Characterization of Foxtail Millet, *Setaria italica* (L.) P. Beauv., in Pakistan Based on Intraspecific Hybrid Pollen Semi-sterility

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Summary

Sixteen strains of foxtail millet, *Setaria italica* (L.) P. Beauv., originally collected from Pakistan were crossed with three strains which had previously been used as testers for classifying six landrace groups based on hybrid pollen sterility. Wide variation was observed in pollen fertility of the F₁ hybrids, ranging from 14.2 % to 94.7 %, while pollen fertility of all the selfed Pakistani strains and testers was normal. Pakistani strains were classified into type C (8 strains), type BC (2) and type X (6) on the basis pollen fertility of more than 75 % is normal fertility. No A, B or AC type strains were detected. Type C was found in landraces collected from Punjab Province, the southern part of the North-West Frontier Province (N. W. F. P.) and Baltistan. Type X was distributed in the northern part of N. W. F. P. and an adjacent area of the western part of Gilgit Agency, in the northeastern part of Gilgit Agency and in Baltistan. Different landrace groups were suggested among the strains of type X by the variation in hybrid sterility observed with each tester. This observation was strongly supported by an almost sterile hybrid between type X strains from N. W. F. P. and the northeastern part of Gilgit Agency. Type BC was found in Gilgit Agency and Baluchistan. Landrace groups classified by hybrid pollen fertility did not show any clear relationship with morphological groups which had a different geographical distribution. The present results imply that *S. italica*, with various phylogenetic backgrounds, might have been introduced into this region several times, and subsequently evolved into three distinct morphological groups.

Key Words : *Setaria italica*, foxtail millet, hybrid sterility, landrace groups, phylogenetic differentiation, crop evolution, Pakistan.

Introduction

Foxtail millet, *Setaria italica* (L.) P. Beauv., is a minor cereal of Eurasian origin and has a long history in cultivation. Various landraces or local cultivars of this crop, originating from different areas, were classified into six landrace groups designated as types A, B, C, AC, BC and X using hybrid pollen sterility as an indicator of genetic differentiation (Kawase and Sakamoto 1987). In *S. italica*, the intraspecific hybrid pollen sterility

is probably under genetic control rather than due to structural changes of chromosomes. Afghanistan to India has been suggested as an important region in the domestication and evolution of *S. italica* based on the distribution of landrace groups. Limited information is available on the landraces of *S. italica* in Pakistan. Cultivation of *S. italica* is mainly for home-consumption and is restricted to remote villages, particularly, in northern mountainous areas. Scheibe (1943) investigated landraces collected by the German Hindukush Expedition mission in 1937 and 1938 from the North-West Frontier Province (N. W. F. P.) of British India (now in Pakistan) and adjacent areas of Afghanistan and reported that there was considerable variation in landraces of *S. italica*. Recently, Ochiai *et al.* (1994) studied morphological variation of landraces collected from northern Pakistan, and recognized three groups, i.e., the Chitral group, the Baltistan group and the Dir group, which were named after the main areas where they are grown. The Chitral group was characterized by an extremely short plant having many tillers with small panicles and elliptic caryopsis with shining lemma and palea. It was distributed in Chitral district of N. W. F. P. and the western part of Gilgit Agency. The Baltistan group had a taller plant stature, one or a few large panicles per plant, dull lemma and palea and round caryopsis, and was distributed in the northeastern part of Gilgit Agency and in Baltistan. The Dir group showed similar characteristics as the Chitral group having many tillers and shining and elliptic caryopsis, but differed by generally being much taller. The Dir group varied greatly in heading date, consequently plant height and panicle length were very variable. The vernacular names for the three groups are also different, which indicates that the farmers growing them had different ethnic backgrounds (Kawase *et al.* 1995).

In the present study, sixteen *S. italica* landraces collected from Pakistan were crossed with three testers. Pollen fertility of the F₁ hybrids produced were examined to determine landrace groups of the strains and to obtain some information on phylogenetic differentiation. Our working hypothesis is that diversity of *S. italica* in Pakistan can be explained by the distribution of groups with independent phylogeny in different areas, if landrace groups classified on the basis of pollen fertility of F₁ hybrids coincides with the morphological groups.

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Materials and Methods

Plant materials used

Sixteen accessions (P 01 to P 16) of *S. italica* landraces collected in Pakistan were used as female parents (Table 1, Fig. 1). Among them, seven accessions (P 01 to P 07) were collected in the North-West Frontier Province (N. W. F. P.), four (P 08 to P 11) in Gilgit Agency, two (P 12 and P 13) in Baltistan, two (P 14 and P 15) in Punjab Province and one (P 16) in Baluchistan Province. They were grown at least once after collecting in experiment fields, and selfed seeds from a selected plant were used as a representative strain for each accession. Three strains that had already been used by Kawase and Sakamoto (1987) as testers A, B and C for classifying landrace groups were used as male parents. Furthermore, eight strains (P 07, P 08, P 10, and P 17 to P 21) were used for crosses between Pakistani landraces of different morphological groups (Table 1, Fig. 1).

Estimation of pollen fertility

Artificial hybridization of strains and classification of landrace groups were performed as described by Kawase and Sakamoto (1987). Emasculation was carried

out just during anthesis in the early morning using an air-sucking pump, and then the florets were immediately pollinated with a tester. Pollen fertility was used as a criteria for landrace classification, since seed fertility shows a similar, but less clear tendency, compared with pollen fertility (Kawase and Sakamoto 1987). Pollen fertility was measured as the percentage of pollen grains stained normally with glycerin carmine among at least one thousand pollen grains observed for each individual of parental strains and/or F_1 hybrids. When more than one individual are available for a strain or a cross combination, a mean value was calculated. Mean pollen fertility of more than 75 % was regarded as normal for the determination of landrace groups.

Results

Pollen fertility of selfed plants of all the Pakistani strains used was normal being more than 93.2 % (Table 2). Testers A, B and C showed slightly lower pollen fertility, 90.6 %, 81.0 % and 89.6 %, respectively. On the other hand, a wide variation was observed in pollen fertility of the F_1 hybrids ranging from 14.2 % to 94.7 %. Similar fertility was observed among the plants for the same cross combination when more than two plants were examined.

Table 1. *S. italica* landraces used

Code	Collection No.	Collection locality (altitude) or source	Note ¹⁾
Pakistani landraces as female parents			
P01	COL/PAKISTAN/1991/IBPGR/2860(1)	Toghsaray, 16 km W from Hangu, Kohat district, N. W. F. P. (900 m)	(3)
P02	87-10-26-1-1	Besham, 55 km N from Battagram towards Chilas, N. W. F. P. (520 m)	(1)
P03	COL/PAKISTAN/1991/IBPGR/2855(1) b	Almas Shou, 37 km E from Dir, Dir district, N. W. F. P. (1,350 m)	(3)
P04	87-9-24-2-2	Bambreet, 31.6 km S from Chitral, Chitral district, N. W. F. P. (1,760 m)	(1)
P05	COL/PAKISTAN/1991/IBPGR/2849(2) a	Susoom, 42 km N from Chitral, Chitral district, N. W. F. P. (2,860 m)	(3)
P06	COL/PAKISTAN/1991/IBPGR/2837(1)	Barpish, 25 km S from Booni, Chitral, N. W. F. P. (1,790 m)	(3)
P07	COL/PAKISTAN/1991/IBPGR/2840(2)	Mastuj, Chitral district, N. W. F. P. (2,280 m)	(3)
P08	87-9-28-1-1	Teru, 112.4 km from Booni, towards Gilgit, Gilgit Agency (3,010 m)	(1)
P09	COL/PAKISTAN/1989/IBPGR/2524(1)	Nilt Nagar, 70 km N from Gilgit towards Hunza, Gilgit Agency (1,900 m)	(2)
P10	COL/PAKISTAN/1989/IBPGR/2528(1)	Aliabad, Hunza, 101 km N from Gilgit, Gilgit Agency (2,180 m)	(2)
P11	COL/PAKISTAN/1991/IBPGR/2819(1)	Gayar, 10 km N from Sazin, Gilgit Agency (1,130 m)	(3)
P12	87-10-6-2-2	Shut, 139.4 km from Gilgit towards Skardu, Baltistan (1,780 m)	(1)
P13	87-10-8-5-1	Kunis, 50±km from Skardu towards Machulo, Baltistan (2,370 m)	(1)
P14	COL/PAKISTAN/1989/IBPGR/2544(1)	Dhrama, 31 km from Khoshad towards Sargodha, Punjab (190 m)	(2)
P15	COL/PAKISTAN/1989/IBPGR/2569(3)	Ahmadpur Kohna, Basti Mahar, 3 km from Ahmadpur Sharkia towards Rahim Yar Khan, Punjab (70 m)	(2)
P16	COL/PAKISTAN/1989/IBPGR/2583(6)	Pishin, near Quetta, Baluchistan (1,450 m)	(2)
Testers			
A	74-12-6-3	Shiiba, Higasi-usuki-gun, Miyazaki, Japan	(4)
B	77-10-7-9	Lan Hsü Island, Taiwan	(5)
C	75-7-7-1b	provided by Dienst voor Parken En Plantsonen, Antwerpen, Belgium	
Other Pakistani strains used for supplemental crosses			
P17	87-9-26-2-2	Kogoz, 20 km NE from Chitral, Chitral district, N. W. F. P. (1,500 m)	(1)
P18	87-9-26-6-3	47.5 km NE from Chitral, Chitral district, N. W. F. P. (1,700 m)	(1)
P19	87-10-2-3-1	Skandarbad, 56.8 km N from Gilgit, Gilgit Agency (1,740 m)	(1)
P20	87-10-8-4-4	Goon, 50±km from Skardu towards Machulo, Baltistan (2,230 m)	(1)
P21	87-10-9-3-1	Barah, 10±km W from Khaplu towards Skardu, Baltistan (2,380 m)	(1)

¹⁾ (1) Sakamoto (1989); (2) Nakagahara *et al.* (1990); (3) Kawase *et al.* (1992); (4) collected by S. Sakamoto; (5) collected by K. Moriguchi

Fig. 1. A map of Pakistan showing the localities where the *S. italica* strains used were originally collected.

Pakistani strains used were classified into type C (8 strains), type BC (2) and type X (6) regarding pollen fertility of more than 75 % as normal fertility. No strain was found which corresponded to type A, type B or type AC. Type C was found in three strains (P 01, P 02 and P 03) collected from the southern part of N. W. F. P., in two strains (P 14 and P 15) from Punjab Province and one (P 13) from Baltistan. Two strains of type C (P 02 and P 03) from N. W. F. P. showed relatively high pollen fertility (66.6 % and 70.3 %, respectively) in the F_1 hybrids, even when crossed with tester A. One strain (P 11) from Gilgit Agency and another (P 16) from Baluchistan were identified as type BC. The former strain showed relatively high pollen fertility (71.1 %) in the F_1 hybrid when crossed with tester A. Two strains (P 06 and P 07) from the northern part of N. W. F. P. (Chitral district), one (P 08) from the western part of Gilgit Agency, two (P 09 and P 10) from the northeastern part of Gilgit Agency and one (P 12) from Baltistan belonged to type X. Pollen fertility of the strains classified as type X showed variable pollen fertility among testers. For example, P 06 showed nearly normal pollen fertility (73.0 %) in the F_1 hybrid when

crossed with tester C.

In the additional crosses between the Chitral group and the Baltistan group both fertile and sterile F_1 hybrids were produced (Table 3). Fertile hybrids were obtained in two cross combinations (P 18 $\times$ P 21 and P 20 $\times$ P 17). However, two combinations (P 07 $\times$ P 10 and P 08 $\times$ P 19) produced almost completely sterile hybrids having indehiscent anthers, of which pollen fertility was quite low (15.7 % and 36.8 %) and few F_2 seeds were produced.

Discussion

Types C, BC and X were recognized in Pakistani land-races of *S. italica*. Half of the strains used were identified as type C. It was previously reported that strains of type C are distributed, particularly in western Europe and rarely in Nepal, Afghanistan and Indonesia (Kawase and Sakamoto 1987). They are also found sporadically in India (Kawase, unpublished data). Results presented here for type C show no correspondence with morphological characteristics reported by Ochiai *et al.* (1994). According to the classification of Ochiai *et al.*

Table 2. Pollen fertility of the F₁ hybrids between Pakistani landraces and testers A, B and C

Code	Mean pollen fertility (No. of plants observed) (range)				Landrace group classified	Morphological group ¹⁾ (or note)
	Selfed plant	Tester (♂) crossed				
		A	B	C		
P01	99.4 (2) (99.3, 99.6)	18.8(1)	38.7(3) (30.0–44.8)	82.2 (1)	type C	(like the Dir group)
P02	97.8 (3) (97.4–98.1)	66.6(1)	15.4(2) (14.8, 16.1)	78.9 (3) (77.3–81.6)	type C	the Dir group
P03	97.3 (1)	70.3(1)	56.9(1)	81.4 (2) (79.7, 83.1)	type C	the Dir group
P04	94.0 (2) (91.2, 96.9)	28.4(3) (24.6–33.1)	38.7(3) (33.9–46.1)	89.5 (1)	type C	the Chitral group
P05	95.1 (2) (95.0, 95.3)	41.3(1)	29.0(2) (23.2, 34.9)	85.4 (1)	type C	the Chitral group
P06	97.5 (2) (97.3, 97.8)	63.9(1)	43.4(1)	73.0(2) (71.0, 75.1)	type X	the Chitral group
P07	97.7 (1)	43.8(2) (30.9, 56.7)	21.0(1)	38.1(2) (39.9, 36.3)	type X	the Chitral group
P08	95.9 (1)	39.3(2) (34.9, 43.8)	14.2(2) (10.1, 18.3)	51.2(2) (50.0, 52.5)	type X	the Chitral group
P09	97.9 (1)	44.0(3) (37.1–50.3)	42.7(3) (37.9–48.7)	54.7(3) (49.3–61.1)	type X	the Baltistan group
P10	93.9 (2) (91.3, 96.6)	33.6(3) (32.9–34.9)	38.1(1)	65.0(1)	type X	the Baltistan group
P11	94.3 (2) (90.2, 98.4)	71.1(2) (69.8, 72.3)	85.9 (2) (82.8, 89.1)	91.2 (1)	type BC	
P12	94.7 (1)	29.6(1)	39.7(1)	62.7(2) (60.2, 65.1)	type X	the Baltistan group
P13	93.2 (2) (39.5, 52.1)	45.8(2)	40.6(1)	81.0 (3) (79.5–83.5)	type C	the Baltistan group
P14	97.9 (2) (97.6–98.3)	31.8(2) (31.8, 31.8)	44.3(2) (41.6, 47.0)	94.7 (3) (86.4–99.1)	type C	(like South Indian landraces)
P15	99.0 (3) (98.6–99.2)	31.0(3) (29.9–32.1)	54.2(3) (51.1–57.2)	91.3 (1)	type C	(like South Indian landraces)
P16	98.2 (1)	48.2(1)	90.1 (1)	92.1 (3) (89.0–94.8)	type BC	

Mean pollen fertility of more than 75% is shown in **bold type** for each cross combination.¹⁾ Based on the classification by Ochiai *et al.* (1994).**Table 3.** Pollen fertility of the F₁ hybrids from supplemental crosses between Pakistani landraces

Cross combination (♀ × ♂)	No. of plants observed	Mean Pollen fertility (%)	Seed ¹⁾ set (%)	Notes
the Chitral group × the Baltistan group				
P07 × P10	2	15.7	0.0	indehiscent anthers
P08 × P19	1	36.8	3.2	indehiscent anthers
P18 × P21	1	88.3	40.8	
the Baltistan group × the Chitral group				
P20 × P17	1	88.9	53.7	

¹⁾ Seed set of a F₁ hybrid was calculated as the percentage of fully ripened grains among spikelets that had flowered.

(1994), two type C strains (P 02 and P 03) belong to the Dir group, another two (P 04 and P 05) to the Chitral group, one (P 13) to the Baltistan group. The remaining two strains (P 14 and P 15) from Punjab Province showed a morphological similarity to South Indian landraces in plant shape and panicle shape through the cul-

tivation in the present study. Therefore, the strains of the Chitral group, which are characterized by extremely short plant height, many tillers with small panicles and shining and elliptic caryopsis, were classified into types C and X. Ochiai *et al.* (1994) also pointed out that the Chitral group shared several common characters with

Afghan strains. Landraces collected from Afghanistan were, however, determined as types A, AC and C by means of hybrid pollen sterility (Kawase and Sakamoto 1987). Two type C strains of the Dir group showed almost normal pollen fertility in the F_1 hybrids when crossed with tester A, which may imply a close relationship with type AC. The same tendency was previously observed in a type C strain from Afghanistan and in a few strains from western Europe (Kawase and Sakamoto 1987). No strain of true type AC was found in Pakistani landraces, even though the type C strains in both the Chitral group and the Dir group may have morphological similarity with Afghani landraces. On the other hand, a strain (P 13) of type C was found in the Baltistan group which had quite different characteristics from the other two groups.

The present results may not make it clear whether P 13 is an exceptional type C strain in Baltistan or type C is commonly distributed in this area since a limited number of landraces from Baltistan was used. Fertile hybrids obtained in two crosses ($P 18 \times P 21$ and $P 20 \times P 17$) between the Chitral group and the Baltistan group, however, suggested that the same type, probably type C, is distributed both in the northern part of N. W. F. P. and Baltistan. Type C was found in the Dir group, the Chitral group and the Baltistan group as well as in landraces collected from Panjab Province. It is likely that various strains of type C were introduced into Pakistan through different pathways rather than morphological differentiation after being introduced.

Six of the Pakistani strains used were classified as type X, which was a higher frequency than expected. It will be necessary to reconsider the world-wide distribution of type X. Kawase and Sakamoto (1987) reported that type X, which always showed hybrid pollen sterility in all the cross combinations with the three testers, were predominantly found in Lan Hsü Island of Taiwan and the Batan Islands of the Philippines and were sporadically distributed in other regions such as China, Southeast Asia, India and Europe. The present results as well as the previous report (Kawase and Sakamoto 1987) suggest that type X might include more than one type, because different kinds of pollen fertility arrays in the three cross combinations are recognized among the strains classified as type X. In other words, a cross between two strains of type X may or may not produce a semi-sterile hybrid.

There is a possibility that type X strains of the Chitral group phylogenetically differ from those of the Baltistan group. The almost completely sterile hybrid having indehiscent anthers in a cross combination ($P 07 \times P 10$) indicates that there are at least two different landrace groups within the type X in Pakistan. It is supported by another almost sterile hybrid from a cross between P 08 (type X, the Chitral group) and P 19 (the

Baltistan group) although the landrace group of P 19 has not yet been determined. Re-classification using a new tester and/or investigation of other genetic characters is necessary within the type X strains to clarify their genetic differences.

One (P 11) strain from Gilgit Agency and another (P 16) from Baluchistan were identified as type BC which had been previously reported only in southern Indian landraces. The former strain produced a F_1 hybrid which was not fully fertile but showed sub-normal pollen fertility when crossed with tester A. This is the first time a strain has been found with such high fertility in both of the cross combinations with testers A and B.

The results presented here on hybrid pollen sterility do not confirm our working hypothesis. Landrace group classification did not correspond with morphological classification and this suggests complicated phylogenetic differentiation in Pakistani landraces. This implies that *S. italica* with various phylogenetic backgrounds might have been introduced through different pathways into this region, and then re-formed into three distinct morphological groups. Further investigation using more strains, another tester(s) and new genetic characters such as DNA polymorphism will be needed to clarify genetic relationships among landraces in Pakistan and neighboring areas.

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