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Genetic variability of foxtail millet (*Setaria italica* P. Beauv.)

Electrophoretic study of five isoenzyme systems

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Summary. The genetic diversity of a world collection of foxtail millet strains (*Setaria italica*) and some samples of wild populations (*Setaria viridis*) was studied by means of electrophoresis on five enzymes (10 loci) Est, Acph, Got, Mdh, Pgd. In spite of an overall limited polymorphism, the diversity appeared to be clearly regionalized. The wild populations collected in France and China introduced new genetic variability to the cultivated forms. However, the interregional diversity within both species was greater than the between species (*S. viridis*/*S. italica*) diversity.

Key words: Foxtail millet – Enzymatic polymorphism – Genetic differentiation – Domestication

Introduction

This paper is a report of the analysis of genetic variability of foxtail millet (*Setaria italica* P. Beauv.) using the approach of enzyme electrophoresis. De Cherisey et al. (1985) have studied the genetic control of seven isoenzyme systems, five of which are treated here. Strains of cultivated *S. italica* and spontaneous green foxtail (*S. viridis* L.) were analyzed. It is commonly recognized that the cultivated species was domesticated from wild green foxtail, and crosses between these taxons have produced semifertile hybrids (Li et al. 1945). This same phenomenon has been observed for hybrids created in our laboratory (unpublished results). De Wet et al. (1979) reported obtaining a completely fertile hybrid.

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The material studied here was sampled from many regions of the world. The principal object of this study was to extract information about genetic variability, both intra- and interregional. Hypotheses pertaining to the domestication process are also discussed.

Materials and methods

Enzymes of 223 strains of *S. italica* and 45 strains of *S. viridis* were studied by electrophoresis. Five isoenzyme systems were observed: Esterase (Est), Acid Phosphatase (Acph), Glutamate Oxaloacetate Transaminase (Got), Malate Dehydrogenase (Mdh) and 6-Phosphogluconate Dehydrogenase (Pgd). All the strains were fixed lines. The *S. italica* samples originated from ten different regions and *S. viridis* from two regions (Table 1).

The methodology of electrophoresis and the genetic control of the enzymes have been described in a separate paper (De Cherisey et al., in press). The principal methods of data analysis used for this paper were the following:

1. The allelic frequencies, the number of polymorphic loci, the average number of alleles per locus, and the genic diversity were calculated in order to study interregional variability. The coefficient of genic diversity was obtained using the formula for heterozygosity proposed by Nei (1978). The coefficient of heterozygosity was initially intended for studying populations mating randomly. Foxtail millet is a cleistogamic plant and the material studied here consisted of samples of stocked seed, thus they are absolutely not randomly mating populations. However, Nei (1973) also suggested using this type of coefficient as a diversity index, with the remark that is would not have the same significance as heterozygosity.

2. Discriminant or Canonical Variable Analysis was used for studying interregional variability (Mardia et al. 1979).

Results

Polymorphism and genetic variability

As previously reported (De Cherisey et al. 1985), 10 loci with 26 alleles were observed for the five isoenzyme systems studied (Table 2).

Table 1. Strains used

Region	No. of strains	Sources
S. italica		
China	38	30 collected in the field in China; 8 from a
Korea	22	All from a
Japan	53	15 collected in Kagoshima, Japan; 35 from a; 13 from b
Okinawa	12	10 from a; 2 from b
Taiwan	27	4 collected in Batani Islands; 8 from Taiwan and Ishigaki Island; 2 from a.
India	12	All from a
Kenya	16	All from a
USSR	11	All from a
Central Europe	16	All from a
France	16	All collected in the field in France
	223	
S. viridis		
China	24	All collected in the field in North China
France	21	All collected in the field in F. France
	45	
Total	268	

[a] Collection of Dr. Myagi Laboratory of Plant Breeding, Faculty of Agriculture, Kagoshima University
[b] Collection of Dr. Sakamoto, Plant Germplasm Institute, Kyoto University

Table 2. Loci and alleles revealed in five isoenzyme systems

Enzyme	Locus	Alleles
Esterase	*Est-1*	*Est-1*[a], *Est-1*[b], *Est-1*[c], *Est-1*[d]
	Est-2	*Est-2*[a], *Est-2*[b], *Est-2*[c], *Est-2*[d]
	Est-3	*EST-3*[a], *Est-3*[b]
Acid phosphatase	*Acph-1*	*Acph-1*[a], *Acph-1*[b]
Glutamate oxaloacetate Transaminase	*Got-1*	*Got-1*[a], *Got-1*[b]
	Got-2	*Got-2*[a], *Got-2*[b]
Malate dehydrogenase	*Mdh-1*	*Mdh-1*[a], *Mdh-1*[b], *Mdh-1*[c]
	Mdh-2	*Mdh-2*[a], *Mdh-2*[b]
6. Phosphogluconate dehydrogenase	*Pgd-1*	*Pgd-1*[a], *Pgd-1*[b]
	Pgd-2	*Pgd-2*[a], *Pgd-2*[b], *Pgd-2*[c]

Regional allele frequencies were calculated for *S. italica* and *S. viridis* and are shown in Table 3. Of the 26 alleles observed, four were present only in *S. viridis* (*Est-1*^{c}, *Est-1*^{d}, *Est-2*^{d} and *Mdh-1*^{c}) and three were present only in *S. italica* (*Est-1*^{b}, *Got-1*^{b} and *Mdh-2*^{b}): these were rare alleles. Many regions had many monomorphic loci. In order to study intraregional genetic variability: the number of polymorphic loci, the average number of alleles per locus, and the genic diversity for each region are shown in Table 4. This parameters showed that *S. viridis* was more polymorphic than *S. italica*. Even though only two regions of origin of *S. viridis* were analyzed, it could be seen that in both regions, *S. viridis* had more polymorphic loci, a higher average number of alleles per locus, and higher genic diversity.

Brown (1978) classified alleles according to their frequency and their distribution into five classes:

1) Common: at least one sample with a frequency over 10%. a) Widespread: common occurrences in more than 2 regions. b) Sporadic: common occurences in 2 regions. c) Localized: common occurrence in only one region.
2) Rare: never occurs with a frequency higher than 10%. d) Widespread: in more than one region. e) Localized: in only one region.

The alleles observed in *S. italica* can accordingly be classified as follows (Table 5).

We will find below that the common sporadic alleles characterize groups of regions.

Regional classification of S. italica

Interregional variability was studied by discriminant analysis and led to a grouping of regions. Interpretations were drawn from graphical representations of the regional centres of gravity and from a Mahalanobis distance matrix. The alleles characterizing the different groups can be found in the allele frequency table (Table 3).

Regional centres of gravity are represented in Fig. 1. Only the first three canonical variables will be discussed, as they accounted for 88% of total inertia. The Mahalanobis squared distances (D^2) between regions are shown in Table 6. The distance of Mahalanobis is equal to the distance on the canonical plan.

Simultaneous interpretation of the position of the regions in the three-dimensional canonical space, Mahalanobis distances and regional allele frequencies provided excellent information for establishing groups of regions and for grouping the alleles that characterized these groups (Table 7). It was found that the alleles which characterized the regions were the sporadic ones.

Discriminant analysis of all the data obtained from *S. italica* and *S. viridis* electrophoresis was used to investigate the genetic variability between the two taxons. There are only two regions, China and France, for which *S. italica* and *S. viridis* were both studied.

The populations differed regionally rather than taxonomically. The distances measured between two taxons originating from the same region were not always superior to the interregional distances between

Table 3. Allele frequencies of the 26 alleles of the 10 loci studied

		S. italica										*S. viridis*	
		China	Korea	Japan	Okinawa	Taiwan	India	Kenya	USSR	Europe	France	China	France
Est-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	0.96	0.85
	b[b]	0	0	0	0	0	0	0	0	0	0.06	0	0
	c[a]	0	0	0	0	0	0	0	0	0	0	0.04	0
	d[a]	0	0	0	0	0	0	0	0	0	0	0	0.16
Est-2	a	0.90	0.96	0.98	1.00	1.00	1.00	1.00	0.46	0.44	0.81	0.64	0.76
	b	0.10	0.05	0.02	0	0	0	0	0.55	0.44	0.13	0.08	0.05
	c	0	0	0	0	0	0	0	0	0.12	0.06	0.24	0.10
	d[a]	0	0	0	0	0	0	0	0	0	0	0.04	0.10
Est-3	a	0.97	1.00	1.00	1.00	1.00	1.00	1.00	0.82	0.31	0.19	1.00	0.43
	b	0.03	0	0	0	0	0	0	0.18	0.69	0.81	0	0.57
Acph-1	a	0.90	0.73	0.74	0.92	0.74	0.83	1.00	1.00	0.88	0.94	0.88	0.81
	b	0.11	0.27	0.26	0.08	0.26	0.17	0	0	0.12	0.06	0.12	0.19
Got-1	a[a]	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	0.81	1.00	1.00	1.00
	b[b]	0	0.05	0	0	0	0	0	0	0.19	0	0	0
Got-2	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	0.88	0.80	0.43
	b	0	0	0	0	0	0	0	0	0.06	0.13	0.20	0.57
Mdh-1	a	0.79	0.82	0.77	1.00	1.00	1.00	1.00	1.00	0.88	1.00	0.88	0.86
	b	0.21	0.18	0.23	0	0	0	0	0	0.12	0	0.04	0.10
	c[a]	0	0	0	0	0	0	0	0	0	0	0.08	0.05
Mdh-2	a	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b[b]	0.03	0	0	0	0	0	0	0	0	0	0	0
Pgd-1	a	0.97	0.96	1.00	1.00	1.00	1.00	1.00	1.00	0.94	0.94	0.88	0.91
	b	0.03	0.05	0	0	0	0	0	0	0.06	0.06	0.12	0.10
Pgd-2	a	0.90	0.86	1.00	0.50	0.30	0.42	0.53	0.64	0.94	1.00	0.92	0.65
	b	0.10	0.14	0	0.50	0.63	0	0.07	0.36	0.06	0	0	0.35
	c	0	0	0	0	0.07	0.58	0.40	0	0	0	0.08	0
No. strains		38	22	53	12	27	12	15	11	16	16	24	21

[a] Allele present only in *S. viridis*
[b] Allele present only in *S. italica*

Table 4. Intraregional variability

Region	No. polymorphic loci[a]	Average no. of alleles per locus	Genic diversity[b]
S. italica			
1. China	4	1.7	0.11
2. Korea	3	1.6	0.11
3. Japan	2	1.3	0.08
4. Okinawa	2	1.2	0.07
5. Taiwan	2	1.3	0.09
6. India	2	1.2	0.08
7. Kenya	1	1.2	0.06
8. USSR	3	1.3	0.13
9. Central Eur.	8	1.9	0.22
10. France	6	1.7	0.12
(all *S. italica*)	10	2.2	
S. viridis			
1. China	6	2.0	0.18
2. France	8	2.1	0.12
(all *S. viridis*)	8	2.4	

[a] A locus is considered polymorphic if the frequency of the most common allele is lower than 95%
[b] Coefficient of genic diversity calculated according to Nei (1978)

Table 5. Classification of alleles according to their frequencies and distribution

	No. alleles	Allele	Regions
Common			
Widespread	15	All others	
Sporadic	3	*Pgd-2*[c]	India, Kenya
		Est-2[c]	Central Europe, France
		Est-3[b*]	
Localized	–		
Rare			
Widespread	2	*Got-1*[b]	Central Europe, Korea
		Got-2[b]	Central Europe, France
Localized	2	*Est-1*[b]	France
		Mdh-2[b]	China

* Common occurrence in more than 2 regions, but only in Europe

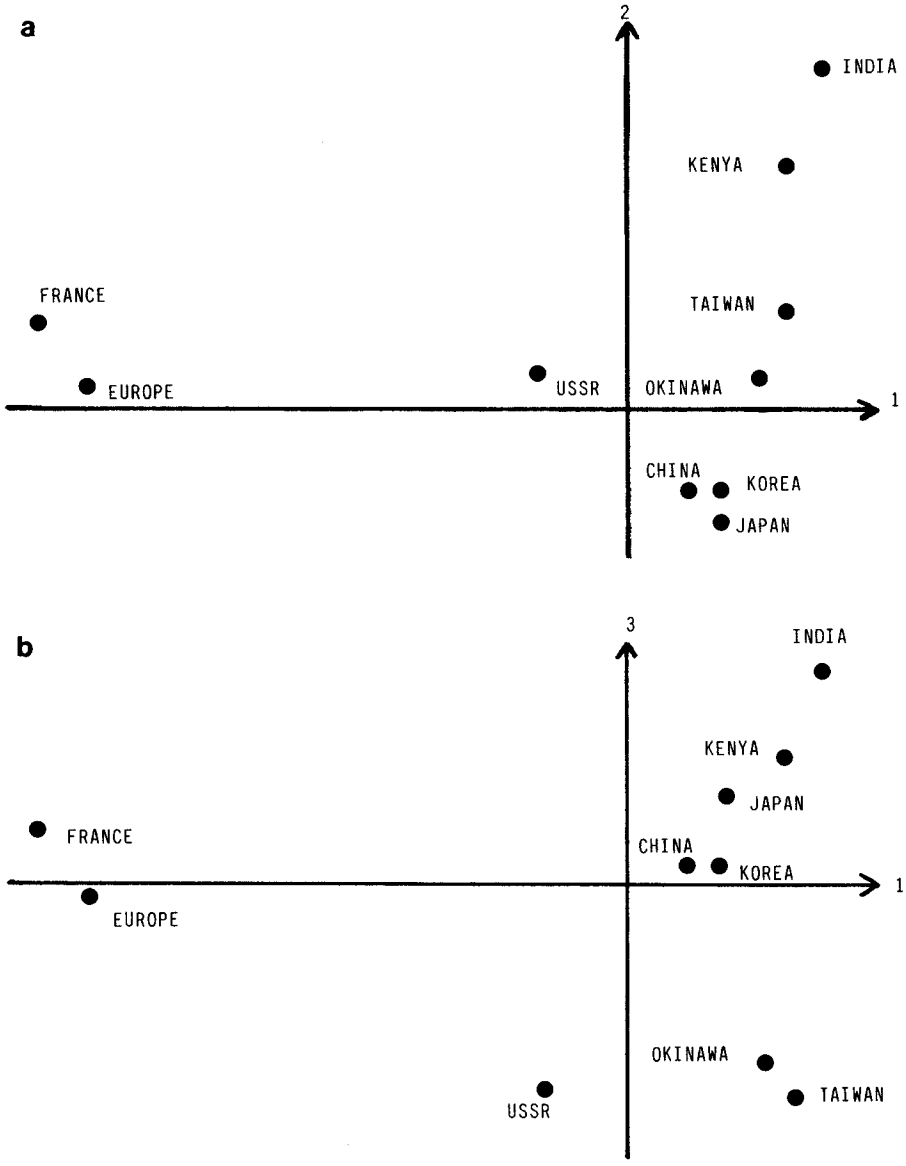

Fig. 1a, b. Graphical representation of the regions on the canonical plans engendered by the first three axes. **a** Axes 1 and 2; **b** Axes 1 and 3

Table 6. Mahalanobis squared distances between the 10 regions of origin of *S. italica* studied

	China	Korea	Japan	Okinawa	Taiwan	India	Kenya	USSR	Europe
Korea	0.75								
Japan	0.83	0.70							
Okinawa	1.69	1.63	1.99						
Taiwan	2.14	2.01	2.37	0.79					
India	3.09	3.05	3.07	3.24	3.12				
Kenya	2.25	2.26	2.34	2.34	2.42	1.06			
USSR	2.33	2.70	2.89	2.39	2.61	3.93	3.22		
Europe	3.93	4.01	4.20	4.48	4.67	5.16	4.71	3.54	
France	4.28	4.45	4.48	4.71	4.94	5.32	4.86	4.16	2.41

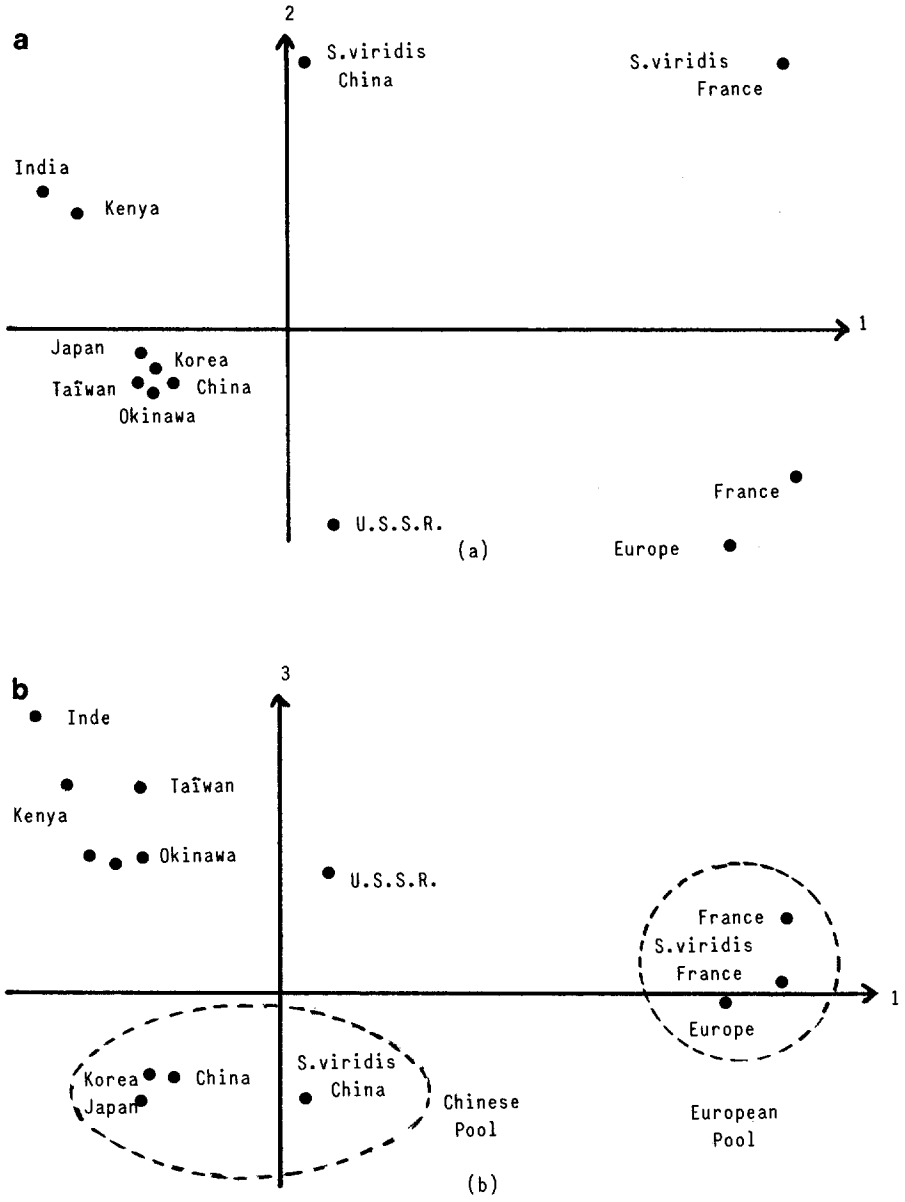

Fig. 2a, b. Graphical representation of the regions of *S. italica* and *S. viridis* on the canonical plans engendered by the first three axes. **a** Axes 1 and 2; **b** Axes 1 and 3

Table 7. Grouping of regions and their characteristic alleles

Group of regions	Important alleles [a]
China–Korea–Japan	*Est-2*[a] *Est-3*[a] *Pgd-2*[a]
Taiwan–Okinawa	*Est-2*[a] *Est-3*[a] *Pgd-2*[b]
India–Kenya	*Est-2*[a] *Est-3*[a] *Pgd-2*[c]
USSR	*Est-2*[b] *Est-3*[a] *Pgd-2*[a]
Central Europe	*Est-2*[b] *Est-3*[b] *Pgd-2*[a]
France	*Est-2*[a] *Est-3*[b] *Pgd-2*[a]

[a] The most important alleles are underlined

strictly *S. italica* strains (Table 8). Figure 2a principally shows the separation between *S. viridis* and *S. italica,* but a certain resemblance in the patterns of distribution of the two taxons is also noticeable. The first eigenvector separated spontaneous and cultivated strains in the same way, with the Asian plants more to the left and the European plants to the right, with the exception of USSR millet, which took a position nearer to the Asian regions.

On Fig. 2b, the regional separation is particularly evident. Two groups, called pools, were found, in which

Table 8. Mahalanobis squared distances between the regions of origin of the 10 *S. italica* strains studied and the 2 *S. viridis* populations

	S. italica										*S. viridis*
	China	Korea	Japan	Okinawa	Taiwan	India	Kenya	USSR	Europe	France	China
S. italica											
Korea	0.79										
Japan	0.84	0.70									
Okinawa	1.69	1.64	2.04								
Taiwan	2.12	1.99	2.40	0.77							
India	3.12	3.06	3.10	3.25	3.17						
Kenya	2.31	2.31	2.41	2.37	2.47	1.04					
USSR	2.37	2.69	2.93	2.32	2.53	4.00	3.30				
Europe	3.57	3.60	3.81	4.00	4.14	4.84	4.39	3.26			
France	3.83	3.99	3.99	4.10	4.29	4.86	4.39	3.80	2.40		
S. viridis											
China	2.34	2.31	2.38	2.87	3.14	3.33	2.79	3.29	3.87	4.11	
France	3.96	3.98	4.10	4.05	4.14	4.49	4.54	4.17	3.53	3.27	3.38

spontaneous and cultivated material coming from the same regions were classed (discontinuous circles). Chinese *S. viridis* and cultivated *S. italica* from China, Korea and Japan made up the first, "Chinese", pool, and the second, "European", pool, was composed of *S. viridis* originating from France and cultivated foxtail millet from Central Europe and France. The other regions, which were represented by cultivated crops only, were located farther away from these two pools.

Discussion

According to the data presented in this report, *S. italica* had little intraregional variability for enzyme alleles. Most of the regions had less than 50% polymorphic loci, and in all regions the average number of alleles per locus was lower than 2 (Table 4). The only region showing relatively higher values for the three parameters of intraregional variability studied was Central Europe, but it must be noted that the sampled material originating from this region was collected from many different countries of North and Central Europe. Lack of intraregional variability was also observed by De Wet et al. (1979), who reported that Chinese cultivars were uniform for storage proteins. As for interregional variability, the analysis presented here has revealed the existence of regional groups (Table 8). Each group was characterized by specific alleles, which were also classed as sporadic alleles.

It should be noted that although observations in our laboratory on morphological variability (Nguyen and Pernes 1984) were not entirely concordant with isoenzyme variability, there were analogies between them. Generally, foxtail millet could be morphologically divided into two races, *moharia* and *maxima*. Primitive cultivars were classified as *moharia* the more modern crops were grouped as *maxima* (De Wet 1979). In our laboratory, the strains of the groups China–Korea–Japan, Taïwan–Okinawa and most of France were observed to fall into the *maxima* group, and the strains of the India–Kenya group were *moharia*. Some of the strains from Central Europe and the USSR were classed *maxima* and others *moharia*. Until now, no special study comparing isoenzymes of *moharia* and *maxima* had been carried out, so not conclusions could be drawn on enzyme differences between the two races. However, from our data, it is not evident that two regions dominated by the same race would have lower distances. For example, India and Kenya, whose millets were classed as *moharia*, were nearer to Asian regions than to the European regions, which also had many *moharia* strains. However, our data did show analogies in variability between enzymatic and morphological genetic variability. It was noted above that the only region presenting important genic diversity and many polymorphic loci was Central Europe. As in this region there were both *moharia* and *maxima* strains, there must be important morphological variability among them as well.

The comparative study between *S. italica* and *S. viridis* reported in the last section of the Results showed that the differentiation was rather regional than taxonomical. The distances between the strains of the two taxons originating in the same regions were not superior to the distances between different regions of *S. italica*. Differences in genetic structure between crop strains coming from two different regions can be the effect of adaptation or selection processes, but in the case of foxtail millet we presume that the differences observed were due to two independent domestications. The presence of two pools (Fig. 2 b) showed the resem-

blance of the patterns of genetic variability in spontaneous green foxtail and cultivated foxtail millet in each of two regions, China and Europe. It is therefore possible that there were independent domestications in these two sites. The genetic structural differences between the two regional cultivated foxtail millets were possibly derived from differences in the genetic structures of the spontaneous green foxtails from which these cultivated crops had been domesticated. It is possible that the specific alleles of *S. italica* in certain regions reflect a similar allelic situation in the *S. viridis* of these same regions. Unfortunately, not enough material was available to compare overall patterns of the genetic variability in *S. italica* and *S. viridis.*

Many loci of *S. italica* are monomorphic, and some loci are nearly fixed for one allele. For each of the loci *Est-1* and *Mdh-2* only one in 223 strains studied presented the rare alleles *Est-1*^{b} or *Mdh-2*^{b}. It is important in the conservation of germplasm to give special treatment to such loci or rare alleles. It was also observed that some rare alleles are present only in the spontaneous plants, for example *Est-1*^{c}, *Est-1*^{d}, *Est-2*^{d} and *Mdh-1*^{c}. There is one allele which was qualified as rare in *S. italica* and which had a non-negligable frequency in *S. viridis, Got-2*^{b}. Therefore, integrating spontaneous plants into a collection can increase its polymorphism.

References

Brown AHD (1978) Isozymes, plant population genetic structure and genetic conservation. Theor Appl Genet 52: 145–157

Cherisey H de, Barreneche T, Jusuf M, Ouin C, Pernes J (1985) Inheritance of some marker genes in *Setaria italica* (L.) P. Beauv. Theor Appl Genet 71:57–60

Ho PT (1969) The loss and the origin of Chinese agriculture. Am Hist Rev 75:1–36

Jusuf M (1983) Variabilité des *Setaria italica,* en relation avec la distribution géographique et la domestication. Approche par l'étude des polymorphismes enzymatiques. Thèse 3ième cycle. Univ Paris-XI-Orsay

Li HW, Li CH, Pao WK (1945) Cytological and genetical studies of the interspecific cross between the cultivated foxtail millet, *Setaria italica* (L.) Beauvais and the green foxtail *S. viridis.* J Am Soc Agron 37:32–54

Mardia KV, Kent JT, Bibby JM (1979) Multivariate analysis. Academic Press, New York

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 76:379–390

Nguyen E, Pernes J (1984) Genetic diversity of foxtail millet (*Setaria italica*). In: Plant population biology symposium. Springer, Berlin Heidelberg New York Tokyo, pp 31–40

Wet JMJ de, Oestry-Stidd LL, Cubero JI (1979) Origins and evolution of foxtail millet (*Setaria italica*). J Agric Trad Bot Appl 26:53–64