N.V. and Smith D.L. (1993) *Plant J.* 3(2), 273-289.
3. McBride K.E. and Summerfelt K.R. (1990) *Plant Mol. Biol.* 14, 269-276.
4. Odell J.T., Nagy F. and Chua N.-H. (1985) *Nature* 313, 810-812.
5. Stomp A.-M. (1992): Part 3 in GUS Protocols (Gallagher S.R. Ed.), Academic Press, Inc.
6. Bechtold N. and Pelletier G. (1998) *Methods Mol. Biol.* 82, 259-266