Note

Effects of 3-Amino-1,4-dimethyl-5H-pyrido(4,3-b)indole (Trp-P-1) and 3-Amino-1-methyl-5H-pyrido(4,3-b)-indole (Trp-P-2) on Somatic Mutation in Soybean $Y_{11}y_{11}$ Plants

Yoshinobu KATO and Yoshio SANO*

*Life Science Division,
Hokkaido Institute of Public Health,
Sapporo, Hokkaido 060, Japan*** National Institute of Genetics,
Mishima, Shizuoka 411, Japan*

Received December 17, 1990

The marker Y_{11} gene in strain T-219 of the soybean (*Glycine max*) is incompletely dominant so that the leaves of heterozygotic $Y_{11}y_{11}$ plants are light green. By somatic mutation, two simple leaves and the first compound leaf of the heterozygous plants showed occasional dark green

spots adjacent to, and almost a mirror image of, a yellow spot (Fig. 1A and 1B). Besides these twin spots, two types of single spots, viz dark green and yellow were also observed (Fig. 1C). These spots most likely arise from somatic crossing-over (twin spot), and forward mutation and backward mutation (single spot).^{1,2)} The literature cited above indicates several kinds of genetic and/or chromosomal abnormalities caused by chromosomal structural changes, somatic crossing-over, or forward and backward mutations to be detectable by the appearance of variously colored spots on the leaves of heterozygotic $Y_{11}y_{11}$ plants.

Trp-P-2 in pyrolysates of broiled meat and fish is strongly mutagenic in the Ames assay systems in the presence of hepatic microsomes,³⁾ and has carcinogenic effects on mammalian test systems.^{4,5)} Trp-P-1 is also a mutagen, but its mutagenicity is slightly lower than that of Trp-P-2.³⁾

An examination was thus made of the effects of Trp-P-1 and Trp-P-2 on somatic mutation in soybean $Y_{11}y_{11}$ plants.

Trp-P-1 and Trp-P-2 were obtained from Wako Pure Chemicals. The chemicals were dissolved in methanol and then diluted to various concentrations with distilled water immediately before treatment. The original strain, T-219, of soybeans was soaked in 20 ml of various concentrations of Trp-P-1 or Trp-P-2 for 24 hr at 20°C in the dark. The solutions were almost completely absorbed by the soaked seeds. The treated seeds were then rinsed with distilled water, sown in soil, and kept in a greenhouse for several

Fig. 1. Somatic Mutation in the Leaves of Soybean ($Y_{11}y_{11}$ Plants).

(A) dark green spot; (B) yellow spot; (C) double spot.

Table I. TYPES AND FREQUENCIES OF SPOTS ON THE LEAVES OF $Y_{11}Y_{11}$ PLANTS AFTER EXPOSURE TO 3-AMINO-1,4-DIMETHYL-5H-PYRIDO(4,3-*b*)INDOLE (Trp-P-1) OR 3-AMINO-1-METHYL-5H-PYRIDO(4,3-*b*)INDOLE (Trp-P-2)

Chemicals ($\mu\text{g/ml}$)	Number of leaves observed	Types and frequencies of spots (Number of spots/leaf)			
		Yellow	Dark green	Double	Total $\pm$ S.E.*
First experiment					
Control	48	0.68	0.45	0.19	1.31 ± 0.13
Trp-P-1					
0.05	142	1.73	1.06	0.18	2.97 ± 0.14
0.1	135	2.62	1.50	0.18	4.30 ± 0.18
Trp-P-2					
0.05	157	2.06	1.10	0.16	3.32 ± 0.11
0.1	108	2.54	1.26	0.13	3.93 ± 0.14
Second experiment					
Control	64	1.06	0.67	0.20	1.94 ± 0.10
Trp-P-1					
0.01	108	0.90	0.90	0.19	1.99 ± 0.09
0.025	118	1.53	1.12	0.23	2.87 ± 0.12
0.05	91	1.88	1.32	0.23	3.43 ± 0.18
0.1	84	2.02	1.74	0.17	3.94 ± 0.22
Trp-P-2					
0.01	93	1.18	0.68	0.16	2.02 ± 0.10
0.025	108	1.34	0.89	0.13	2.36 ± 0.15
0.05	87	1.66	1.11	0.16	2.93 ± 0.19
0.1	98	2.02	1.36	0.14	3.52 ± 0.20

* Standard error.

weeks (15–25°C). Mutation frequency was measured by the average number of mutated spots per leaf. Two simple leaves and the first compound leaf at the stage of full expansion were used for counting spots.

In the experiment on soybeans, Trp-P-1 and Trp-P-2 concentrations of 0.01, 0.025, 0.05, and 0.1 $\mu\text{g/ml}$ were used. Most of the leaves showed normal growth at these concentrations. Table I shows the types and frequencies of spots on the leaves of heterozygous plants treated with Trp-P-1 or Trp-P-2. An increased frequency of spots per leaf was observed at 0.025 $\mu\text{g/ml}$ or higher. An almost linear reaction was noted between spot frequency and chemical concentration (Trp-P-1; $\gamma=0.939$, Trp-P-2; $\gamma=0.995$). An appreciable increase (Chi-square, $p<0.01$) in frequency per leaf was evident at 0.025 $\mu\text{g/ml}$ treatment of Trp-P-1 and Trp-P-2. Trp-P-1 and Trp-P-2 are thus shown to induce somatic mutations in soybeans at concentrations exceeding 0.025 $\mu\text{g/ml}$.

As shown in Table I, the spot frequency after Trp-P-1 or Trp-P-2 treatment differed from that of the control. The number of yellow and dark green spots markedly increased at greater concentrations of Trp-P-1 and Trp-P-2. With 0.1 $\mu\text{g/ml}$ treatment of Trp-P-1 in the second experiment,

the number of yellow and dark green spots per leaf increased by about 2 and 2.6-fold respectively, and by Trp-P-2 treatment, this number increased by about 2-fold, compared with the control. The number of twin spots did not increase like single spots. Trp-P-1 and Trp-P-2 thus induce mostly yellow and dark green spots. Trp-P-1 and Trp-P-2 may have enhanced the induction of forward and/or backward mutation on the leaves of soybean $Y_{11}Y_{11}$ plants.^{1,2)}

References

- 1) B. K. Vig, *Mutat. Res.*, **32**, 49 (1975).
- 2) B. K. Vig, R. A. Nilan and P. Arenaz, *Env. Exp. Botany*, **16**, 223 (1976).
- 3) S. Takayama, T. Hirakawa and T. Sugimura, *Proc. Jpn. Acad.*, **54**, 418 (1978).
- 4) T. Sugimura, *Mutat. Res.*, **150**, 33 (1985).
- 5) T. Kusuge, K. Tsuji, K. Wakabayashi, T. Okamoto, K. Shudo, Y. Iitaka, A. Itai, T. Sugimura, T. Kawachi, M. Nagao, T. Yahagi and Y. Seino, *Chem. Pharm. Bull.*, **26**, 611 (1978).