PEROXIDASE ISOZYMES IN FOUR STRAINS OF MORNING GLORY*

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Recently, intra- and interspecific variations of zymograms have been studied in plants and animals from genetic and evolutionary viewpoints (Kikkawa, 1964; Yoshitake and Akiyama, 1964; Ogita and Kasai, 1965; Chu, 1967; Wall, 1968). In Japanese morning glory (*Pharbitis nil* Choisy), distributions of peroxidase isozymes and their indoleacetate oxidase activity were investigated (Yoneda and Endo, 1967, 1969). Most peroxidases were found to have destructive activity of indoleacetic acid, a hormone controlling many aspects of plant growth, after removal of the inhibitory substances of the enzyme (or the auxin protectors). In order to study physiological functions of the isozymes, surveys of the peroxidase zymograms in many mutant strains of Japanese cultivars and wild strains of different geographical origins were conducted, which indicated that some strains had different patterns of zymograms. In this study, four strains of different geographical origins were selected for peroxidase zymography from the collected stocks of morning glory at the National Institute of Genetics, Misima, in order to estimate their mutual similarity. The genes controlling two cathodal isozymes were also analysed.

MATERIALS AND METHODS

The following four strains were used.

1. TO16: A substrain of TKS (Tokyo Kokei Standard), selected by the late Dr. Y. Takenaka from Japanese cultivars.
2. Tendan: Collected at Peking by Dr. H. Kihara in 1938 (Takenaka, 1958).
3. N-1: Collected in Nepal by Dr. S. Nakao in 1952 (Takenaka, 1958).
4. WOO1: Collected in Guinea by Dr. K. Furusato in 1959 (pers. commun.). This strain has small, light blue flowers, dense hairs on the stems, capsules (Figs. 1 and 2) as well as seeds, and "retracted" mutant-like leaf (Fig. 3).

All these strains were raised in porous pots in a greenhouse if not otherwise noted. In winter season, the plants were grown under a long day condition to promote vegetative growth.

The starch gel electrophoresis described by Endo (1968) was used. Leaves, stems, roots and other organs were dissected and immediately ground in a mortar and the exudate was absorbed by a rectangular piece of filter paper, Toyo No. 50, which was then inserted in the gel. Electrophoresis was run under constant 200 voltage drop

* This study was partly conducted at the National Institute of Genetics, Misima.

Fig. 1-3. African strain. Fig. 1, Peduncle and capsule covered with dense hairs. Fig. 2, Stem and calyx covered with dense hairs. Fig. 3, Leaf.

(10 V/cm gel) at 8-10°C for 4 hours. After that, the gel was sliced into two halves, and the cut surface was subjected to the peroxidase stain.

RESULTS

1. *Peroxidase zymograms*

As previously reported, TKS strains have at least 7 anodal isozyme bands (1A to 7A) (Yoneda and Endo, 1969). TO16 substrain has a sharp cathodal band, 1C (Endo, 1966). These isozyme bands were classified into organ-specific, age-specific and non-specific types (Yoneda and Endo, *in press*).

Old leaves contain a large amount of mucoidal substance, which disturbs the separation of isozymes, leading to blurs or smears in the 1A to 6A region, but they were suitable for observing 6A to 7A as well as the cathodal bands.

As shown in Fig. 4, similar isozyme patterns were observed in the leaves and stems of both TO16 and Tendan. However, the Nepalese strain had at least one more band between 6A and 7A, other bands being similar to those of TO16 (Fig. 5). The leaves of the African strain showed at least 3 more bands in the 6A-7A region. These bands were clear and intense in aged tissues, though they did not appear in young tissues. This indicates their age dependency.

TO16 had a sharp cathodal band, 1C, located at a 18mm distance from the origin in leaves, stems and roots, except seeds and young cotyledons. This band appeared in both young and old tissues. The same band was also detected in Tendan and Nepalese strains. A similar sharp band observed in the African strain, designated as 2C (Yoneda, 1968), had a migrating distance of 22mm and could be clearly distinguished from 1C in other strains. For distinguishing between 1C and 2C, since the difference in migration distance was small, two pieces of filter paper absorbing the exudates of different materials were placed in the same gel block side by side.

Bands 1A, 2A, 5A and 6A were also detected in the roots of all four strains.

Fig. 4. Peroxidase zymograms of old stems (left) and leaves (right) in 4 strains of morning glory. O denotes the origin. A, TO16. B, Tendan. C, Nepalese. D, African.

2. 1C and 2C isozymes in hybrids and their descendants

In order to examine the genetic behavior of the 1C and 2C bands, in the summer of 1968, several plants of the African strain, WOO1, were grown in a greenhouse and were crossed with TO16. The F_1 seeds of TO16 $\times$ WOO1, sown in November, gave rise to 14 F_1 plants. All of them had both 1C and 2C in the leaves (Yoneda, 1968). This indicates that the two cathodal bands are controlled by different genes. The F_1 plants of Tendan $\times$ WOO1 had also a 1C-2C type, while TO16 $\times$ Nepalese and Tendan $\times$ Nepalese had 1C only.

The TO16 $\times$ WOO1 F_1 plants set abundant seeds by self-fertilization. In 1969, 140 F_2 seeds were sown in the field and 124 F_2 plants were obtained. They were examined for the peroxidase isozymes by the above-mentioned two paper piece method (Fig. 6). The F_2 plants segregated into 28 1C, 67 1C-2C and 29 2C types, giving a good fitness to a 1 : 2 : 1 ratio ($\chi^2=0.82$; $0.80 > P > 0.50$). It is then concluded that the two cathodal bands are controlled by two alleles at the same locus. These two alleles are denoted

Fig. 5. Peroxidase zymograms of old stems in 3 strains of morning glory. O denotes the origin. Left, TO16. Middle, Neplese. Right, African. 7A of TO16 and Neplese stems are weakly stained, probably due to their young age.

Fig. 6. Leaf peroxidase zymograms in TO16 x African F_2 segregants. O denotes the origin. No. 1, gel block, 1C-2C left and 1C right. No. 2, 1C and 1C-2C. No. 3, both 1C-2C. No. 4, 1C-2C and 2C. No. 5, both 2C. No. 6, 2C and 1C-2C.

by Pe^{1C} and Pe^{2C} , respectively. As shown in Fig. 6, the 1C and 2C types had a densely stained single band, while the 1C-2C type had relatively weakly stained two bands.

It is worthy to note that substrain TO36 of TKS had neither 1C nor 2C. The F_1 plants, TO46 (1C type) $\times$ TO36 and its reciprocal both had 1C (Endo, 1966). Many cultivated varieties of Japanese morning glory were later found to be null form in respect to the cathodal isozyme. In 1968, the F_2 of sr (side reduced; null form) $\times$ TO16 (1C type) was zymographically analyzed. Of 50 F_2 plants, 38 had 1C, whereas the remaining 12 were null forms. This gives a good agreement with a 3 : 1 ratio ($\chi^2=0.027$; $0.95>P>0.80$), indicating that the null form is controlled by an inactive allele Pe^o at the Pe locus. The genotype of sr or TO36 is assumed to be Pe^o/Pe^o and that of the F_1 of $sr \times$ TO16 to be Pe^{1C}/Pe^o . To summarize, the presence or absence of cathodal bands 1C and 2C is controlled by multiple alleles Pe^o , Pe^{1C} and Pe^{2C} .

DISCUSSION

The Japanese morning glory is believed to have been introduced from China some one thousand years ago (Miyake and Imai, 1934). Since then, this plant has been cultivated in Japan for pharmaceutical and ornamental purposes. The native habitat of morning glory has been assumed to be south-west China to Himalayan mountains and South-east Asia, including Borneo and Celebes (Takenaka, 1958). Morphological and physiological characters of "Violet" (a Japanese strain), Tendan and Nepalese strains were compared by Muramatsu and Sakamoto (1956). They suggested that the latter strains have similar characters to the putative strain first introduced to Japan. It may be compared with these characters that in peroxidase isozyme patterns TO16 and Tendan were similar in all respects, while those of the Nepalese strain showed minor differences.

The African strain has small, funnel-shaped flowers with light blue corolla and white tubes. These characters are very similar to those of Nepalese and Tendan strains. Its leaf shape resembles that of the "retracted" (*re*) mutant of a Japanese cultivar (Fig. 3). The stem, calyx and capsule are densely covered with long, brown hairs (Figs. 1 and 2). This extreme hairiness may be a unique character of the African strain. Its black and small seeds also have dense hairs. The average seed weight was 30 mg/grain for this strain, while it was 55, 64 and 37 mg/grain for TO16, Tendan and Nepalese, respectively. In Shizuoka, the African strain flowers in late September. Although it morphologically differs from the other three, it can be easily crossed with Asian strains and the hybrids set seeds abundantly, suggesting that it may be fairly closely related to the Asian ones.

It is also interesting to find that TO16, Tendan and Nepalese strains had the peroxidase 1C isozyme, while the African strain had a different isozyme 2C. This band appeared in many different organs, the intensity being not influenced by the age, in the same manner as 1C. These two isozymes were found to be controlled by alleles which are probably monomeric.

As described previously, a Japanese strain seemed to have a null form for 1C. Such a null form was already found in many other isozyme mutants such as acid phosphatase mutants of silkworm (Yoshitake and Akiyama, 1964).

SUMMARY

Peroxidase isozymes in four geographical strains of *Pharbitis nil*, TO16 (Japanese), Tendan (Chinese), Nepalese and African, were compared. Though they had similar zymograms, the Nepalese and African strains showed one and three bands in addition to those found in TO16, respectively. The African strain had a cathodal band, 2C, while the others had 1C. Genetic analysis showed that these two isozymes were controlled by alleles Pe^{1C} and Pe^{2C} , respectively. A Japanese strain seemed to have a null form for 1C controlled by an inactive allele, Pe^o at the same locus.

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