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Ribosomal DNA variation in foxtail millet, *Setaria italica* (L.) P. Beauv., and a survey of variation from Europe and Asia

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Abstract Restriction fragment length polymorphism (RFLP) and the structure of ribosomal RNA genes (rDNA) were investigated in 117 landraces of foxtail millet, *Setaria italica* (L.) P. Beauv. Five RFLP phenotypes were found when the genomic DNA was digested with *Bam*HI; these were named types I–V. Of these types I, II and III were the most frequent. Type I was mainly distributed in the temperature zone, type II in the Taiwan-Philippines Islands and type III in South Asia. Restriction mapping of the cloned rDNA and comparison with RFLP phenotypes showed that the different types originated from a polymorphism in the length within the intergenic spacer (IGS) and *Bam*HI site changes within the IGS.

Key words Foxtail millet · rDNA · RFLP · Restriction mapping · Geographical distribution

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Introduction

Foxtail millet, *Setaria italica* (L.) P. Beauv is an old staple crop in Europe and Asia and is thought to have been a major cultivated cereal in early agriculture. Landraces of this millet are still cultivated on a small scale in scattered locations throughout Europe and Asia (Kawase and Sakamoto 1984).

The variation and distribution of phenol color reaction, esterase isozymes, hybrid pollen sterility and other characters have been studied to determine the evolution and domestication history of *S. italica* (Kawase and Sakamoto 1982, 1984, 1987). Cytological studies indicated that the wild ancestor of foxtail millet is *S. viridis* (Kihara and Kishimoto 1942; Li et al. 1945). The geographical origin of domesticated *S. italica* has not been determined from the distribution of *S. viridis*, which is found in various parts of Europe and Asia. Vavilov (1926) stated that the principal center of diversity for *S. italica* is eastern Asia, including China and Japan. Harlan (1975) suggested that this millet was independently domesticated in China and Europe. Recently, Sakamoto (1987) suggested that foxtail millet originated somewhere in the region of Central Asia, Pakistan, Afghanistan and India. In this region, strains with less restricted comptability are distributed in Afghanistan and India, and strains with primitive morphological traits are found especially in Afghanistan.

Nuclear ribosomal RNA genes (rDNA) are organized in tandemly repeated units. The copy number of these units varies from a few hundred to thousands in plants (Rogers and Bendich 1987). Each repeat unit contains a sequence coding for mature 18S, 5.8S and 25S rRNA and transcribed spacers and is separated from the next repeat unit by an intergenic spacer (IGS). Intraspecific variation in IGS length has been reported in barley (Saghai Maroof et al. 1984; Zhang et al. 1992), wheat (Appels and Dvorak 1982), rice (Cordesse et al.

1990; Sano and Sano 1990), sorghum (Springer et al. 1989) and many other plants.

In the study reported here, we studied restriction fragment length polymorphism (RFLP) of rDNA and the geographical distribution of rDNA variants in foxtail millet. The structure of rDNA was also investigated to clarify the genetic differentiation of rDNA genes among strains of *S. italica*. On the basis of the results obtained, a possibility was implied that the strains from Afghanistan and those from India originated independently.

Materials and methods

Plant materials

A total of 117 strains were investigated (Table 1). Most of the strains were those used for the classification of landrace groups by means of intraspecific hybrid pollen sterility (Kawase and Sakamoto 1987). All of the strains were maintained by selfing at the Plant Germplasm Institute. Faculty of Agriculture, Kyoto University.

Table 1 Materials used and their rDNA types

Country	Region	Code no.	rDNA type
East Asia			
Japan	Iwate Pref.	031	I
	Nagano Pref.	032	I
	Yamanashi Pref.	033	IV
	Toyama Pref.	034	I
	Kanagawa Pref.	035	I
	Nara Pref.	036	III
	Hyogo Pref.	037	II
	Kochi Pref.	012	III
	Kumamoto Pref.	038	I
	Nagasaki Pref. (Tsushima Is.)	039	I
	Miyazaki Pref.	099	I
	Kagoshima Pref.	013	I
Nansei Islands of Japan	Okinawa Pref. (Ikema Is.)	020	I
	Okinawa Pref. (Ishigaki Is.)	021	II
	Okinawa Pref. (Iriomote Is.)	022	II
Korea	Kangwon-do	040	IV
	Kyonggi-do	041	IV
	Chungchong-pukto	042	I
	Chungchong-namdo	043	I
	Kyongsang-pukto	044	I
	Kyongsang-namdo	045	I
	Cholla-pukto	046	I
	Cholla-namdo	047	I
	Cheju-do	048	I
ex-USSR	Primorskaya Prov.[a]	083	V
China	Heilungkiang Prov.	091	I
	Heilungkiang Prov.	092	I
	110001[b]	049	I
	110002[b]	050	I
	110003[b]	051	I
	110005[b]	052	II
	110007[b]	053	I
	110008[b]	054	II
	110009[b]	055	I
	110015[b]	056	I
	Yunnan Prov.	015	I
	Anhui Prov.	121	III
Mongolia	Uncertain[a]	095	III
Taiwan	Taiwan Is. (Pington)	057	IV
	Taiwan Is. (Pington)	058	III
	Taiwan Is. (Pington)	059	II
	Taiwan Is. (Pington)	060	I
	Taiwan Is. (Taitung)	061	II
	Taiwan Is. (Taitung)	062	II
	Taiwan Is. (Hwalien)	063	II
	Taiwan Is. (Nantou)	064	II
	Taiwan Is. (Nantou)	065	II
	Lan-Hsü Is.	098	I
	Lan-Hsü Is.	02	II
	Lan-Hsü Is.	04	II
	Lan-Hsü Is.	05	II
	Lan-Hsü Is.	06	II
Southeast Asia			
The Philippines	Itbayat Is.	07	I
	Batan Is.	08	II
	Batan Is.	09	II
	Batan Is.	010	II
	Batan Is.	011	II
Indonesia	Halmahera Is.	03	II
	Sulawesi Is.	016	I
Thailand	A village near Cheng-mai	01	IV
Burma	Shan Prov.	024	III
South Asia and its northwestern vicinity			
Nepal	Dhuche	017	I
	Bhargu	018	IV
	Bhargu	019	III
	Kathmandu	026	III
Bangladesh	Jumalpur	100	III
India	South India[c]	066	III
	South India[c]	067	III
	South India[c]	068	III
	South India[c]	069	III
	South India[c]	070	III
	South India[c]	071	III
	South India[d]	072	III
	South India[d]	073	III
	South India[d]	074	III
	South India[d]	075	III
	Bihar	117	III
	Orissa	118	III
	Madhya Pradesh	119	III
	Madhya Pradesh	120	III
Pakistan	N.W.F.P.	101	III
	N.W.F.P.	102	I
	N.W.F.P.	103	I
	N.W.F.P.	104	I
	N.W.F.P.	105	I
	N.W.F.P.	106	I
	N.W.F.P.	107	I
	Gilgit agency	109	I
	Gilgit agency	110	I
	Gilgit agency	111	I
	Baltistan	112	I
	Baltistan	113	I
	Punjab	114	IV
	Punjab	115	V
	Baluchistan	116	IV

Table 1 Continued

Country	Region	Code no.	rDNA type
Afghanistan	Jabalsalaj-Zenya	076	I
	Takhar	077	I
	Takhar	078	I
	Badakhshan	079	I
	Badakhshan	080	I
Central Asia			
exUSSR	Novosibirk[a]	094	II
	Kirghizia[a]	081	I
	Uzbekistan[a]	082	I
	Georgia[a]	028	I
	Georgia[a]	029	I
	Ukraine[a]	093	I
Europe			
Bulgaria	Uncertain[a]	096	I
Czechoslovakia	Uncertain[e]	030	I
France	Cuon, Maine et Loire	088	I
France	Cuon, Maine et Loire	089	I
France	Cuon, Maine et Loire	090	I
Belgium	Uncertain[c]	086	I
Belgium	Uncertain[c]	087	II
Belgium	Uncertain[c]	097	II
Germany	Uncertain[g]	085	I
Spain	Canges de Narcea	084	I
Africa			
Kenya	Embu District	027	III

[a] All were provided by the N.I. Vavilov All-Union Institute of Plant Industry, Leningrad, the exUSSR
[b] All were provided by the National Institute of Agricultural Science, Japan
[c] All were provided by the University of Agricultural Science, Bangalore
[d] All were provided by Tamil Nadu Agricultural University, Coimbatore, India
[e] It was provided by the Institute of Genetics and Plant Breeding, Prague-Rizyne, Czechoslovakia
[c] All were provided by Dienst voor Parken En Platsonen, Antwerp, Belgium
[g] It was provided by Karl Marx Universitaet, Lepzig, DDR

DNA extraction and RFLP analysis

Total DNA was extracted from leaves of bulked seedlings as described by Murray and Thompson (1980) with some modification. Approximately 2 μg of DNA from each strain was digested with *Bam*HI following the manufacturer's instruction (Takara). The fragments were separated by electrophoresis on 0.8% agarose gel in TAE buffer (Maniatis et al. 1982). The DNA was transferred to a nylon membrane (Boehringer Mannheim) employing the technique of Southern (1975). A cloned rice rDNA, pRR217 (Takaiwa et al. 1984) digested with *Bam*HI into three fragments was used as a probe for gel blot hydridization (Southern 1975). The enhanced chemiluminescence (ECL) direct labeling and detection kit (Amersham) was used following the manufacturer's manual.

Cloning of rDNA

An accession (73-10-26-15, code no. 012) collected from Kochi Prefecture, Japan, was used for cloning rDNA. Approximately 100 μg of total DNA was digested with *Pst*I, which cuts the rDNA into single-repeat units. Fragments of 7–9 kb were extracted after 0.8% agarose gel electrophoresis in TAE buffer. Methylene blue was used to visualize the fragments. The fragments were ligated into pUC118 using ligation kit ver. 2 (Takara) and transformed into competent *E. coli* cells (strain XL1-Blue MRF′). Recombinant clones were selected on plates containing ampicillin, X-gal and Isopropyl-*β*-D-thiogalactopyranoside (IPTG). The clones were screened using pRR217 as a probe.

Restriction mapping

A possible clone of rDNA obtained from foxtail millet was digested with eight restriction enzymes (*Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, *Kpn*I, *Nde*I, *Sal*I and *Xba*I) and double-digested with combinations of these enzymes. After the size of each fragment was determined using 1.2% and 1.5% agarose gel electrophoresis, DNA was transfered to a nylon membrane. Cross hybridizations were carried out with pRR217 fragments to identify the regions corresponding to the 25S, 17S and 5.8S genes, and the IGS.

Results

RFLP analysis

Five different patterns of rDNA in foxtail millet were observed in the RFLP analysis with pRR217; these were named types 1–V (Fig. 1A). Type-I strains were distributed mainly in the temperature zone of Europe and Asia and sporadically in Taiwan Island and Lan-Hsü Island of Taiwan, Batan Islands of the Philippines, Indonesia and Nepal. Approximately one-half of the strains investigated were of this type. Strains of type II were found mainly in the Taiwan-Philippines Islands, i.e. Nansei Islands of Japan, Taiwan Island and Lan-Hsü Island of Taiwan and Batan Islands in the Philippines. Strains of type III were distributed mainly in South Asia (India, Nepal and Bangladesh). All of the Indian strains were of this type, and this type was not

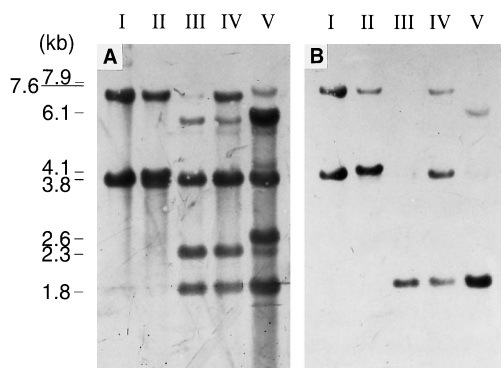

Fig. 1A, B RFLP patterns of genomic rDNA digested with *Bam*HI in foxtail millet, types I–V **A** with pRR217 as probe (type I: 7.6 + 3.8 kb, type II: 7.9 + 4.1 + 3.8 kb, type III: 6.1 + 3.8 + 2.3 + 1.8 kb, type IV: 7.6 + 6.1 + 3.8 + 2.3 + 1.8 kb, type V: 6.1 + 3.8 + 2.6 + 2.3 + 1.8), **B** with pSIR012.1 as probe

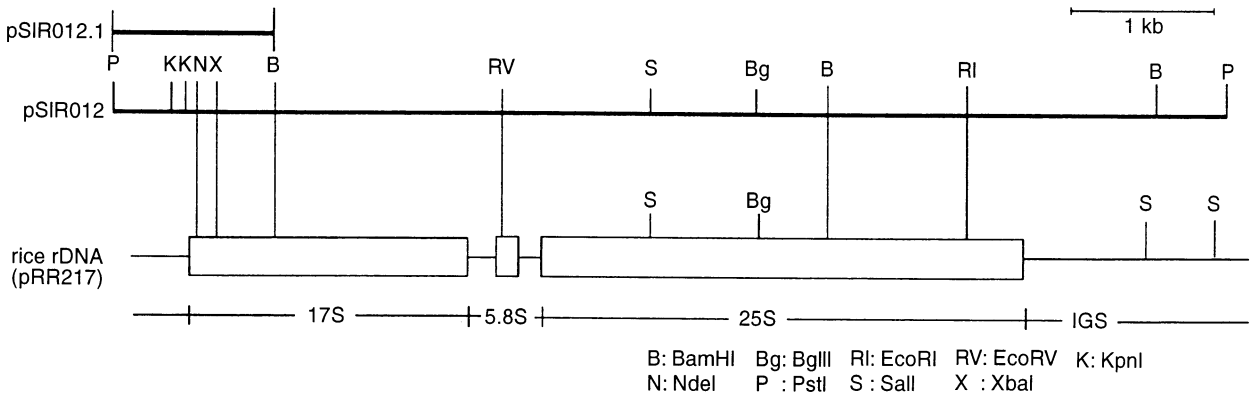

Fig. 2 The restriction mapping of a cloned rDNA, pSIR012, in foxtail millet compared with the restriction mapping of one of rice, pRR217 (Takaiwa et al. 1984, 1985a, b 1990)

found in Afghanistan, Central Asia or Europe. Of the 117 strains investigated, 107 strains belonged to above-mentioned three types. The type-IV pattern was rare and was found in Japan, Korea, Taiwan, Thiland and southern Pakistan. Type V was also rare and was found in southern Pakistan and the Primorskaya Province of exUSSR (Table 1).

Cloning and restriction mapping of rDNA

The strain used for cloning displayed type-III rDNA. A 7.9-kb *Pst*I fragment cloned in pSIR012 hybridized strongly with pRR217 and was thus identified as an rDNA repeat unit. Single sites for *Bgl*II, *Eco*RI, *Eco*RV, *Nde*I and *Xha*I, two sites for *Kpn*I and three sites for *Bam*HI were detected in the cloned fragment. The relative positions of these sites were determined by double-digestions and further hybridization tests. After hybridizations with digested pRR217 fragments, the locations of the 25S, 17S and 5.8S genes and the IGS were determined (Fig. 2). In pSIR012, single *Nde*I, *Xba*I, *Eco*RV and *Eco*RI sites and two *Bam*HI sites clearly corresponded to the sites for these enzymes in the coding regions of pRR217. *Sal*I and *Bgl*II sites were also located in similar positions to those in pRR217.

Genomic southern hybridization carried out using pSIR012 as a probe showed a band pattern similar to that obtained with a pRR217 probe. Genomic gel blot hybridization was also re-examined using a subcloned fragment, pSIR012.1, as a probe (Fig. 1B). This fragment consists of a part of the IGS and the 5′ end of the 25S gene. There was a decreased number bands compared with the RFLP profile detected with pSIR012, but polymorphism was still observed.

Discussion

RFLP and geographical distribution of rDNA phenotypes

Type I was widely distributed in the temperature zone from the Far East to Europe, type II mostly in the Taiwan-Philippines Islands and type III predominantly in South Asia. It is interesting to note that the geographical distribution of types I, II and III shows clear correspondence with that of other genetic characters. An analysis of the rDNA will be helpful in solving phylogenetic problems in *S. italica*.

The distributions of plants displaying different phenol color reactions have been reported by Kawase and Sakamoto (1982). Strains showing a positive phenol color reaction were rarely found in the temperate zone but were frequently found in South Asia and the Taiwan-Philippines Islands. The distribution of type-I rDNA coincided with the regions where those showing a positive phenol color reaction were rarely found. In contrast, types II and III were found in the areas where the positive phenotype of phenol color reaction was frequently distributed. This distribution of phenol color-reaction phenotypes is consistent with the present observation of two gaps in rDNA distributions between the temperate zone and India and between the temperate zone and the Taiwan-Philippines Islands.

Intraspecific hybrid pollen sterility was used to classify *S. italica* into 6 landrace groups, A, B, C, X, AC and BC using three tester strains (Kawase and Sakamoto 1987). Distribution of the landrace groups classified showed some correspondence with that of the rDNA phenotypes. Distribution of type-I rDNA coincided with the distributions of types A, C and AC, that of type II with the regions where types B and X were found and that of type III with the area of type BC.

The structure of rDNA in foxtail millet

The structure of the coding regions of pSIR012 was quite similar to that of a rice DNA, pRR217. Springer

et al. (1989) reported a similar structure of rDNA in maize, sorghum and sugarcane. These crops belong to subfamily Panicoideae, to which foxtail millet also belongs. These crops have single sites of *Nde*I, *Xba*I, *Eco*RV, *Bgl*II and *Eco*RI and two of *Bam*HI on quite similar positions on the coding regions. It is interesting to one that rice (subfamily Oryzoideae) and the above-mentioned crops belonging to subfamily Panicoideae share all these sites.

Interpretation of RFLP phenotypes

Major phenotypic bands were commonly detected in the analysis using both pSIR012 and pSIR012.1, as shown in Fig. 1. The cloned type-III rDNA showed only a 1.8-kb fragment when pSIR012.1 was used as a probe. A similar result was obtained when a 0.5-kb *Pst*I-*Bam*HI fragment of pSIR012 was used as a probe (data not shown). These results suggest that a 1.8-kb band in genomic gel blot hybridization corresponds to the 1.3-kb and 0.5-kb *Bam*HI-*Pst*I fragments in pSIR012. Two bands, 2.3 kb and 3.8 kb, in genomic Southern hybridization presumably correspond to the 2.3-kb and 3.8-kb fragments in pSIR012, respectively. Type I was deduced to consist of two 3.8-kb *Bam*HI fragments. One of the two 3.8-kb fragments was estimated to contain the complete IGS. This type lacks a *Bam*HI site on the IGS and differs from types II and III in the length of the IGS. Type II consists of 4.1- and 3.8-kb fragments. The 4.1-kb fragment appears to contain the complete IGS because a 3.8-kb and was not detected in genomic hybridization using pSIR012.1 as a probe. This type also lacks a *Bam*HI site on the IGS.

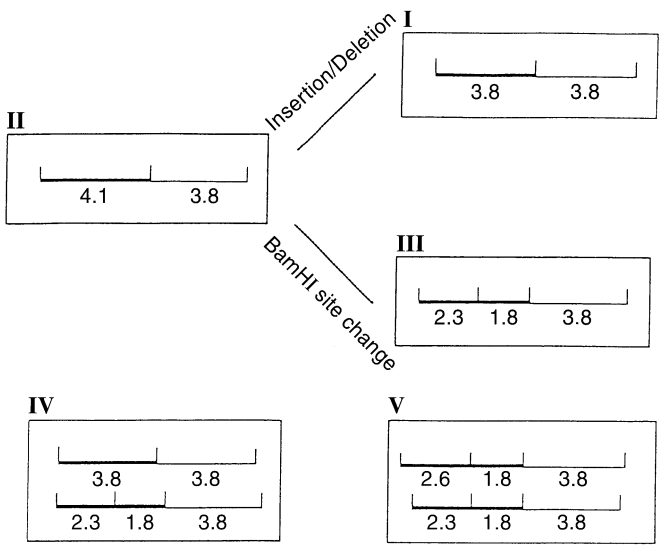

Fig. 3 Interpretation of RFLP phenotypes. Each *bar* shows a repeat unit with *Bam*HI sites. *Thick bars* show the regions which contain the IGS

Type IV has both types of the repeat units of types I and III because both types of bands of types I and III were observed in gel blot hybridization using pSIR012 as a probe. Upper bands, 7.6 kb of types I and IV, 7.9 kb of type II and 6.1 kb of types III, IV and V, may have arisen from the incomplete digestion due to the methylation of *Bam*HI sites. A similar phenomenon was also reported in rice (Olmedilla et al. 1984). Type IV can be distinguished from type III by the presence of a 7.6-kb band.

In the differentiation of rDNA phenotypes, IGS length polymorphism (insertion-deletion) and *Bam*HI site changes (point-mutation) were strongly suggested (Fig. 3). All the repeat units have 3.8-kb fragments in common which contain most of the coding regions, but not the IGS. Each of types I–III consists of a single type of repeat unit. On the other hand, both types IV and V consist of two different repeat units. Therefore, the RFLP patterns appear to represent different combinations of four different repeat units.

Domestication and dispersal

The geographical distribution of different rDNA patterns, which shows clear correspondence with that of other characters such as the phenol color reaction and hybrid pollen sterility, may reflect some important aspects of phylogenetic differentiation. A major point of interest is that the strains from Afghanistan and India have different rDNA phenotypes, yet these regions were included in the area suggested by Sakamoto (1987) as the geographical origin of *S. italica*. The strains from Afghanistan and India may have differentiated from each other before domestication, but many other explanations are possible. To clarify the process of domestication and differentiation of foxtail millet, we are now planning analyses of the wild ancestor, *S. viridis*.

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