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Variation, geographical distribution and genetical analysis of esterase isozymes in foxtail millet, *Setaria italica* (L.) P. Beauv.*

M. Kawase and S. Sakamoto

Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University, Mozume, Muko, Kyoto 617, Japan

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Summary. The esterase isozymes of 432 strains of foxtail millet, *Setaria italica* (L.) P. Beauv., collected from different areas throughout Eurasia, were investigated by gel isoelectric focusing. Five phenotypes were recognized, based on the combination of five major activity bands. Cross experiments among different phenotypes revealed these isozymes to be controlled by two codominant alleles and a null allele on the locus, *Est-1*, and three codominant alleles on another independent locus, *Est-2*. On locus *Est-1*, 388 strains had *Est-1*^{a}, 41 had *Est-1*^{b} and three had *Est-1*^{null} alleles. *Est-1*^{a} was widely distributed throughout Eurasia, while the distribution of *Est-1*^{b} and *Est-1*^{null} was distinctly restricted. On locus *Est-2*, 417 strains had *Est-2*^{a}, nine had *Est-2*^{b} and six had *Est-2*^{c} alleles. *Est-2*^{a} was widely distributed throughout Asia to Czechoslovakia, but was not detected in the western part of Europe. *Est-2*^{b} was found in all of the strains from the western part of Europe and in one of the Indian strains. *Est-2*^{c} was rarely found in Japan and China. The distribution of *Est-2*^{a} and *-2*^{b} might indicate some degree of phylogenetic differentiation between the Asian and the European strains. Polymorphism in both loci was observed only in Chinese strains.

Key words: Foxtail millet – *Setaria italica* – Esterase isozyme – Geographical distribution – Phylogenetic differentiation

Introduction

Foxtail millet, *Setaria italica* (L.) P. Beauv. is an old staple crop in Eurasia, and is thought to have been one of the main cultivated cereals in the early period of agriculture, especially in East Asia. Various endemic races of *S. italica* are now cultivated sporadically on a small scale in scattered local areas throughout Eurasia. *S. italica* is, therefore, considered as an ideal material for studies on phylogenetic differentiation and related problems.

Biochemical characters such as phenol color reaction and isozymes, which do not serve as a direct means for artificial selection in agricultural practices, might be useful in studies on the phylogenetic differentiation of cultivated cereals. Variation, geographical distribution and genetical analysis studies of phenol color reaction in *S. italica* have already been reported (Kawase and Sakamoto 1982). The present study was, therefore, conducted to investigate the variation, geographical distribution and genetical basis of esterase isozymes in *S. italica* by gel isoelectric focusing.

Materials and methods

Strains

In the present study, 432 strains of foxtail millet, *Setaria italica* (L.) P. Beauv. were used (Table 1). They were originally collected from Japan, Korea, China, Taiwan, the Philippines, Indonesia, Thailand, Nepal, India, Afghanistan, the USSR, Czechoslovakia, Germany, Belgium, France and Spain, as listed in Table 1. Of the 432 strains, 348 were collected directly from the local villages where they were being cultivated. The remaining 43 strains from China, 30 from India, seven from the USSR, one from Czechoslovakia and three from Belgium were kindly provided by the various institutes described in Table 1. All strains are being maintained at the Plant Germplasm Institute, Kyoto University.

Gel isoelectric focusing

Fifty mg of matured grains (13–50 grains) of each strain, which had been placed on moist filter paper in a petri-dish at

* Contribution No. 30 from the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University, Kyoto, Japan

Table 1. Strains used

Region	No. of strains used	Collection locality or source (no. of strains)
Japan	122	Iwate (2); Niigata (1); Nagano (5); Yamanashi (6); Gifu (2); Kanagawa (1); Mie (3); Nara (21); Hyogo (3); Kochi (21); Tokushima (5); Ehime (4); Kumamoto (10); Oita (1); Miyazaki (6); Kagoshima (8); Okinawa (23)
Korea	128	Kangwon-do (7); Kyonggi-do (16); Chungchong-pukto (7); Chungchong-namdo (5); Kyongsang-pukto (24); Kyongsang-namdo (8); Cholla-pukto (18); Cholla-namdo (27); Cheju-do (16)
China	46	Tohoku Nat. Agr. Exp. Sta., Japan (20); Nat. Inst. Agr. Sci., Japan (23); Unnan (1); Kweichow (1); Hunan (1)
Taiwan	44	Pington (12); Taitung (13); Hwalien (1); Lan Hsu Isls. (11); Nantou (7)
Philippines	14	Batan Isls. (13); Luzon Is. (1)
Indonesia	7	Halmahera Is. (5); Sulawesi Is. (2)
Thailand	1	A village near Cheng-mai (1)
Nepal	3	Dhunche (1); Bhargu (2)
India	30	Univ. Agr. Sci., Bangalore, India (20); Tamil Nadu Agr. Univ., Coimbatore, India (10)
Afghanistan	21	Jabalsalaj-Zenya (3); Kabul (8); Takhar (4); Badakhshan (6)
USSR	7	N. I. Vavilov All-Union Inst. Plant Industry, Leningrad, USSR [Dagestan (1); Kirghizia (2); Uzbekistan (1); Primorskaya Prov. (1); Georgia (1); Ukraine (1)]
Czechoslovakia	1	Inst. Genet. Plant Breed., Prague-Ruzyne, Czechoslovakia (1)
Germany	1	Karl-Marx-Universität, Leipzig, DDR (1)
Belgium	3	Dienst voor Parken en Plantsonen, Antwerpen, Belgium (3)
France	3	Cuon (3)
Spain	1	Canges de Narcea (1)
Total	432	

28 °C for 24 h, were homogenized in a 0.05 M potassium phosphate buffer (pH 7.0) containing 20% sucrose, 5% Tween 80 and 1% ampholine (pH 3.5–10). 0.2 ml of the crude extract was directly placed on the gel. The polyacrylamide gel with 7.5% monomer concentration containing 1% ampholine (pH 3.5–10) was prepared as described by Nakai and Tsunewaki (1971). The isoelectric focusing was conducted at 240 V with 0.02 M HCl as an anode and 0.02 M ethylenediamine as a cathode, for 4 h at 4 °C. The activity bands of esterase were developed in an 1/15 M phosphate buffer (pH 7.0) containing 0.1% Fast blue RR salt and 5 mM α-naphthyl acetate.

Cross experiment

To analyze the genetical basis, strains with different phenotypes of esterase isozymes were crossed using the method of Takahashi (1942). The genotypes of the F_1 hybrids and F_2 generations were also estimated by gel isoelectric focusing of the extracts from the grains produced on the F_1 and F_2 plants by selfing, respectively.

Results

Variation and geographical distribution of esterase isozymes

Five major bands representing distinctly high activity and several minor bands were produced by gel isoelectric focusing. The minor bands, which were slightly stained, were not included in the present analysis. The five major activity bands were designated as Est-1^a, -1^b, -2^a, -2^b and -2^c (Fig. 1). Est-1^a and -1^b were more intensely stained than Est-2^a, -2^b and -2^c. Based on the band combination, five phenotypes were recognized. They were designated as phenotype A (containing Est-1^a and -2^a), phenotype B (Est-1^a and -2^b), phenotype C (Est-1^a and -2^c), phenotype D (Est-1^b and -2^a) and phenotype E (Est-2^a only).

In the 432 strains examined in the present study, 373 were phenotype A, nine were phenotype B, six were phenotype C, 41 were phenotype D and three were phenotype E (Table 2). As shown in Table 2, the phenotype A strains, the most frequent type, were widely distributed except in the western part of Europe. Of the nine strains with phenotype B, on the other

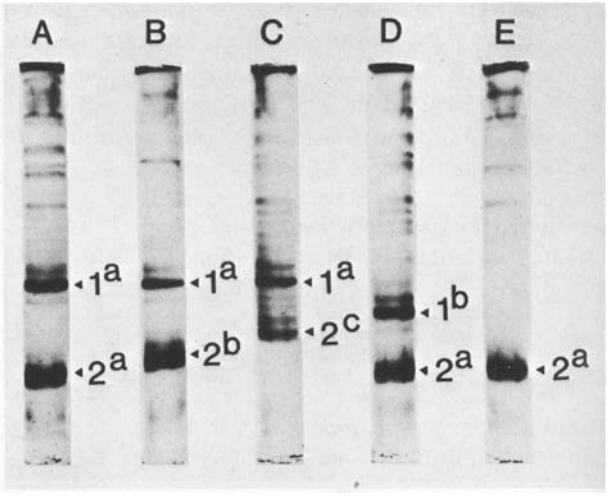

Fig. 1. Five phenotypes (*A, B, C, D* and *E*) of esterase isozymes in *Setaria italica*

Table 2. Variation and geographical distribution of phenotypes (a) and alleles on *Est-1* and *Est-2* (b) of esterase isozymes in *S. italica*

Region	No. of strains examined	(a) Phenotype					(b) Allele					
		A	B	C	D	E	*Est-1*			*Est-2*		
							-1^a	-1^b	-1^{null}	-2^a	-2^b	-2^c
Japan	122	119	–	3	–	–	122	–	–	119	–	3
Korea	128	119	–	–	8	1	119	8	1	128	–	–
China	46	11	–	3	32	–	14	32	–	43	–	3
Taiwan	44	43	–	–	1	–	43	1	–	44	–	–
Philippines	14	14	–	–	–	–	14	–	–	14	–	–
Indonesia	7	7	–	–	–	–	7	–	–	7	–	–
Thailand	1	1	–	–	–	–	1	–	–	1	–	–
Nepal	3	3	–	–	–	–	3	–	–	3	–	–
India	30	29	1	–	–	–	30	–	–	29	1	–
Afghanistan	21	19	–	–	–	2	19	–	2	21	–	–
USSR	7	7	–	–	–	–	7	–	–	7	–	–
Czechoslovakia	1	1	–	–	–	–	1	–	–	1	–	–
Germany	1	–	1	–	–	–	1	–	–	–	1	–
Belgium	3	–	3	–	–	–	3	–	–	–	3	–
France	3	–	3	–	–	–	3	–	–	–	3	–
Spain	1	–	1	–	–	–	1	–	–	–	1	–
Total	432	373	9	6	41	3	388	41	3	417	9	6

hand, eight were collected from the western part of Europe and the remaining one was found in the Indian strains. The phenotype C strains were collected from Japan and China. Phenotype D was found in the strains from Korea, China and the Lan Hsu Islands of Taiwan. Phenotype E was found in the Korean and Afghan strains.

Genetical analysis

The F_1 hybrids and the F_2 generations obtained from the crosses of phenotype C×D, B×A and E×A were examined.

a) Phenotype C×Phenotype D. The F_1 phenotype consisted of Est-1^a, -1^b, -2^a, -2^c and two additional bands, one (designated as Est-$1^{a/b}$) in the intermediate position between Est-1^a and -1^b and the other (Est-$2^{a/c}$) between Est-2^a and -2^c (Fig. 2, c). These intermediate bands seemed to represent active hybrid enzymes and to suggest the dimeric nature of these enzymes.

The segregation of nine phenotypes of band patterns in the F_2 generation indicated that these enzymes are controlled by two independent loci without linkage, designated as *Est-1* and *Est-2* (for test of independency: $x^2_{(4)}=4.964$, $P=0.30-0.20$) (Table 3). Est-1^a and -1^b enzymes are controlled by two codominant alleles on the *Est-1* locus, designated as $Est\text{-}1^a$ and -1^b; Est-2^a and -2^c by two codominant alleles on the *Est-2* locus, designated as $Est\text{-}2^a$ and -2^c. The F_2 segregation for each locus showed no significant deviation from the expected ratio of 1 : 2 : 1 (for *Est-1*: $x^2_{(2)}=5.172$, $P=0.10-0.05$; for *Est-2*: $x^2_{(2)}=2.922$, $P=0.30-0.20$) (Table 3).

b) Phenotype B×Phenotype A. The F_1 hybrid showed Est-1^a, -2^a, -2^b and a hybrid band (Est-$2^{a/b}$) between Est-2^a and -2^b (Fig. 2, f). In the F_2 generation, 14 plants

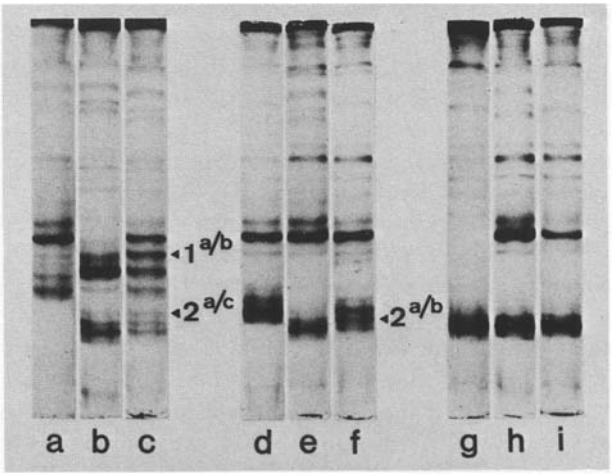

Fig. 2. Esterase isozymes in the parents and in the F_1 hybrids of the crosses among different phenotypes. *a:* phenotype C; *b:* phenotype D; *c:* F_1 phenotype of C×D; *d:* phenotype B; *e* and *h:* phenotype A; *f:* F_1 phenotype of B×A; *g:* phenotype E; *i:* F_1 phenotype of E×A

Table 3. Phenotypic segregation and estimated genotypes in the F_2 generation of phenotype C × phenotype D

Est-1 bands observed (estimated genotype)	Est-2 bands observed (estimated genotype)			
	-2^a (*Est-2*$^{a/a}$)	-2^a, $-2^{a/c}$, -2^c (*Est-2*$^{a/c}$)	-2^c (*Est-2*$^{c/c}$)	Subtotal
-1^a (*Est-1*$^{a/a}$)	9	11	14	34
-1^a, $-1^{a/b}$, -1^b (*Est-1*$^{a/b}$)	18	37	18	73
-1^b (*Est-1*$^{b/b}$)	7	7	7	21
Subtotal	34	55	39	128 (total)

were pheontype B, 29 were F_1 phenotype and 21 were phenotype A. This was not a significant deviation from the expected ratio of 1:2:1 ($\chi^2_{(2)} = 2.094$, $P = 0.50-0.30$). Est-2^b was indicated to be controlled by a codominant allele, *Est-2*^{b}.

c) Phenotype E × Phenotype A. The F_1 phenotype showed Est-1^a and -2^a and was phenotype A (Fig. 2, i), but Est-1^a was considerably more weakly stained in the F_1 phenotype than in phenotype A. In the F_2 generation, 14 plants were phenotype E, 28 were F_1 phenotype and 22 were phenotype A. This was not a significant deviation from the expected ratio of 1:2:1 ($X^2_{(2)} = 3.000$, $P = 0.30-0.20$). A null allele, *Est-1*^{null}, was indicated in phenotype E.

Discussion

Genetical basis of esterase isozymes

The present results demonstrate that the major esterase isozymes detected by gel isoelectric focusing are controlled by two codominant alleles and a null allele on the locus, *Est-1*, and three codominant alleles on another independent locus, *Est-2*.

The genotypes for the five phenotypes recognized are as follows: *Est-1*$^{a/a}$ and *Est-2*$^{a/a}$ for phenotype A, *Est-1*$^{a/a}$ and *Est-2*$^{b/b}$ for phenotype B, *Est-1*$^{a/a}$ and *Est-2*$^{c/c}$ for phenotype C, *Est-1*$^{b/b}$ and *Est-2*$^{a/a}$ for phenotype D and *Est-1*$^{null/null}$ and *Est-2*$^{a/a}$ for phenotype E. Of the nine possible combinations of homozygous alleles on these two independent loci, only five were observed. No strain showed heterozygosity in these loci, which is an expected phenomenon because *S. italica* is a predominantly self-fertile plant (Takahashi and Hoshino 1933). Homozygosity might be promoted through maintenance cultivation in the institutions.

Geographical distribution of alleles on Est-1 and Est-2

Concerning *Est-1*, among 432 strains examined, 388 strains had *Est-1*^{a}, 41 had *Est-1*^{b} and 3 had *Est-1*^{null} (Table 2). *Est-1*^{a} was predominantly found in all of the regions so far examined, thus showing the widest geographical distribution throughout Eurasia. On the other hand, *Est-1*^{b} was found in the strains from Korea, China and the Lan Hsu Islands of Taiwan, and *Est-1*^{null} was rare and found only in the Korean and Afghan strains. It is likely that *Est-1*^{b} originated in China where it was found in a high frequency and only subsequently was introduced into Korea and Lan Hsu Islands in the neighborhood of China. *Est-1*^{null} is thought to have been raised independently in Korea and in Afghanistan.

Concerning locus *Est-2*, 417 strains had *Est-2*^{a}, nine had *Est-2*^{b} and six had *Est-2*^{c} alleles (Table 2). *Est-2*^{a} was widely distributed throughout Asia to Czechoslovakia but it was not found in the western part of Europe. *Est-2*^{c} was sporadically found in Japan and China. It is rather likely that *Est-2*^{c} originated in China and was later introduced into Japan since all of the three Japanese strains carrying *Est-2*^{c} were collected from the southwestern part of Kyushu Island, near China. The distribution of *Est-2*^{b} shows an interesting pattern. Of the nine strains with *Est-2*^{b}, eight were collected in the western part of Europe, where strains with *Est-2*^{a} or *-2*^{c} were not found. In Asia, only one of the Indian strains was exceptionally shown to carry *Est-2*^{b}. This clear difference in the geographical distribution of *Est-2*^{a} and *-2*^{b} might indicate some degree of phylogenetic differentiation between the Asian and the European strains.

As shown in Table 2, the polymorphism in *Est-1* and *-2* was characterized in the Chinese strains. Strains from other regions showed smaller variations or almost monomorphism.

The time and place of the domestication of *S. italica* remains obscure. Vavilov (1926) stated that the principal center of diversity in *S. italica* is Eastern Asia, including China and Japan, where it is cultivated as a cereal for food. Based on archaeological evidence, de Wet and Harlan (1975) suggested the possibility that *S. italica* was independently domesticated at least in Central Europe and China. The diversity of genetic polymorphism of esterase loci in Chinese strains is not in conflict with the statement of Vavilov (1926). Although the distribution of *Est-2*^{a} and *-2*^{b} might indicate some degree of phylogenetic differentiation between the Asian and the European strains, it is not thought to be enough to give support to the view of de Wet and Harlan (1975).

Positive and negative phenotypes of phenol color reaction were recognized in *S. italica* (Kawase and Sakamoto 1982). It is interesting to note that Chinese strains which showed only negative phenol color reaction were genetically diversified in esterase loci, while the strains from Taiwan, the Philippines or India, in which both phenotypes of phenol color reaction were observed, showed little or no diversity in esterase loci. Although the cause of the difference in distributional patterns of the two biochemical characters is not yet clear, these characters might reflect some aspect of phylogenetic differentiation. Further comparative analyses on other biochemical characters, therefore, should be made to clarify the phylogenetic differentiation in *S. italica.*

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