Original Article

Molecular Basis of Photobleaching Herbicide Resistance in Tobacco

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The resistant mechanism of the tobacco cell line ETR-245 to the photobleaching herbicide, pyraflufen-ethyl, was analyzed at the molecular level. Wild-type cDNAs of protoporphyrinogen oxidase (Protox), the target enzyme of photobleaching herbicides, were isolated from tobacco leaves and sequenced by the genetic complementation. Using the sequence information, Protox cDNAs were amplified by RT-PCR from normal and ETR-245 cells. The sensitivities of each Protox gene product to pyraflufen-ethyl were compared. Although mitochondrial Protox gene products showed almost equivalent sensitivities to pyraflufen-ethyl, the chloroplastic Protox gene product encoded by the cDNA from ETR-245 cells was more than 4000-fold resistant than that encoded by the cDNA from normal cells. The nucleotide sequence analysis of these chloroplastic Protox cDNAs revealed single-point mutation in the Protox cDNA from ETR-245, which accompanied with one amino acid substitution. From these results, it was clarified that this mutation was the cause for the resistance in ETR-245.

Key words: protoporphyrinogen oxidase, herbicide resistance, photobleaching herbicide, cDNA cloning, genetic complementation, *Nicotiana tabacum*.

INTRODUCTION

The application of photobleaching herbicides leads to the peroxidative destruction of cellular membrane and bleaching of tissues in plant¹⁾ in the presence of light.²⁾ In treated tissues, these herbicides cause the accumulation of protoporphyrin IX (Proto IX)³⁾ known as a strong photosensitizer to generate singlet oxygen. This accumulation of Proto IX was shown to be the result of the inhibition of the protoporphyrinogen oxidase (Protox) by these herbicides.^{4,5)} Protox catalyzes the oxidization of protoporphyrinogen IX (Protopogen) to Proto IX, the final common precursor in the heme and chlorophyll synthesis.⁶⁾ Surprisingly, the inhibition of the Protox by photobleaching herbicides causes the accumulation of not the substrate but the product. At present, the mode of action of photobleaching herbicide is hypothesized as follows. When the photobleaching herbicide inhibits chloroplastic Protox, Protopogen is exported from chloroplast to cytoplasm and rapidly oxidized to Proto IX by herbicide-resistant Protox-like activity associated with plasma membrane.^{7,8)}

Protox is known to be localized in mitochondria in microorganisms and animals,⁹⁾ whereas in plants, Protox activity was found in both mitochondria and chloroplasts.¹⁰⁾ The role of Protox in mitochondria in

the mode of action of photobleaching herbicide remains to be clarified,¹¹⁾ because both of the plant Protox enzymes are strongly inhibited *in vitro* by photobleaching herbicides.¹²⁻¹⁴⁾

Recently, Protox genes were isolated from several organisms. As a Protox gene, *hemG* of *Escherichia coli*¹⁵⁾ and *hemY* of *Bacillus subtilis*¹⁶⁾ were isolated in 1993 and 1994, respectively. Human¹⁷⁾ and mouse¹⁸⁾ Protox cDNAs were isolated in 1995. As concerned with plants, one kind of Protox cDNA was isolated from *Arabidopsis thaliana*¹⁹⁾ in 1996. In 1997, two kinds of Protox cDNA were isolated from tobacco and the subcellular localization of each gene products in mitochondria and chloroplast was clarified.²⁰⁾

Various herbicide resistant plants were produced by genetic engineering techniques and some of them have been already commercialized.²¹⁾ Glyphosate resistant crops were produced by the introduction of the gene encoding for herbicide resistant target enzyme, 5-enolpyruvyl-shikimate-3-phosphate synthase.²²⁾ Bromoxynil²³⁾ and phosphinothricin²⁴⁾ resistant crops were produced by the introduction of detoxifying enzymes which metabolize herbicides into inactive forms. As the first step toward genetic engineering for the photobleaching herbicide resistant crops, we attempted to isolate the photobleaching herbicide resistant gene.

We had already isolated the tobacco cell line ETR-245 resistant to a photobleaching herbicide, pyraflufen-ethyl.²⁵⁾ Pyraflufen-ethyl (formerly known as ET-751) is a 3-phenylpyrazole with photobleaching herbicidal activity²⁶⁾ synthesized in Nihon Nohyaku Co., Ltd.,²⁷⁾ which is now on developmental works for the market. It was shown that the resistance in ETR-245 was caused by the alteration of Protox to the herbicide resistant form.²⁵⁾ In this paper, we analyzed Protox genes in the cell line at the molecular level. At first, wild-type Protox cDNAs were isolated by the genetic complementation of the *E. coli* strain defective in *hemG* gene. From the sequence information of the isolated genes, Protox cDNAs were isolated from resistant cell line and analyzed to reveal molecular basis of photobleaching herbicide resistance.

MATERIALS AND METHODS

1. Chemicals

Pyraflufen-ethyl [ethyl 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetate] was synthesized in Nihon Nohyaku Co., Ltd. Other herbicides were purchased from commercial suppliers.

2. Tobacco Cell Lines

Normal and ETR-245 tobacco (*Nicotiana tabacum* cv. Xanthi NC) cell lines were established and maintained in MS agar medium as described previously.²⁵⁾

3. Bacterial Strains and Vectors

E. coli strains SASX38¹⁵⁾ and VSR800¹⁷⁾ defective in *hemG* (Protox gene of *E. coli*) were generously gifted from Dr. A. Sasarman at Montreal University and Dr. H. Inokuchi at Kyoto University, respectively. *E. coli* strains Y1090(r⁻) and XL-1 blue were purchased from Life Technologies and Stratagene, respectively. Phage vector λ gt22A, phagemid vector pBluescript II SK(−) and plasmid vector pCR^{TM}2.1 were purchased from Life Technologies, Stratagene and Invitrogen, respectively.

4. Isolation of Wild-type Protox cDNAs from Tobacco Leaves

Total RNA was extracted from leaves of tobacco plants (*N. tabacum* cv. SR1) by SDS-phenol method and LiCl precipitation. mRNA was isolated from total RNA using mRNA purification kit (Pharmacia). cDNA library was constructed using Superscript Lamda System (Life Technologies). Synthesized cDNAs were ligated directionally with λ gt22A *Not*I-*Sal*I arms and packaged *in vitro* using Gigapack II Gold Packaging Extracts (Stratagene). The primary library containing 1,900,000 independent clones was infected to *E. coli* Y1090(r⁻) and amplified.

Protox cDNAs were isolated by genetic complementation as described by Nishimura *et al.*¹⁷⁾ *E. coli* SASX38

defective in *hemG* precultured in the LB liquid medium containing 10 mM MgSO₄ and 0.2% maltose was infected with amplified tobacco cDNA library at 37°C for 30 min, and spread onto the LB agar plates containing IPTG to induce gene expression. After incubation for 2 days at 37°C, large colonies were subcultured in the LB liquid medium. Recombinant phage particles were released from the cultured medium by the addition of chloroform, plaque-purified and reinfected to *E. coli* SASX38 to reconfirm the ability to restore the growth of the *E. coli*.

cDNA insert in the recombinant phage was amplified by PCR using TaKaRa EX Taq (TaKaRa Medicals), forward primer for λ gt22A (5'-ATT GGT GGC GAC GAC TCC TGG AG-3') and reverse primer for λ gt22A (5'-CCA GAC CAA CTG GTA ATG GTA GCG-3'). Amplified cDNAs were analyzed by restriction enzyme digestion and agarose gel electrophoresis. The cDNAs were divided into two groups. The largest cDNA in each group was inserted into *Eco*RV site in pBluescript II SK(−) using TA cloning method.

5. Isolation of Protox cDNAs from Normal and ETR-245 Cells

Protox cDNAs were amplified by RT-PCR from cultured cells. mRNA isolated from normal and ETR-245 cells was reverse transcribed into cDNA using oligo(dT) 12-18 (Life Technologies) as primer by SuperscriptII^{TM} RNaseH⁻ Reverse Transcriptase (Life Technologies). Synthesized cDNA recovered was used as templates for PCR. PCR primers used for Protox cDNA amplifications were (5'-GCG GTC TAC AAG TCA GGC AGT CAT-3') and (5'-CAT GCC AAT TTT CCC AAG GCA TGA TCG TAC T-3') for chloroplastic one, (5'-TAT GGC TCC TTC TGC CGG AGA AG-3') and (5'-ACA AAC ATG GTC GCA GAG TTA AGA AGG CAA-3') for mitochondrial one, respectively. Amplified cDNA fragments were subcloned into pCR^{TM}2.1 (Invitrogen) by TA-cloning method.

6. Measurements of Sensitivities of Each Protox Gene Products to Photobleaching Herbicides Using Complemented *E. coli*

E. coli SASX38 complemented with plasmids containing each Protox cDNA were precultured in LB liquid medium containing ampicillin at 28°C over night. Same amount of precultured *E. coli* was added to the LB liquid medium containing ampicillin and various concentrations of photobleaching herbicide and incubated with vigorous shaking at 28°C. A portion was collected periodically and the growth of *E. coli* was measured photometrically. The I₅₀ values were calculated as the concentration of the herbicides that caused 50% inhibition of the growth of each *E. coli*.

7. DNA Sequencing

cDNAs in pBNtPX-1 and pBNtPX-2 were sequenced by the generation of nested deletions using deletion kit for kilo sequence (TaKaRa Medicals). cDNAs in pCR-HC and pCR-RC were sequenced by the primer walking method. Sequencing reactions were performed using cycle sequencing kit AmpliTaqFS (Perkin-Elmer) and ABI PRISM 310 autosequencer (Perkin-Elmer). The cDNA sequence in each plasmid was analyzed by genetic analysis software GENETYX (SDC).

RESULTS

1. Isolation of Protox cDNAs from Tobacco Leaves

In order to facilitate the structural analysis of the resistant Protox gene in ETR-245, wild-type Protox cDNAs were isolated from tobacco leaves. Directional cDNA library in λ gt22A was infected to *E. coli* SASX38 defective in *hemG* and gene expression was induced. After incubation, several larger colonies were observed among dwarf colonies. Recombinant phage was recovered from larger colonies and reinfected to *E. coli* SASX38 to confirm the ability to complement the poor growth. cDNAs in confirmed phages were amplified by PCR, analyzed by restriction enzyme digestion and agarose gel electrophoresis. The cDNAs were divided into two groups, one with *EcoRV* site and the other with *XbaI* site. The largest cDNA in each group was subcloned into the phagemid vector pBluescript II SK(−). The plasmid in which cDNA with *EcoRV* or *XbaI* site was inserted in the positive direction to the *lacZ* promoter and complemented the poor growth of the *E. coli* was designated as pBNtPX-1 or pBNtPX-2, respectively. The nucleotide sequence of cDNAs in pBNtPX-1 and

pBNtPX-2 (deposited in the GenBank database, accession numbers AF044128 and AF44129) were almost the same as the sequences of plastidal and mitochondrial Protox cDNAs recently reported by Lermontova *et al.*,²⁰ respectively, though some differences were observed. Two base substitutions were observed in pBNtPX-1, which accompanied with two amino acid substitutions (Fig. 1A). Two base substitutions were observed in pBNtPX-2, which did not accompany with any amino acid substitutions (data not shown).

2. Isolation of Protox cDNAs from ETR-245 Cells

mRNAs from normal and ETR-245 cells were reverse transcribed into cDNAs, and mitochondrial and chloroplastic Protox cDNAs were amplified by PCR using PCR primers designed from wild-type Protox cDNA sequences. Amplified cDNAs were subcloned into pCR^{TM}2.1 in frame with *lacZ* gene. The plasmids, in which mitochondrial Protox cDNAs from normal and ETR-245 cells were inserted, were designated as pCR-HM and pCR-RM, respectively. The plasmids, in which chloroplastic Protox cDNAs from normal and ETR-245 cells were inserted, were designated as pCR-HC and pCR-RC, respectively. Each plasmid was introduced into *E. coli* SASX38 and confirmed to complement the poor growth of the *E. coli*.

3. Sensitivity of Each Gene Product to Pyraflufen-ethyl

The inhibitory effect of pyraflufen-ethyl on the growth of *E. coli* SASX38 complemented with each plasmid described above was evaluated to compare the sensitivity of each gene product encoded by the plasmids. As

A

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1  MTTTPIANHP NIFTHQSSSS PLAFLNRTSF IPFSSISKRN SVNCNGWRTR CSVAKDYTVP
61  SSAVDGGPAA ELDCVIVGAG ISGLCIAQVM SANYPNLMVT EARDRAGGNI TTVERDGYLW
121 EEGPNSFQPS DPMLTMAVDC GLKDDLVLGD PNAPRFVLWK GKLRPVPSKL TDLFFDLMS
181 IPGKLRAFVG AIGLRPSPPG HEESVEQFVR RNLGGEVFER LIEPFCSGVY AGDPSKLSMK
241 AAFGKVVWLE ETGGSIIIGT FKAIKERSST PKAPRDPRLP KPKGQTVGSF RKGLRMLPDA
301 ISARLGSKLK LSWKLSSITK SEKGGYHLTY ETPEGVVSQ SRSIVMTVPS YVASNILRPL
361 SVAAADALSN FYYPPVGAVT ISYPQEAIRD ERLVDGELKG FGQLHPRTQG VETLGTIYSS
421 SLFPNRAPKG RVLLLNYYIG AKNPEILSKT ESQLEVVDR DLRKMLIKPK AQDPLVVGVR
481 VWPQAIPQFL VGHLDTLSTA KAAMNDNGLE GLFLGGNYVS GVALGRCVEG AYEVASEVTG
541 FLSRYAYK

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B

			Ala
pCR-HC	GTT TAT	GCT GGT GAT	
pCR-RC	GTT TAT	GTT GGT GAT	
		Val	
amino acid		231	

Fig. 1 (A) Amino acid sequence of chloroplastic Protox cDNA in pBNtPX-1. The underlines in amino acids 174 and 382 denote the amino acid substitutions in pBNtPX-1 compared with the sequence reported by Lermontova *et al.*²⁰ The double underline in amino acids 231 denotes the mutation site in pCR-RC. (B) Single-point mutation in pCR-RC.

shown in Table 1, I_{50} values of pyraflufen-ethyl against *E. coli* SASX38 (pCR-HM) and *E. coli* SASX38 (pCR-RM) were 70 and 90 nM, respectively, indicating that both gene products encoded by the mitochondrial Protox cDNAs from normal and ETR-245 cells were similarly sensitive to pyraflufen-ethyl. Although I_{50} value of pyraflufen-ethyl against *E. coli* SASX (pCR-HC) was 2.5 nM, that against *E. coli* SASX38 (pCR-RC) was more than 10,000 nM, indicating that the gene product encoded by the chloroplastic Protox cDNA from ETR-245 was more than 4000-fold resistant than that from normal cells.

Cross-resistance of resistant chloroplastic Protox gene product encoded by pCR-RC from ETR-245 to other photobleaching herbicides such as oxadiazon, S-23142 and acifluorfen was also evaluated. As shown in Table 2, the resistant gene products exhibited high resistance to all the photobleaching herbicides tested.

4. Sequence Analysis of Chloroplastic Protox Gene from ETR-245

Nucleotide sequences of sensitive and resistant chloroplastic Protox cDNAs in pCR-HC and pCR-RC, respectively, were compared to reveal molecular basis for the

Table 1 Sensitivity of the Protox gene products to pyraflufen-ethyl measured as the inhibitory effect on the growth of the *E. coli* complemented with the Protox cDNAs.

<i>E. coli</i>	I_{50} of pyraflufen-ethyl (nM)	R/S ratio
SASX38(pCR-HM ^a)	70	1.3 ^e)
SASX38(pCR-RM ^b)	90	
SASX38(pCR-HC ^c)	2.5	> 4000 ^f)
SASX38(pCR-RC ^d)	> 10,000	

^a) The plasmid with mitochondrial Protox cDNA from normal cells. ^b) The plasmid with mitochondrial Protox cDNA from ETR-245 cells. ^c) The plasmid with chloroplastic Protox cDNA from normal cells. ^d) The plasmid with chloroplastic Protox cDNA from ETR-245 cells. ^e) b)/a). ^f) d)/c).

resistance. Nucleotide sequence of the cDNA in pCR-HC was exactly the same as that of wild-type Protox cDNA from tobacco leaves in pBntPX-1. On the other hand, single-point mutation was observed in the nucleotide sequence of the cDNA in pCR-RC. The codon corresponding to amino acid 231 was mutated from GCT to GTT, which resulted in the substitution of alanine for valine at the position (Fig. 1B).

DISCUSSION

When we had attempted to isolate the resistant Protox gene from ETR-245, no sequence information about tobacco Protox gene was available. Therefore, we isolated wild-type tobacco Protox cDNA by the genetic complementation using *E. coli* strain SASX38¹⁵⁾ defective in *hemG* by ourselves. The isolated Protox cDNAs also complemented *E. coli* strain VSR800¹⁷⁾ defective in *hemG*. The sequences of wild-type Protox cDNAs thus derived were almost the same as the sequence of tobacco Protox cDNA recently reported by Lermontova *et al.*,²⁰⁾ though some differences were observed (Fig. 1A). They reported that each Protox cDNA is encoded in small subfamilies, therefore, it was thought that the wild-type Protox cDNA isolated from tobacco leaves in this study might be in the other subfamily.

Utilizing sequence information of wild-type Protox cDNAs, chloroplastic and mitochondrial Protox cDNAs were amplified by RT-PCR from normal and ETR-245 cells. The sensitivities of each Protox gene product were compared by measuring the inhibitory effect of pyraflufen-ethyl on the growth of *E. coli* SASX38 complemented with plant Protox cDNAs. This method using complemented *E. coli* SASX38 was preferred to the extraction of the crude enzyme from *E. coli* and measuring the enzyme activities *in vitro*, because extraction of the active Protox enzyme from *E. coli* was rather difficult as shown by Lermontova *et al.*²⁰⁾ This method was very sensitive and almost reflected the sensitivity of the Protox enzyme to pyraflufen-ethyl measured *in vitro*. Mitochondrial Protox gene products encoded by pCR-HM and pCR-RM from normal and ETR-245 cells, respectively, showed almost equivalent sensitivities to

Table 2 Sensitivity of chloroplastic Protox gene products to several photobleaching herbicides measured as the inhibitory effect on the growth of *E. coli* complemented with each Protox cDNA.

Herbicide	I_{50} (nM)		
	SASX38(pCR-HC ^a)	SASX38(pCR-RC ^b)	R/S ratio ^c)
Oxadiazon	550	> 100,000	> 180
S-23142	2.5	1,400	560
Acifluorfen	1800	17,000	9.4

^a) The plasmid with chloroplastic Protox cDNA from normal cells. ^b) The plasmid with chloroplastic Protox cDNA from ETR-245 cells. ^c) b)/a).

pyraflufen-ethyl, whereas chloroplastic Protox gene product encoded by pCR-RC from ETR-245 cells was more than 4000-fold resistant than that encoded by pCR-HC from normal cells (Table 1). It was clarified from these results that the resistance in ETR-245 to photobleaching herbicides was caused by the alteration of the chloroplastic Protox gene product from sensitive form to resistant one. Because the alteration of the mitochondrial Protox gene product was unnecessary for the resistance in ETR-245, it was suggested that the target enzyme of photobleaching herbicides would not be both of mitochondrial and chloroplastic Protox but solely chloroplastic Protox.

Although the nucleotide sequences of sensitive chloroplastic Protox cDNA in pCR-HC from normal cells was exactly the same as wild-type Protox cDNA from tobacco leaves, single-point mutation was observed in the nucleotide sequence of resistant Protox cDNA in pCR-RC from ETR-245, which accompanied with one amino acid substitution (Fig. 1B). When the same nucleotide substitution was introduced into sensitive chloroplastic Protox cDNAs in pCR-HC from normal cells by the site directed mutagenesis, the gene product was converted from sensitive form to resistant one (data not shown), confirming that this mutation was the cause for the resistance in ETR-245.

The resistant gene products exhibited high resistance to all the photobleaching herbicides tested (Table 2). Therefore, it was suggested that photobleaching herbicides would bind at or near this mutation site in Protox enzyme. Because photobleaching herbicides are competitive inhibitors of Protox,^{28,29)} the substrate of Protox, i.e. Protogen, would also bind at or near this mutation site in Protox enzyme. Further study using other resistant Protox enzymes would reveal the binding site and interaction of photobleaching herbicides and the substrate. Such a resistant Protox enzymes were reported in soybean³⁰⁾ and tobacco.³¹⁾ In tobacco, Protox enzyme extracted from S-23142 resistant cell line exhibited 20-fold increase in tolerance to S-23142, whose increase was not so high as reported in this paper. Therefore, the different mutation might be involved in the resistance in S-23142 resistant cell line.

At present, we are introducing the resistant Protox gene from ETR-245 into tobacco plants to evaluate the expression of resistance in a whole plant. If the resistance is expressed in a whole plant, this gene would be valuable not only for producing photobleaching herbicide resistant crops but also as a selectable marker in transforming plants with exogenous genes, because this gene is one of plant origin.

REFERENCES

- G. L. Orr & F. D. Hess: *Plant Physiol.* **69**, 502 (1982)
- S. Matsunaka: *J. Agric. Food Chem.* **17**, 171 (1969)
- M. Matringe & R. Scalla: *Plant Physiol.* **86**, 619 (1988)
- M. Matringe, J. M. Camadro, P. Labbe & R. Scalla: *Biochem. J.* **260**, 231 (1989)
- D. A. Witkowski & B. P. Halling: *Plant Physiol.* **90**, 1239 (1989)
- R. Poulson: *J. Biol. Chem.* **250**, 1269 (1975)
- J. M. Jacobs, J. M. Wehner & N. J. Jacobs: *Pestic. Biochem. Physiol.* **50**, 23 (1994)
- H. J. Lee & S. O. Duke: *J. Agric. Food Chem.* **42**, 2610 (1994)
- J. C. Deybach, V. da Silva, B. Grandchamp & Y. Nordmann: *Eur. J. Biochem.* **149**, 431 (1985)
- J. M. Jacobs & N. J. Jacobs: *Biochem. J.* **244**, 219 (1987)
- M. Matringe, J. M. Camadro & N. Brouillet: "Proceedings 1993 Brighton Crop Protection Conference—Weeds," pp. 703–712, 1993
- J. M. Camadro, M. Matringe & R. Scalla: *Biochem. J.* **277**, 17 (1991)
- J. M. Jacobs, N. J. Jacobs, S. F. Borotz & M. L. Guerinet: *Arch. Biochem. Biophys.* **280**, 369 (1990)
- M. Matringe, J. M. Camadro, P. Labbe & R. Scalla: *FEBS Lett.* **245**, 35 (1990)
- A. Sasarman, J. Letowski & G. Czaika: *Can. J. Microbiol.* **39**, 1155 (1993)
- T. A. Dailey, P. Meissner & H. A. Dailey: *J. Biol. Chem.* **269**, 813 (1994)
- K. Nishimura, S. Taketani & H. Inokuchi: *J. Biol. Chem.* **270**, 8076 (1995)
- S. Taketani, T. Yoshinaga, T. Furukawa, H. Kohno, R. Tokunaga, K. Nishimura & H. Inokuchi: *Eur. J. Biochem.* **230**, 760 (1995)
- S. Narita, R. Tanaka, T. Ito, K. Okada, S. Taketani & H. Inokuchi: *Gene* **182**, 169 (1996)
- I. Lermontova, E. Kuruse, H.-P. Mock & B. Grimm: *Proc. Natl. Acad. Sci. USA* **94**, 8895 (1997)
- S. O. Duke: "Herbicide Resistant Crops," Lewis Publishers, Boca Raton, 1995
- S. R. Padgett, D. B. Re, G. F. Barry, D. E. Eichholtz, X. Delannay, R. L. Fuchs, G. M. Kishore & R. T. Fraley: "Herbicide Resistant Crops," ed. by S. O. Duke, Lewis Publishers, Boca Raton, pp. 53–84, 1995
- D. M. Stalker, K. E. McBride & L. D. Malyj: *Science* **242**, 419 (1988)
- M. De Block, J. Botterman, M. Vandewiele, J. Dockx, C. Thoen, V. Gossele, N. Rao Movva, C. Thompson, M. Van Montagu & J. Leemans: *EMBO J.* **6**, 2513 (1987)
- M. Horikoshi & T. Hirooka: *J. Pesticide Sci.* **24**, 13 (1999)
- S. Murata, A. Yuda, A. Nakano, Y. Kimura, K. Motoba, T. Mabuchi, Y. Miura, H. Nishizawa & S. Funayama: "Proceedings 1995 Brighton Crop Protection Conference—Weeds," pp. 243–248, 1995
- Y. Miura, M. Ohnishi, T. Mabuchi & I. Yanai: "Proceedings 1993 Brighton Crop Protection Conference—Weeds," pp. 35–40, 1993
- M. Matringe, R. Mornet & R. Scalla: *Eur. J. Biochem.* **209**, 861 (1992)
- R. Varsano, M. Matringe, N. Magnin, R. Mornet & R. Scalla: *FEBS Lett.* **272**, 106 (1990)
- T. Pornprom, H. Matsumoto, K. Usui & K. Ishizuka: *Pestic. Biochem. Physiol.* **50**, 107 (1994)
- K. Ichinose, F. S. Che, Y. Kimura, A. Matsunobu, F. Sato & S. Yoshida: *J. Plant Physiol.* **146**, 693 (1995)

要 約

タバコにおける光要求型除草剤耐性の分子的基盤

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大腸菌の protoporphyrinogen oxidase (Protox) 遺伝子欠損変異株 (SASX38) を用いた遺伝的相補法によって, タバコ葉から葉緑体及びミトコンドリア型 Protox cDNA を単離し, 全塩基配列を解明した. 得られた塩基配列情報を利用し, 光要求型除草剤 pyraflufen-ethyl 耐性タバコ細胞 ETR-245 株及び感受性株から, RT-PCR によって各々2種の Protox cDNA を増幅した. これら Protox cDNA によ

て生育を相補した大腸菌 SASX38 株に対する pyraflufen-ethyl の生育阻害度を測定し, 各遺伝子産物の pyraflufen-ethyl に対する感受性を比較した. ミトコンドリア型遺伝子産物には感受性の差は認められなかったが, 葉緑体型遺伝子産物では ETR-245 株由来のものは感受性株由来のものに比べ, 4000 倍以上の耐性を示した. さらに, これら葉緑体型 Protox cDNA の全塩基配列を比較した結果, 1 塩基にのみアミノ酸置換を伴う塩基の変異が認められた. したがって, この変異が ETR-245 株における光要求型除草剤耐性の原因であることが明らかとなった.