JARQ 30, 61-67 (1996)

Genetic Differentiation of Rice Germplasm Collected in Northern Pakistan

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Abstract

In 1909 and 1991 Japan and Pakistan undertook collaborative explorations in Pakistan. We report on rice cultivation in northern Pakistan and results from evaluation of rice varieties collected. The rice germplasm collected from northern Pakistan reflects the ecogeographic adaptation to this mountainous region and variation in cultural practices. Rice diversity is also related to the elevation. Japonica varieties with round grain are dominate at the elevations above 1,200 m. Indica varieties with slender grains were found below 1,200 m and both types are grown at elevations between 400 and 1,200 m. Landraces collected in the Chitral district are either broadcasted or transplanted, each group showing distinct differences in seed and germination characteristics. Many landraces have disappeared from northern Pakistan and they were replaced with improved varieties during the past decade. Seeds are preserved at the National Genebank of NIAR and PGRI-PARC and available for distribution.

Discipline: Crop production
Additional key words: genetic resources, Oryza sativa, variation

Introduction

Rice (*Oryza sativa*) is one of the most important crops in Pakistan. Annual per capita consumption of milled rice is 16 kg. Rice is a staple food and also used in dishes for special occasions[2]. Fine, aromatic Basmati cultivars are highly appreciated as high quality rice in Pakistan. These varieties are also an important export crop.

Rice covers about two million ha in Pakistan, accounting for approximately 10% of the arable land. Rice cultivation is concentrated in four distinct agro-ecological zones. The first zone consists of northern mountainous areas including the North-West Frontier Province (NWFP). In the NWFP rice is mainly cultivated in cooler, high altitude areas of mountainous valleys. The second zone lies in the broad strip of irrigated land between the Ravi and Chenab Rivers in Punjab Province. The climate is subtropical and suitable for growing fine aromatic varieties such as Basmati. Rice is grown on the west bank of the Indus River and in the Indus delta in the Sind Province, where the subtropical or arid climate is suitable for growing coarse rice varieties[1].

Pakistan is climatically and ecogeographically diverse. Consequently crop germplasm shows a considerable genetic variation. Japanese and Pakistani scientists carried out collaborative explorations in Pakistan in 1989 and 1991 with the financial support from IBPGR (presently IPGRI; International Plant Genetic Resources Institute). Missions focused on surveying and collecting germplasm of rice, grain legumes and millets. The first missions in 1989 surveyed most of the country except for the Sind Province. They found that landraces are most commonly cultivated in the northern mountains[4]. Consequently, the mission in 1991 concentrated on this area. During the two missions, crop germplasm from 57 genera and 15 families including 249 samples of rice were collected[4,5].

The Himalayan and Hindu Kush mountains, which rise to an elevation of more than 6,000 m, dominate the landscape of northern Pakistan. The elevation of the rice-growing villages ranges from 200 m in Punjab to 2,250 m in the Chitral district.

The mountains isolate villages from one valley to another. Northern Pakistan is located at the intersection between West and East Asian countries and the silk road used to pass through the area.

In this report, we describe the diversity of rice varieties cultivated in northern Pakistan based on the explorations organized in 1989 and 1991.

Materials and methods

Two hundred and four rice samples consisting of 300 morphotypes, collected in northern Pakistan during the explorations covering a 2-year period, were evaluated. The samples were collected in NWFP, Baltistan, Gilgit Agency and northern Punjab.

Seed samples were grown and evaluated in the isolation greenhouse of the National Institute of Agrobiological Resources (NIAR), Tsukuba, Japan. Samples collected in 1989 and 1991 were sown on May 15, 1991 and 1992, respectively. They were grown in the greenhouse under natural light conditions. Esterase zymograms were analyzed from leaf samples at the 7th leaf-stage by agar gel electrophoresis. Freshly harvested seeds were used for the measurement of grain size and amylose content of the endosperm. Grain size was measured to the nearest 0.1 mm value for 10 grains per sample and grain shape was examined. Amylose content of single milled grain was determined by the I_2-KI coloration method with three replications per sample.

Fifty five strains out of 204 samples evaluated in 1991 and 1992 were sown on April 26 and transplanted on May 15, 1993. These samples included landraces collected from 28 villages in the Chitral district and its vicinity. Dormancy of these samples was estimated using 50 freshly harvested seeds. They were sown in 9 cm petri dishes with filter paper No.2. Dormancy was evaluated after 7 days of incubation at 30°C in the dark by recording the number of germinated seeds. Grain size and amylose content were analyzed for grains as described above. Phenol reaction was evaluated based on the color of the hull after 12 h of soaking in 1.5% phenol solution. Measurement of seedling traits was undertaken after incubation of the dry seeds at 45°C for 20 days to break dormancy. Seeds were sterilized with 1% sodium hypochlorite solution for 30 min and subsequently rinsed with tap water for 2 h. Twenty seeds per accession were placed in a glass tube 3 cm in diameter with 2 ml water. They were grown in the dark for 9 days at 30°C and mesocotyl and coleoptile length was measured.

Results and discussion

1) Rice varieties growing in northern Pakistan

The distribution of rice cultivation in northern Pakistan is shown in Fig. 1. The list of the varieties

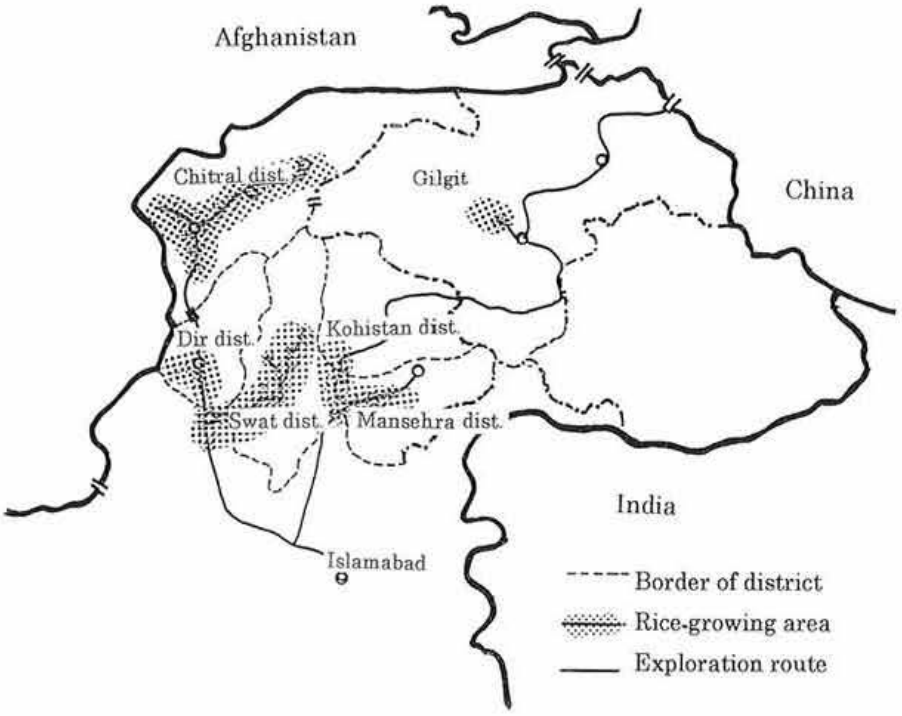


Fig. 1. Rice-growing area in northern Pakistan

Table 1. Rice varieties grown in northern Pakistan

District	Variety name	Range of elevation (m)	Characteristics
Chitral	Nali	1,200–2,035	For transplanting, long awn, pigmented and straw awn types, pigmented and straw hulls, local variety
	Byene, Bayan	1,430–2,250	For broadcasting, local variety
	Basmati	1,200–1,400	Introduced from Peshawar, slender-shaped grain, very low productivity, awned and awnless types
Dir	IRRI-Pak	715–1,300	IRRI line (IR 8), introduced by Dep. of Agric.
	Munjai	880, 900	Round-shaped grain, introduced from Swat valley
	Sela	715	Slender-shaped grain, introduced by Dep. of Agric.
Swat	Begami	760–1,320	Round-shaped grain, introduced by Dep. of Agric.
	Sela	650–780	Slender-shaped grain, introduced by Dep. of Agric.
	Lawangai	1,180	Aromatic, slender-shaped grain, local variety
	Chini	840–1,410	Round-shaped grain
	Motia	1,200–1,410	Round-shaped grain, introduced by Dep. of Agric.
	Garrara	990–1,410	Round-shaped grain, introduced by Dep. of Agric.
	Kashmiri	630–1,170	Round-shaped grain, introduced by Dep. of Agric.
	Japani	630–1,170	Round-shaped grain, introduced by Dep. of Agric.
	Khush-bu-dar	940	Aromatic, slender-shaped grain, local variety
	Niki Dhan	940	Slender-shaped grain, local variety
	Basmati	840	Slender-shaped grain, introduced by Dep. of Agric.
	Johna	940	Introduced by Dep. of Agric.
Gilgit Agency	Bueu, Brue	1,450, 1,640	Round-shaped grain, local variety
Punjab Prov.	Basmati 385	190–240	Improved semi-dwarf variety

grown in the area is summarized in Table 1.

Landraces are still dominant in the Chitral district. Two kinds of landraces, Nali (transplanting) and Byene or Bayan (broadcasting) are adapted to these areas due to their high and stable productivity. The varieties display a round grain and are morphologically similar to japonica varieties. Basmati, which has a slender grain and is aromatic, is grown at lower elevations. Basmati varieties are characterized by a low productivity in most fields. In the Chitral district, rice is cultivated in terraced fields located at elevations ranging from 1,000 to 2,250 m. Rice suffers from the cool climate and also irrigation water is cold since it consists of snowmelt water from the surrounding mountains. During the mission in Chitral, sterility due to cold was frequently observed. As most of the improved varieties are not cold-tolerant, in Chitral farmers still grow landraces.

In the Dir district, rice is grown on either side of the Panjkora River valley. The elevation of rice fields ranges from 800 to 1,500 m. The improved variety, IRRI-Pak (IR 8), was introduced to this area by the Department of Agriculture in the 1980s. Prior to IRRI-Pak, the local variety Shugai was grown in this district. Shugai is a variety with a round-shaped grain and red pericarp. It was collected from a field where it was mixed with IRRI-Pak.

The Swat valley and Malakand region are the largest rice-producing areas in the NWFP. Rice is grown in a broad area along river valleys at elevations ranging from 600 to 1,300 m. The main cultivated varieties are Begami (JP-5) and Sela. Both are improved varieties introduced by the extension service. Local varieties have disappeared gradually because of their low productivity compared with these introduced varieties. One landrace collected called Lawangai is characterized by slender grains and a strong aroma. This variety was found only in a small field and is used for home consumption.

In the Kagan valley, Mansehra district, rice grows in small terraced paddy fields along the Kundar River at elevations below 1,400 m. From Mansehra to Pattan in the Kohistan district, rice is grown along the river. The elevation ranges from 600 to 1,400 m. In this area, improved varieties have been cultivated for the past 10 years. Two landraces were collected there, Niki Dhan and Khush-bu-dar, which are characterized by slender grains. Khush-bu-dar is an aromatic variety.

In a village located close to Gilgit, Bueu was collected. It is a landrace characterized by round grains. The rice-growing area in the valley decreased

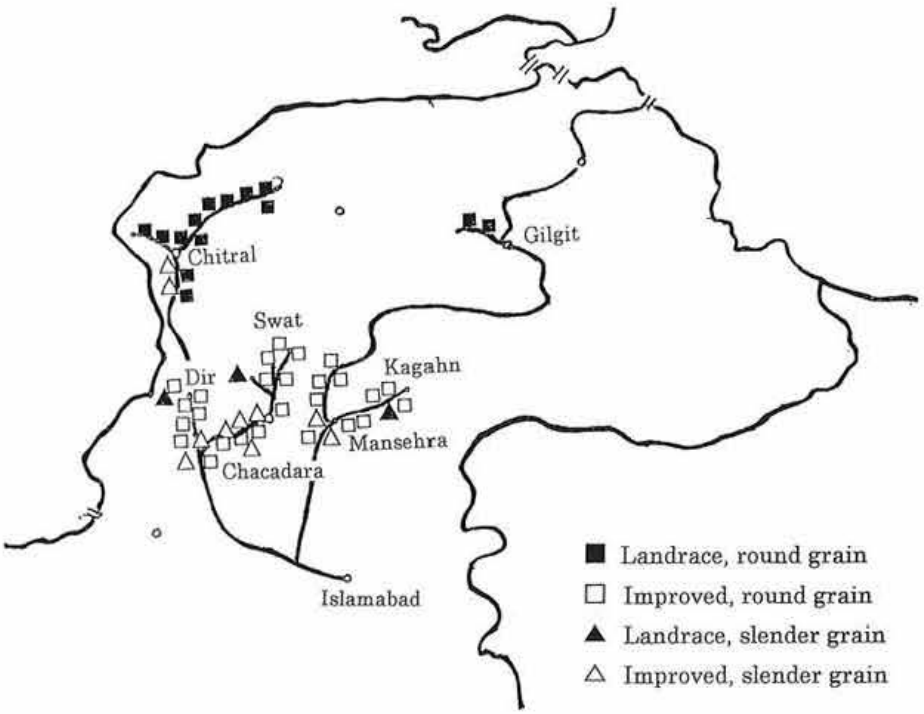


Fig. 2. Distribution of varieties in northern Pakistan

markedly during the past decade in this area. Maize/wheat and rice/wheat crop rotations are practiced in this area. Since the maize/wheat rotation is more productive, rice is gradually being replaced by maize throughout the area. Within a few years rice may not be grown any longer in this area.

The distribution of varieties is shown in Fig. 2. Although most of the landraces have been replaced with improved varieties in much of the area, landraces of different types could be found. Varieties with round grains are grown in the northern area at high elevations while a few landraces collected south at lower elevations have slender grains, suggesting that varietal groups are adapted to different elevations.

2) Variation in principal characteristics

Variation in days to heading ranged from 70 to 141 days and in most of the varieties heading occurred less than 100 days after sowing (Fig. 3). Grain shape, as indicated by the grain length and width ratio, ranged from 1.6 to 4.0 (Fig. 4). Amylose content of endosperm ranged from 15 to 38% (Fig. 5). Varieties with a high amylose content were common

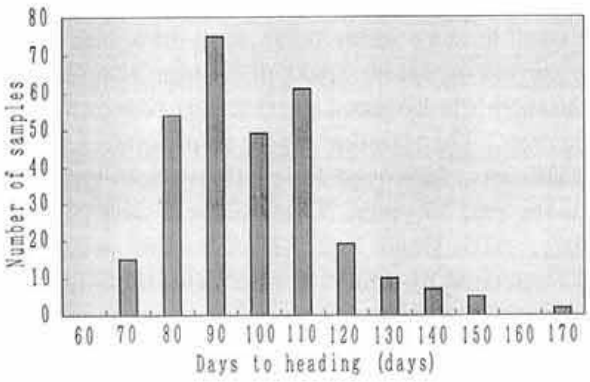


Fig. 3. Histogram of days to heading

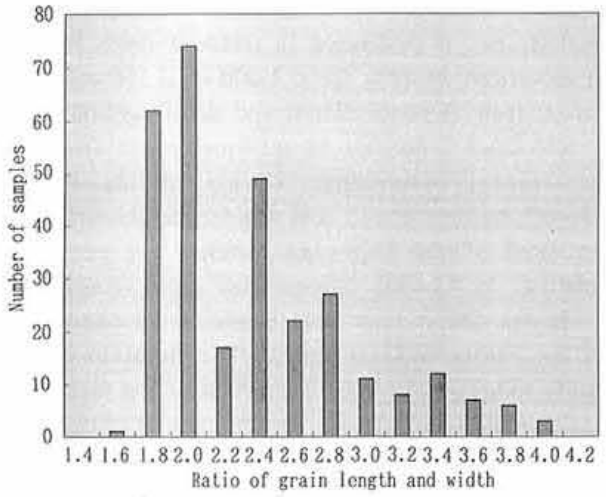


Fig. 4. Histogram showing distribution of grain length/width ratio

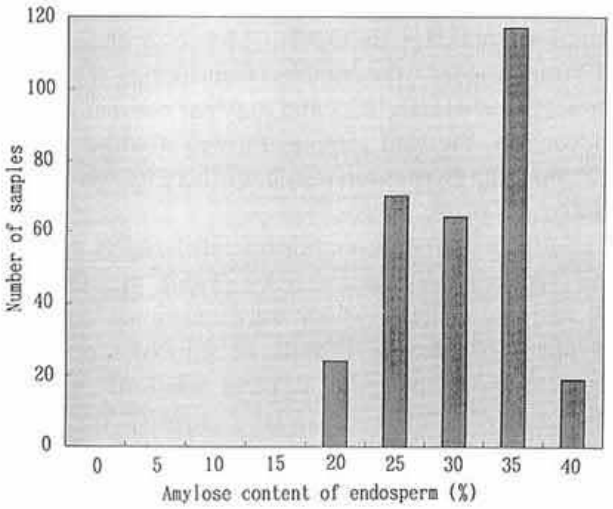


Fig. 5. Histogram of endosperm amylose content

and no glutinous samples were collected.

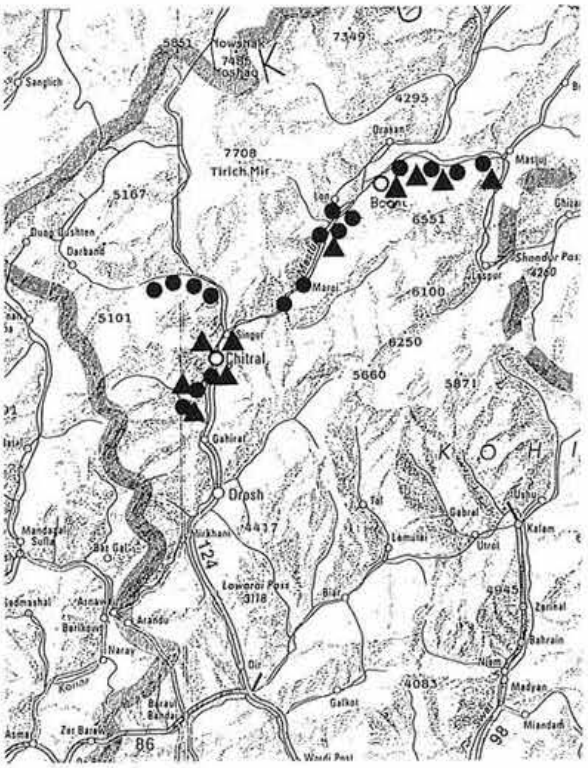
The relationship between these characteristics and the elevation at which the samples were collected was examined (Table 2). At elevations below 400 m, varieties with slender grain and high amylose content were common. At elevations above 1,200 m, grains were usually round and the amylose content was generally lower than 30%. At elevations between 1,200 to 400 m, the varieties showed characteristics of low and high elevation varieties.

The esterase 3 isozymes displayed a slow and a fast band, correlated with rice varietal groups. The fast band of esterase 3 isozyme is common in indica varieties and the slow band is mostly found in japonica varieties[3]. The fast band was observed mainly in varieties from elevations lower than 400 m. The slow and fast bands were found in the varieties collected at an elevation between 400 and 1,200 m. However, only the slow band was observed in varieties collected above 1,200 m.

Variation in the characteristics of the collected rice varieties is related to the elevation at which the varieties are grown. Characteristics of both indica and japonica varieties, such as grain shape and esterase isozyme zymograms were found at an elevation below 1,200 m. However at higher elevations, only japonica varieties were observed. In cold climate at high elevations, growth of indica varieties which are more sensitive to cold than japonica varieties was restricted. Rice varieties in northern Pakistan show a clinal variation in varietal groups as reported in other countries[7].

3) Characterization of landraces in Chitral district

In the Chitral district, the landraces called Nali



● Transplanted variety

▲ Direct-sown variety

Fig. 6. Collection site of landraces in the Chitral district

Table 2. Geographical distribution of collection

Elevation of collection site (m)	Grain length/width[a]			Amylose content (%)[b]			Esterase 3 isozyme genotype	
	~2.0	~3.0	3.1~	<20	20~30	30>	Est_3^S	Est_3^F
~400	0	66	22	0	23	62	24	55
400~800	9	21	3	4	7	22	14	16
800~1,200	32	25	7	12	17	33	20	30
1,200~1,600	31	11	7	2	30	17	27	0
1,600~	64	1	0	1	62	3	18	0

a): Grain length and width of 10 grains.
b): Amylose content of single grain.

and Byene are common at elevations from 1,200 to 2,250 m. According to farmers, these varieties have been cultivated for several hundred years in the Chitral area because of their adaptation to the climatic conditions at high elevations. Nali is a transplanted variety and Byene is directly sown. In the 28 villages we surveyed, 11 farmers grow both varieties. Nali is transplanted into the fields after wheat is harvested. Fallow fields are broadcast with Byene since this variety requires a longer period in the field. Collection sites of these varieties are shown in Fig.6.

At the collection sites, both varieties were very similar morphologically. Culm length was approximately 80 cm and panicle length was about 20 cm, respectively. Spikelets have a long awn which is red or purple at maturity. Grains are round.

Results of morphological characterization are shown in Table 3. Heading date of Nali ranged from 70 to 102 days after sowing. Heading date of Byene ranged from 62 to 75 days. Other characteristics were very similar in both varieties. Culm length, panicle length, grain size, awn frequency and its length were not different statistically. The awn color of Nali was heterogeneous purple, red or straw. The awn color of the Byene samples was straw. Esterase zymogram of both varieties belongs to type 6, which is the most common type in japonica varieties. Amylose content of the endosperm was analyzed in freshly harvested seeds. Amylose content of the Nali varieties ranged from 20.0 to 35.6% and of the Byene samples from 21.9 to 26.9%. Moreover the means of both varieties were similar. Considering the morphological characteristics and amylose content of the endosperm, the Nali samples showed a wider variation than the Byene samples, but otherwise were very similar in plant type.

Nali and Byene showed distinct differences in seed and germination characteristics (Table 4). Of the 44 samples of Nali which were examined for the phenol color reaction of hull, 41 showed a positive reaction and 3 showed a negative reaction. All the 11 samples of Byene showed a negative reaction. Dormancy of seed also was different between the two groups of samples. Nali samples which showed a positive reaction to phenol exhibit dormancy. All the Byene samples and 3 Nali samples were non-dormant and had a negative phenol reaction. The 3 samples with a negative reaction may have been misidentified when collected from farmers' fields. The Nali samples had a longer mesocotyl and shorter coleoptile than the Byene samples.

In addition, disease susceptibility was different between these two varieties. Byene is highly susceptible to leaf blast disease. In rice plants cultivated in Tsukuba, most of the leaves and panicles were infected severely and died. Although Nali was susceptible, the symptoms were less severe than on Byene.

Table 3. Morphological characteristics of local cultivars in the Chitral district

Cultural practice	No. of samples	Days to heading	Culm length (cm)	Panicle length (cm)	Grain shape L/W	Grain size L×W	Awn	Amylose content of endosperm (%)
Transplanting	44	70–102	124[a] (91–143)[b]	22.3 (16.8–29.6)	1.8 (1.8–2.0)	21.2 (18.1–25.5)	Full and long, pigmentation heterogeneous	26.2 (20.0–35.6)
Broadcasting	11	66–75	128 (119–135)	23.4 (14.0–28.0)	1.8 (1.6–2.0)	21.2 (19.5–24.2)	Full and long, no pigmentation	24.4 (21.9–26.9)

a): Means of samples evaluated.
b): Range of samples.

Table 4. Seed and seedling characteristics of local cultivars in Chitral district

	Phenol reaction of hull		Dormancy	Mesocotyl length (mm)	Coleoptile length (mm)
	+	−			
Transplanting	41	3	Dormant	6.4 (0–20)[a]	43.0 (32–52)
Broadcasting	0	11	Non-dormant	3.9 (1–8)	53.3 (50–63)

a): Mesocotyl and coleoptile length was measured on 20 plants of each sample grown in the dark for 9 days at 30°C.
Average of samples and range of mid-value are shown.

These two varieties were clearly distinguished by local farmers as being adapted to different cultural practices. Although the plant type was similar, different seedling characteristics were maintained between these two varieties. Nali and Byene are physiologically distinct varieties which are adapted to different cultural practices.

Conclusion

The rice germplasm from northern Pakistan shows a regional variation that is related to the eco-geographical characteristics of the collection sites. Although landraces have disappeared in most of the area, remaining varieties maintain a considerable level of variation. As for the grain shape, slender to round grain varieties were distributed according to the elevation. Both indica and japonica varieties were growing in the area sampled at lower elevations and japonica varieties were dominant at higher elevations. In the Chitral district, landraces could be differentiated on the basis of seedling characteristics which are related to cultural practices.

Considering the diversity of other crops in northern Pakistan, it was also reported that foxtail millet and common bean showed wide and regional variations. Foxtail millet (*Setaria italica*) could be divided into 3 groups, the Chitral group, the Baltistan group, and the Dir group. Each group was similar to the strains from different regions of Eurasia surrounding northern Pakistan[6]. *Phaseolus vulgaris* in northern Pakistan was analyzed by electrophoresis for total seed protein. All but one of the 7 types identified were found in northern Pakistan. The proportion of these 6 types varied markedly in valleys from east to west[8].

The northern mountainous area of Pakistan lies on the ancient silk road and shares a border with China, Afghanistan, Iran and India. Agriculture in this area has been influenced by surrounding countries. A broad range of varieties was introduced and either became adapted to the area or disappeared. The geographical characteristics of this region prevent easy movement and maintain the diversity of adapted varieties. The diversity of the germplasm collected from this area reflects the regional situation and provides information on crop adaptation.

The original sample seeds are preserved at PGRI-PARC (Plant Genetic Resources Institute, Pakistan Agricultural Research Council), while propagated seeds are preserved at the National Genebank of NIAR and available for distribution.

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(Received for publication, Feb. 22, 1995)