### GENETIC AND GENOMIC Resources for grain cereals Improvement

EDITED BY Mohar Singh and Hari D. Upadhyaya



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### Proso, barnyard, little, and kodo millets



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### 8.1 Introduction

Proso, barnyard, little, and kodo millets belong to the group called small millets, sometimes also referred to as minor millets. Proso millet (*Panicum miliaceum* L.) is commonly known as broomcorn millet, common millet, hog millet, Russian millet, and so on, in different parts of the world. Barnyard millet is generally well-known as Japanese barnyard millet (*Echinochloa crus-galli* (L.) P. Beauv.), Indian barnyard millet (*Echinochloa colona* (L.) Link), cockspur grass, Korean native millet, prickly millet, sawa millet, watergrass, and so on. Kodo millet (*Paspalum scrobiculatum* L.) is also known by different names in different languages in India (kodo in Hindi, khoddi in Urdu, arugu in Telugu, varagu in Tamil), African bastard millet grass, arika, haraka, ditch millet in New Zealand, and mandal in Pakistan. Similarly, little millet (*Panicum sumatrense* Roth. ex. Roem. & Schult.) is also commonly known as samai, gindi, mutaki, kutki, and so on, in different Indian languages.

These crops are cultivated in the marginal areas, and are adapted to a wide range of growing environments. Proso millet is currently grown in Asia, Australia, North America, Europe, and Africa (Rajput et al., 2014), and used for feeding birds and as livestock feed in the developed countries and for food in some parts of Asia. Barnyard millet is mainly grown in India, China, Japan, and Korea for human consumption as well as fodder (Upadhyaya et al., 2014). Kodo and little millets are largely cultivated throughout India by tribal people in small areas. All these crops have superior nutritional properties including high micronutrients, dietary fiber content, and low glycemic index (GI) with potential health prospective (Chandel et al., 2014; Dwivedi et al., 2012; Saleh et al., 2013). Research evidences support that the low-GI carbohydrate diets help in the prevention of obesity, diabetes, and cardiovascular disease (Brand-Miller et al., 2009). Proso, barnyard, kodo, and little millets together with finger and foxtail millets, are used as an ingredient in multigrain and gluten-free cereal products and serve as a major food component for various traditional foods and beverages, such as bread, porridges, and snack foods, while grains are feed to animals, including pigs, fowls, and cage birds.

These crops are under-researched and underutilized compared to foxtail and finger millets and other cereals, and are being neglected in terms of support for production, promotion, research, and development. More research efforts on proso, barnyard, little, and kodo millets are required for developing high-yielding varieties and to diversify food habits for healthy lives and to face the global threats of malnutrition and climate change. In this chapter, we mainly focus on four small millets, that is, proso, little, barnyard, and kodo millets and provide an overview of their origin, history, domestication, and diversity; the status germplasm collections conserved in genebanks worldwide and at ICRISAT; germplasm evaluation for agronomic and nutritional traits, and for biotic and abiotic stresses; way to enhance the use of germplasm through core collection approach; and genomic resources and their use for germplasm characterization, and genomic research in these crops.

### 8.2 Origin, distribution, taxonomy, and diversity

Proso millet is an annual herbaceous plant in the genera Panicum, and it has a chromosome number of 2n = 36 with basic chromosome number of x = 9. Vavilov (1926) suggested China as the center of diversity for proso millet, while Harlan (1975) opined that proso millet probably was domesticated in China and Europe. The earliest records come from the Yellow River valley site of Cishan, China dated between 10,300 cal years BP and 8,700 cal years BP (Lu et al., 2009). Evidence of proso millet also occurs at a number of pre-7000 cal years BP sites in Eastern Europe, in the form of charred grains and grain impressions in pottery (Hunt et al., 2008; Zohary and Hopf, 2000). These two centers of earlier records suggest independent domestication of proso millet in eastern Europe or Central Asia, or may have also originated from domestication within China and then spread westward across the Eurasian steppe (Hunt et al., 2011; Jones, 2004). Most recently, Hunt et al. (2014) used nuclear and chloroplast DNA sequences from proso millet and a range of diploid and tetraploid relatives to unveil the phylogenies of the diploid and tetraploid species, and suggested the allotetraploid origin of P. miliaceum, with the maternal ancestor being Panicum capillare (or a close relative) and the other genome being shared with Panicum repens; however, further studies of the Panicum species, particularly from the Old World are required. Cultivated proso millet can be divided into five races: miliaceum, patentissimum, contractum, compactum, and ovatum (de Wet, 1986). Race miliaceum resembles wild P. miliaceum in inflorescence morphology, characterized by large, open inflorescences with suberect branches that are sparingly subdivided. Race patentissimum is characterized by slender and diffused panicle branches, which is often difficult to distinguish from race miliaceum. Cultivars with more or less compact inflorescences are classified into races contractum, compactum, and ovatum. Cultivars in race contractum have compact, drooping inflorescences while the race compactum have cylindrical inflorescences that are essentially erect, whereas the cultivars with compact and slightly curved inflorescences that are ovate in shape belong to race ovatum (de Wet, 1986).

Little millet belongs to the genus *Panicum* having a chromosome number of 2n = 36, with basic chromosome number of x = 9. It was domesticated in India (de Wet et al., 1983a), particularly in the Eastern Ghats of India, where it forms an important part of tribal agriculture. Little millet is grown across India, Sri Lanka, Nepal, and western Burma. The species *Panicum sumatrense* is divided into subsp. *sumatrense* (cultivated little millet), and subsp. *psilopodium* (wild progenitor). These two subspecies cross where they are sympatric to produce fertile hybrids, derivatives of which are often weed in little millet field (de Wet et al., 1983a). *P. sumatrense* subsp. *sumatrense* has two races, *nana* and *robusta*, and two subraces each, *laxa* and *erecta* in *nana*, and *laxa* and *compacta* in *robusta*. The race *nana* includes plants with decumbent to almost prostrate culms that become erect at the time of flowering. Inflorescences are large, open with the upper branches sometimes clumped and curved at the time of maturity. The *robusta* includes erect plants with large, strongly branched, open, or compact inflorescences (de Wet et al., 1983a).

The genus *Echinochloa* comprises of approximately 25 species and two species, namely, E. crus-galli and E. colona are cultivated as cereals. E. crus-galli is native to temperate Eurasia and was domesticated in Japan around 4000 years ago, while E. colona is widely distributed in the tropics and subtropics of the Old World, and was domesticated in India. Both the cultivated species are hexaploids (2n = 54) and are morphologically related, but hybrid between these two species is sterile. In general, cultivated plants of E. colona are erect or geniculate ascending, often tufted, annual and can grow up to 242 cm tall and awnless spikelets with membranaceous glumes, while plants of E. crus-galli are erect, tufted, annual, grow up to 100 cm tall, and awned spikelets with chartaceous glumes (de Wet et al., 1983b). The species E. crus-galli is classified into two subspecies (crus-galli and utilis) and four races (crus-galli and macrocarpa in subsp. crus-galli, and utilis and intermedia in subsp. utilis). Similarity, E. colona has two subspecies, colona and frumentacea. The subsp. colona has no races and subsp. frumentacea is divided into four races: stolonifera, intermedia, robusta, and laxa (de Wet et al., 1983b). More recently, Wallace et al. (2015) investigated the patterns of population structure and phylogeny among the accessions belonging to two species, E. crus-galli and E. colona, through genotyping-by-sequencing (GBS) approach suggested distinct phylogenetic structure within and between two species, four subpopulations within E. colona accessions, and three such clusters within E. crus-galli.

Kodo millet belongs to the genus *Paspalum*, a diverse genus comprising about 400 species, most of which are native to the tropical and subtropical regions of the Americas, and the main center of origin and diversity of the genus is considered to be South American tropics and subtropics (Chase, 1929). The chromosome number of the kodo millet is reported to be 2n = 4x = 40 (Hiremath and Dandin, 1975). Kodo millet was domesticated in India around 3000 years ago and cultivated by tribal people in small areas throughout India, from Kerala and Tamil Nadu in the south, to Rajasthan, Uttar Pradesh, and West Bengal in the North. It occurs in moist or shady places across the tropics and subtropics of the Old World (de Wet et al., 1983c). Kodo millet has three races, namely, *regularis, irregularis*, and *variabilis*. The most common race is *regularis*, characterized by racemes with the spikelets arranged in two rows on one side of

a flattened rachis. In the case of the race *irregularis*, the spikelets are arranged along the rachis in two to four irregular rows; whereas in the race *variabilis*, the lower part of each raceme is characterized by irregularly arranged spikelets, while spikelets arrangement becomes more regularly two-rowed in the upper part of the raceme (de Wet et al., 1983c).

### 8.3 Erosion of genetic diversity from the traditional areas

Genetic variation found in traditional landraces and wild species is important for continued progress of crop improvement. Genetic erosion refers to the loss of genetic diversity, sometimes used in a narrow sense, that is, the loss of genes or alleles, as well as more broadly, referring to the loss of varieties, and crop species, mainly because of the replacement of traditional landraces by modern, high-yielding cultivars, natural devastations, and large-scale destruction and modification of natural habitats sheltering wild species. Proso, barnyard, little, and kodo millets continue to be grown largely by the traditional practices using traditional landraces under subsistence farming. Area under cultivation of these crops is decreasing around the world mainly due to increasing importance of a few selected crop species causing genetic erosion of small millets and narrowing the food security basket. In China, proso millet continued to be a very important crop until the beginning of the twentieth century, but recently there has been significant reduction in its cultivation due to the adoption of modern high-yielding varieties of major crops like rice, wheat, and maize. However, it is still produced because of their adaptation to areas that are too dry or too cold for other crops (Bonjean, 2010). Drastic decline in cultivated area (5.34 million ha during 1955-1956 to 0.80 million ha during 2011-2012), and production (2.07 Mt during 1955 to 0.46 Mt during 2011-2012) under six small millets (finger, foxtail, proso, little, barnyard, and kodo millets) was noticed in India (NAAS, 2013). About 72% reduction in barnyard millet area was reported from 11 villages in Garhwal Himalayas (Maikhuri et al., 2001). Decline in cultivation of small millets is mainly due to low productivity, nonavailability of high-yielding varieties, lack of production and processing technologies, and introduction of high-yielding commercial crops.

### 8.4 Status of germplasm resource conservation

*Ex situ* conservation is the widely used method to conserve millet genetic resources. Globally >29,000 accessions of proso millet, >8,000 accessions each of barnyard and kodo millets, and >3,000 accessions of little millet have been assembled and conserved (Fig. 8.1). The major genebanks conserving proso, barnyard, kodo, and little millets are presented in Table 8.1. The major collections of proso millet germplasm accessions are assembled in the Russian Federation, China, Ukraine, and India; barnyard millet in Japan and India; kodo millet in India and USA; and little millet in India.



Figure 8.1 Global status of small millet germplasm maintained in different genebanks.

### 8.5 Germplasm evaluation and maintenance

Proso, barnyard, kodo, and little millets are highly self-pollinating crops, so there is no special regeneration and maintenance practice as in the case of cross-pollinated crops like pearl millet. The field used for regeneration should not have grown the same crops in the previous year in order to avoid volunteer plants. Individual accessions can be planted in rows (4 m length) and harvested panicles by hand will be bulked to make up the accession. The ICRISAT Genebank at Patancheru, India conserves 849 accessions of proso millet, 749 accessions of barnyard millet, 665 accessions of kodo millet, and 473 accessions of little millet under medium- (4°C and 30% relative humidity) and long-term ( $-20^{\circ}$ C in vacuum-packed standard aluminum foil pouches) storage conditions.

Limited works on germplasm evaluation for various agronomic and nutritional traits, biotic and abiotic stress tolerance have been reported in these crops. Few studies on evaluation and identification of important traits of economic interest are discussed here.

		Germplasm accessions		
Crop/country	Institute	Cultivated	Wild	Total
Proso millet		` 		
Australia	Australian Tropical Crops and For- ages Genetic Resources Centre (ATCFC)	228		228
Bangladesh	Plant Genetic Resources Centre, BARI (PGRC, BARI)	198		198
Bulgaria	Institute for Plant Genetic Resources "K. Malkov" (IPGR)	489		489
China	Institute of Crop Science, Chinese Academy of Agricultural Sciences (ICS-CAAS)	8451		8451
Czech Republic	Genebank Department, Division of Ge- netics and Plant Breeding, Research Institute of Crop Production (RICP)	171		171
Germany	Genebank, Leibniz Institute of Plant Ge- netics and Crop Plant Research (IPK)	165	1	166
Hungary	Institute for Agrobotany (RCA)	243	1	244
India	AICRP on Small Millets (AICRP-Small Millets)	920		920
	International Crop Research Insti- tute for the Semi-Arid Tropics (ICRISAT)	849		849
	National Bureau of Plant Genetic Resources (NBPGR)	994	4	998
Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences (NIAS)	516		516
Mexico	Estación de Iguala, Instituto Nacio- nal de Investigaciones Agrícolas (INIA-Iguala)	400		400
Poland	Botanical Garden of Plant Breed- ing and Acclimatization Institute (BYDG)	354		354
	Plant Breeding and Acclimatization Institute (IHAR)	359		359
Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR)	8778		8778
Ukraine	Institute of Plant Production n.a. V.Y. Yurjev of UAAS (IR)	1046		1046
	Ustymivka Experimental Station of Plant Production (UDS)	3975	1	3976

### Table 8.1 Major genebanks conserving germplasms of proso,barnyard, kodo, and little millets worldwide\*

		Germplasm accessions		sions
Crop/country	Institute	Cultivated	Wild	Total
USA	North Central Regional Plant In- troduction Station, USDA-ARS, NCRPIS (NC7)	717	4	721
Barnyard mille	t			
Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences	3603	68	3671
India	National Bureau of Plant Genetic Resources	1668	9	1677
	AICRP on Small Millets	868		868
	International Crop Research Institute for the Semi-Arid Tropics	749		749
	The Ramiah Gene Bank, Tamil Nadu Agricultural University, India	232		232
China	Institute of Crop Science, Chinese Academy of Agricultural Sciences	717		717
Kenya	Kenya National Genebank of Kenya, Crop Plant Genetic Resources Centre - Muguga		16	208
Kodo millet				
Argentina	Banco Activo de Germoplasma de		127	127
	Papa, Forrajeras y Girasol Silvestre Instituto de Botánica del Nordeste, Universidad Nacional de Nordeste, Consejo Nacional de Investigaciones Científicas y Técnicas	390		390
Australia	Australian Tropical Crops and For- ages Genetic Resources Centre	54	159	213
Brazil Colombia	Embrapa Pecuária Sudeste (CPPSE) Centro Internacional de Agricultura Tropical (CIAT)	327	155	327 155
Ethiopia	International Livestock Research Institute (ILRI)	3	205	208
India	AICRP on Small Millets, Bangalore International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)	1111 665		1111 665
	National Bureau of Plant Genetic Resources (NBPGR), New Delhi	2170	10	2180
Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences (NIAS)	158		158

### Table 8.1 Major genebanks conserving germplasms of proso,barnyard, kodo, and little millets worldwide\* (cont.)

(Continued)

		Germplasm accessions		sions
Crop/country	Institute	Cultivated	Wild	Total
Kenya	National Genebank of Kenya, Crop Plant	130		130
	Genetic Resources Centre - Muguga			
New Zealand	Margot Forde Forage Germplasm	281		281
	Centre, Agriculture Research			
	Institute Ltd			
Nigeria	National Centre for Genetic Resourc-	294		294
	es and Biotechnology (NACGRAB)			
USA	Plant Genetic Resources Conserva-	1074	249	1323
	tion Unit, Southern Regional Plant			
	Introduction Station, University of			
	Georgia, USDA-ARS			
Uruguay	Facultad de Agronomía	106	446	552
Little millet				
India	AICRP on Small Millets (AICRP-	928		928
	Small Millets)			
	International Crop Research Institute for	473		473
	the Semi-Arid Tropics (ICRISAT)			
	National Bureau of Plant Genetic	1253		1253
	Resources (NBPGR)			
	Regional Station Akola, NBPGR	165		165
	(NBPGR)			
	The Ramiah Gene Bank, Tamil Nadu	108		108
	Agricultural University, India			
USA	North Central Regional Plant In-	226		226
	troduction Station, USDA-ARS,			
	NCRPIS (NC7)			

### Table 8.1 Major genebanks conserving germplasms of proso, barnyard, kodo, and little millets worldwide\* (cont.)

\* Institutes/genebanks with >100 accessions are enlisted.

Source: http://www.fao.org/wiews-archive/germplasm\_query.htm.

### 8.5.1 Germplasm evaluation

#### 8.5.1.1 Agronomic traits

Proso, little, barnyard, and kodo millets germplasm accessions conserved at the ICRI-SAT Genebank show substantial variation for important agronomic traits (Table 8.2). In proso millet, days to 50% flowering ranges from 26 days to 50 days, plant height from 20 cm to 133 cm, basal tiller number from 1 to 32, and inflorescence length from 22 mm to 400 mm (Upadhyaya et al., 2011). The characterization of proso millet germplasm conserved at the ICRISAT collection revealed that most of the early flowering accessions are from Syria and late flowering accessions are from India; dwarf plant height accessions are from Mexico and tall plant height accessions are

	Mean		Range			
Trait	Entire	Core	Entire	Core		
Proso millet	Proso millet					
Days to 50% flowering	34.5	34.9	26–50	28-50		
Plant height (cm)	59.4	61.4	20-133	25-133		
Basal tillers number	4.0	4.1	1–32	1–32		
Flag leaf blade length (mm)	222.7	219.3	80-380	85-380		
Flag leaf blade width (mm)	19.5	18.8	6–30	8–30		
Flag leaf sheath length (mm)	82.1	80.6	30-170	30-170		
Peduncle length (mm)	181.3	179.7	15-400	15-400		
Panicle exsertion (mm)	100.1	102.8	0-320	0-320		
Inflorescence length (mm)	193.1	193.8	22-400	22-400		
No. of nodes	11.4	11.0	2–90	2–90		
Inflorescence primary branches	16.1	15.8	5–29	5–29		
number						
Barnyard millet						
Days to 50% flowering	48.8	49.6	30.9-77.2	33.2-73.2		
Plant height (cm)	93.2	95.8	44.5-196.5	57.4-196.5		
Basal tillers number	7.1	7.0	3.9-20.1	4.2-10.9		
Culm thickness (mm)	5.5	5.5	4.7–7.2	4.9-7.2		
No. of leaves	6.0	6.1	5.4-7.2	5.4-6.9		
Flag leaf blade length (mm)	205.6	207.6	102.8-311.3	127.8-287.9		
Flag leaf blade width (mm)	19.6	19.9	7.4-32.0	11.3-32.0		
Flag leaf sheath length (mm)	88.1	88.9	59.4-156.5	66.9–156.5		
Peduncle length (mm)	144.6	144.4	69.2-277.4	75.4-277.4		
Panicle exsertion (mm)	56.5	55.9	29.8-80.6	33.9-77.7		
Inflorescence length (mm)	155.5	159.2	81.0-257.8	102.7-240.8		
No. of racemes per	26.4	26.7	21.9-30.4	22.5-29.8		
inflorescence						
No. of nodes on primary axis of	10.1	10.2	8.7-12.0	9.4–11.3		
inflorescence						
Length of lowest raceme (mm)	30.0	30.1	25.6-38.5	25.6–38.5		
Little millet						
Days to 50% flowering	65.0	67.3	30.9-139.1	35.0-139.1		
Plant height (cm)	112.7	115.3	58.3-201.7	60.6-201.7		
Basal tillers number	13.0	13.1	10.4–16.9	11.5-16.2		
Culm thickness (mm)	6.0	6.0	5.1-7.1	5.3-6.9		
Flag leaf blade length (mm)	241.0	245.5	175.2-322.9	191.7-322.9		
Flag leaf blade width (mm)	32.1	32.3	22.6-41.3	23.9-41.3		
Flag leaf sheath length (mm)	98.5	99.1	81.5-121.3	88.1-114.4		
Peduncle length (mm)	159.8	159.8	153.3-166.9	154.6-166.7		
Panicle exsertion (mm)	21.2	21.0	6.9-48.2	9.9-48.2		
Inflorescence length (mm)	273.4	275.1	198.5–347.6	218.2-330.7		

### Table 8.2 Diversity in entire and core collections of barnyard, kodo, little, and proso millets conserved at ICRISAT, Patancheru, India

(Continued)

	Mean		Range	
Trait	Entire	Core	Entire	Core
No. of nodes on primary axis of inflorescence	12.7	12.7	11.7–15.0	11.7–14.0
No. of secondary inflorescence branches	21.9	21.9	17.7–30.0	18.6–30.0
Seed length (mm)	2.3	2.3	2.1-2.4	2.1-2.4
Seed width (mm)	1.6	1.6	1.5-1.8	1.5-1.8
Kodo millet				
Days to 50% flowering	78.7	77.7	56.2-117.4	60.0–110.2
Plant height (cm)	54.6	54.5	44.1-69.3	44.3-63.3
Basal tillers number	15.3	15.2	6.5-30.4	9.2-29.9
No. of leaves	5.7	5.7	5.4-6.7	5.5-6.7
Flag leaf blade length (mm)	191.7	191.9	156.1-226.5	156.1-226.5
Flag leaf blade width (mm)	7.2	7.2	5.9-8.4	5.9-8.4
Flag leaf sheath length (mm)	144.8	144.5	137.7–151.6	137.7-149.7
Inflorescence length (mm)	64.1	64.0	55.4-75.7	57.3-75.7
Sterile primary axis length (mm)	108.2	108.0	96.0-123.2	96.0-122.0
No. of racemes above thumb	3.0	3.0	2.7-3.9	2.7-3.9
Thumb length (mm)	56.5	56.5	50.3-66.0	52.4-63.7
Longest raceme length (mm)	28.6	28.6	25.7-32.4	27.3-32.3

'	Table 8.2	Diversity	y in entire	and core	collecti	ons of ba	arnyard,	kodo,
	little, a	nd proso`	millets cou	nserved a	t ICRIS	SAT, Pat	ancheru,	India
(	(cont.)	-				,		

Sources: Upadhyaya et al. (2011, 2014).

from Sri Lanka. Accessions with good exsertion are mostly from Australia and China, and shorter panicle exsertion accessions are from the former USSR, while the longest panicle types are from Nepal (Reddy et al., 2007). Similarly, larger variation of germplasm conserved at ICRISAT for various agronomic traits in barnyard millet (days to flowering from 30.9 days to 77.2 days, plant height from 44.5 cm to 196.5 cm, basal tillers number from 3.9 to 20.1, inflorescence length from 81 mm to 257.8 mm, etc.), little millet (days to flowering from 30.9 days to 139.1 days, plant height from 58.3 cm to 201.7 cm, basal tillers number from 10.4 to 16.9, inflorescence length from 198.5 mm to 347.6 mm, seed length from 2.1 mm to 2.4 mm, etc.), and kodo millet (days to flowering range from 56.2 days to 117.4 days, plant height from 44.1 cm to 69.3 cm, inflorescence length from 55.4 mm to 75.7 mm, etc.) were found (Table 8.2).

Proso millet germplasm accessions conserved at the National Centre for Crop Germplasm Conservation, Beijing, China were evaluated for their agronomic potential, disease resistance, and nutritional content, and elite accessions for specific or multiple traits were identified (Wang et al., 2007). Joshi et al. (2014) reported large variability in the kodo millet landraces collected from seven kodo millet growing districts of Madhya Pradesh, India for agronomic traits and grouped the landraces on

the basis of plant height (dwarf, semidwarf, and tall), days to flowering and maturity (early, medium, and late), basal tillers number (low, medium, and high), degree of culm branching, inflorescence length, number of racemes above thumb, length of longest raceme, grain yield per plant, and 1000 grain weight. They reported promising kodo millet genotypes, dwarf types (RPS# 521, 529, 541, 683, 733, 801, and 926), for extra early maturity (RPS# 540, 541, 546, 632, 681, 687, 696, and 700), higher grain yield (RPS# 503, 556, 639, 649, 710, 712, 769, 775, 780, 798, 859, 910, 967, and 977), and higher 1000 grain weight (RPS# 507, 540, 556, 612, 614, 620, 638, 639, 642, 648, 650, 700, 705, 708, 709, 910, 912). Choi et al. (1991) evaluated barnyard millet at Suwon, South Korea, from 1985 to 1990, revealing that barnyard millet was found to be superior to that of other species as a fodder crop (on par with maize) and identified lines IEc 514 and IEc 515 from ICRISAT for high grain and green fodder yields. Gupta et al. (2009a) collected barnyard millet germplasm throughout the Himalayan region mainly from the hill state of Uttarakhand, India and promising donors for plant height (<120 and >200 cm), productive tillers (>4), inflorescence length (>28 cm), raceme number (>50) and raceme length (>3.1 cm), and grain yield (>16 g) were identified.

#### 8.5.1.2 Nutritional traits

In general, small millet grains are the storehouses of many nutrients, phytochemicals, and nonnutritive plant protective functional constituents (Rao et al., 2011; Saleh et al., 2013). Proso, barnyard, kodo, and little millets have the higher amount of protein, crude fiber, minerals, and vitamins as compared to other cereals like rice and wheat. Particularly, proso millet is rich in protein content (12.5%), while barnyard millet is rich in protein (11%), crude fiber (13.6%), and Fe (18.6 mg per 100 g edible portion) (Saleh et al., 2013). It clearly signifies the importance of these crops in terms of nutritional and health perspective. Incorporation of these crops in the daily routine food habits may help in diversifying the food security basket. However, except for proso millet, there are very few or no studies on assessing the extent of genetic variability for grain nutritional traits involving a large number of germplasm in barnyard, kodo, and little millets. In the case of proso millet, Wang et al. (2007) evaluated 6515 germplasm from 14 provinces of China for grain protein and fat content and reported germplasm with high protein (>15%) and fat (>4%).

#### 8.5.1.3 Biotic stress

Proso, barnyard, little, and kodo millets are said to be less affected by pests and diseases; however, there are a few pests and diseases that cause substantial reduction in grain yield of these crops. Limited number of resistant sources for major diseases and to some extent for pests in proso, barnyard, little, and kodo millets have been reported however, and large-scale exploitation of germplasm resources has not been done in these crops.

In proso millet, very few diseases have been reported and the major diseases are head smut, sheath blight, bacterial spot, and so on. Screening 18 proso millet genotypes for sheath blight under artificial inoculation conditions revealed that none of the genotypes were free from sheath blight; however, resistant (<20% disease severity) and moderately resistant (20-30% disease severity) genotypes were reported (Jain and Tiwari, 2013). Breeding of proso millet for resistance to head smut and melanosis (blackening of the grain under the husk, caused mainly by Pseudomonas syringae and Xanthomonas vasicola pv. holcicola) have been reported (Konstantinov and Grigorashchenko, 1986, 1987; Maslenkova and Resh, 1990; Konstantinov et al., 1989). Economically useful mutants with high yield, large grain, good grain quality, and resistance to smut and melanosis were isolated using chemical mutagens such as N-methyl-N-nitrosourea, dimethyl sulfate and Nethyl-N-nitrosourea at the Ukrainian Institute of Plant Production, Breeding and Genetics (Konstantinov et al., 1989). The smut-resistant mutants, like Mutant 5, Mutant 6, 83-10170, and 83-10146, were used in hybridization and the mutant variety Khar'kovskoe 57 with high yield and good quality was released in Ukraine and Dagestan (Konstantinov et al., 1989). Soldatov and Agafonov (1980) tested 300 varieties of proso millet for resistance to melanosis at the Ural'sk Agricultural Experiment Station, Kazakh Soviet Socialist Republic (KSSR), of which 12 varieties were found fairly resistant. Very limited studies on insect, pest screening have been done in proso millet. Shailaja et al. (2009) screened different prerelease and released varieties against infestation of rice moth (Corcyra cephalonica), and identified TNAU 151 as comparatively resistant. Promising proso millet germplasm accessions and varieties relatively resistant to shoot fly have been reported in India (Murthi and Harinarayana, 1986) (Table 8.3).

Barnyard millet is mostly affected by smut (*Ustilago panici-frumentacei*) and leaf spot (*Colletotrichum graminicola*). Grain smut can cause 6.5–60.8% yield loss. Gupta et al. (2009b) screened 257 accessions of barnyard millet, which includes advanced breeding lines for grain smut tolerance and grouped accessions based on reaction against smut infection and identified highly resistance accessions. Screening of barnyard millet genotypes against resistance to diseases led to the identification of resistant/moderately resistant sources for grain smut, head smut, *Helminthosporium* leaf spot or blight, and banded leaf and sheath blight (BLSB) (Table 8.3). Promising germplasm accessions and varieties of barnyard millet relatively resistant to shoot fly have been reported in India (Murthi and Harinarayana, 1986) (Table 8.3).

Head smut and blight are the major diseases in kodo millet. Many researchers have evaluated germplasm/cultivars and identified resistant sources for important diseases like smut and sheath blight (Table 8.3). Shoot fly is the major pest that causes considerable yield loss of up to 40% (Patel and Rawat, 1982). Screening kodo millet genotypes for shoot fly resistance has led to the identification of highly resistant landraces (Table 8.3). Joshi et al. (2014) reported multiple resistant accessions, namely, RPS# 575, 583, 590, 830, 886, 898, and 910 for head smut, sheath blight, and shoot fly.

Little millet is mainly affected by grain smut and sheath blight, and donors for resistance sources have been identified (Table 8.3). Little millet production is quite often affected by pests like shoot fly resulting in heavy loss to the crop. Morphologic characters are found to be associated with resistance to shoot fly in little millet. Tolerant genotypes showed higher trichome length and density, which offer mechanical

Crop/biotic		
stress	<b>Resistant/tolerant sources</b>	References
Proso millet		
Smut	K8763, Saratovskoye 2, Saratovs- koye 3, Saratovskoye 6, Veselepo- dolyankoye 632, Barnaulskoye 80, Gorlinka, "II"Inovskoe' Kh86, MS1316, Orenburgskoe 9, Khar'kovskoe 86	Ilyin et al. (1993); Konstantinov et al. (1986); Krasavin and Usmanova (1988); Konstantinov et al. (1991); Sharma et al. (1993); Zolotukhin et al. (1998)
Banded leaf and sheath blight	TNAU 137, GPUP 22 and RAUM 8	Jain and Tiwari (2013)
Melanosis	K8789, K8773, K8790, K8740 and K7606, UNIIZ670, Solnech- noe and Krasnoe Toidenskoe 215 (KT215), Orenburgskoe 9, Khar'kovskoe 57	Krasavin and Usmanova (1988); Konstantinov et al. (1989); Konstanti- nov et al. (1991)
Shoot fly	GPMS # 101, 102, 105, 108, 112, 114, 115, 117, 122, 123, 124, 125, 126, 135, 136, 138, 148, 152, 153, 155, 156, 157, 159, 164, RAUm# 1, 2, 3, MS # 1307, 1316, 1437, 1595, 4872, PM 29-1, BR 6, Co 1	Murthi and Harinarayana (1986)
Rice moth	TNAU 151	Shailaja et al. (2009)
Fall army warm	PI 176653	Wilson and Courteau (1984)
Barnyard millet		
Grain smut	PRB 402, S 841, TNAU# 92, 141, 155, VL# 216, 219, PRB# 901, 903, Co 1	Kumar and Kumar (2009); Kumar (2013); Muthusamy (1981);
Head smut ABM 4-1, K 1, RAU 8, RBM 7-1, TNAU# 82, 86, 92, 96, 99, 101, 116, 128, 130, PRB# 401, 402, VL# 29, 172, 202, 205, 207, 208, 215, 216, 219, 220, 221, 222		Kumar and Kumar (2009); Kumar (2012)
Leaf spot or blight	TNAU # 116, 130, VL# 221, 222, 172, 29	Kumar (2012)
172, 29           Brown spot         ABM 4-1, K 1, PRB# 401, 402, S           841, TNAU 82, 86, 92, 96, 99, 101, 116, 128, VL# 29, 172, 202, 205, 207, 208, 209, 216, 215, 220, RAU# 8, 12, RBM# 7, 7-1, VMBC 248		Kumar and Kumar (2009)

# Table 8.3 Germplasm/cultivars identified as resistance/tolerance sources for various biotic stresses in proso, barnyard, kodo, and little millets

(Continued)

Crop/biotic	Desistant/talayant sources	Deferences
stress	Resistant/toierant sources	Kelerences
Banded leaf and sheath blight (BLSB)	TNAU# 128, 130, VL# 29, 220, RBM 12	Jain and Gupta (2010)
Shoot fly	GECH# 102, 106, 108, 111, 120, 123, 127, 142, 149, 151, 157, 180, 205, 210, 218, 224, 226, 227, 230, 235, 240, 241, 246, 247, 248, 250, 260, 276, 288, VL# 8, 13, 21, 24, 30, 31, 32, ECC # 19, 18, 20, 21, RAU 7, KE 16, K1, PUNE 2386, Bhageshwar Local 2	Murthi and Harinarayana (1986)
Kodo millet		
Head smut	RPS# 539, 575, 581, 583, 590, 804, 818, 820, 830, 859, 886, 898, 910, 977, JK 13, GPLM# 78, 96, 176, 322, 364, 621, 641, 679 720, Acc. no 64, 348, 424	Jain (2005), Joshi et al. (2014); Jain et al. (2013)
Sheath blight	RPS# 502, 503, 508, 510, 516, 529, 531, 535, 543, 548, 550, 556, 566, 575, 577, 579, 585, 593, 607,609, 621, 629, 634, 646, 649, 661, 662, 689, 691, 694, 695, 708, 739, 753, 755, 787, 789, 814, 830, 867, 881, 883, 918, 919, 923, 929, 956, 961	Joshi et al. (2014)
Shoot fly	RPS# 515, 583, 612,628,642, 685, 763, 806, 810, 811, 822, 823, 834, 842, 846, 871, 872, 901, 902, 904, 905, 909, 910, 914, 915, 917, 918, 921, 925, 927, 929, 930, 933, 934, 938, 939, 941, 943, 944, 945, 946, 948, 951, 953, 967, 968, 970, 974, GPUK 3, JK13, GPLM # 6, 11, 20, 21, 29, 32, 39, 42, 45, 50, 60, 106, 110, 113, 117, 119, 120, 121, 131, 142, 155, 158, 160, 170, 172, 173, 178, 180, 185, RPS# 40-1, 40-2, 62-3, 61-1, 69-2, 72-2, 75-1, 102-2, 107-1, 114-1, 120-11, IQS 147-1, Co 2, Keharpur	Joshi et al. (2014); Murthi and Harinarayana (1986)

# Table 8.3 Germplasm/cultivars identified as resistance/tolerance sources for various biotic stresses in proso, barnyard, kodo, and little millets (cont.)

Crop/biotic		
stress	<b>Resistant/tolerant sources</b>	References
Little millet		
Grain smut	IPmr 841, 1061	http://www.dhan.org/ smallmillets/docs/ report/1_Advances_in_ Crop_Improvement_of_ Small_Millets.pdf
Head smut	GPMR# 65, 82, 67, 105, 70, 73, 80, 83, 92; OLM 36, 40, 203, TNAU# 89, 98, RLM# 13, 14, VMLC# 281, 296, Varisukdhara	Jain (2003); Jain and Tripa- thi (2007)
Shoot fly	GPMR# 164, 274, 236, 243, 110, 213, 584, 66, 683, 569, 189, 241, 98, 163, 324, 670, 598, 192, 96, 583, 161, 596, 95, 190, GPMR # 7, 17, 18, 20, 22, 26, 46, 53, 78, 84, 92, 98, 101, 104, 106, 107, 112, 114, 115, 116, 117, 124, 132, 134, 136, 141, 148, 149, 163, 169, 170, 171, 172, 175; PRC # 2, 3, 7, 8, 9, 10, 11, 12, RPM# 1-1, 8-1, 12-1, 41-1, RAU# 1, 2, K1, Co 2, Dindori 2-1	Gowda et al. (1996a); Murthi and Harinarayana (1986)

# Table 8.3 Germplasm/cultivars identified as resistance/tolerance sources for various biotic stresses in proso, barnyard, kodo, and little millets (cont.)

obstruction to young larvae in reaching their feeding sites (Gowda et al., 1996b). Field evaluation of little millet germplasm accessions for shoot fly resistance led to identification of highly resistant accessions (Table 8.3).

### 8.5.1.4 Abiotic stress

Proso, little, barnyard, and kodo millets are also affected by abiotic stresses, though they are generally considered well-adapted to abiotic stresses as compared to most other cereals (Dwivedi et al., 2012). Barnyard millet is reported to be tolerant to drought and waterlogging (Zegada-Lizarazu and Iijima, 2005), while proso millet is susceptible to drought (Seghatoleslami et al., 2008). Lodging is a constraint in many crops, including proso, little, barnyard, and kodo millets, causing substantial losses in grain yield and quality. Use of lodging-resistant cultivars along with good crop husbandry is the most effective way to minimize losses due to lodging. Proso millet lines developed in the United States have had strong selection for lodging resistance (Baltensperger et al., 1995a, 1995b, 2004). Sources for salinity tolerance have been reported (Acc. No. 008211, 008214, and 008226) (Sabir et al., 2011) in proso millet. Heavy metal tolerance (copper and zinc) at seedling stage was found highest in kodo millet followed by proso millet (Arora and Katewa, 1999).

### 8.6 Use of germplasm in crop improvement

The large size of germplasm particularly in the case of low research priority crops like proso, barnyard, little, and kodo millets reduce use of germplasm in breeding programs due to extremely low funding for research and development as compared to other crops. Developing representative subset of the entire collection of the species is a more economical and efficient way of utilizing germplasm to screen and identify the potential genetic resources for various economically important traits. At ICRISAT, Upadhyaya et al. (2011, 2014) developed core collections in proso, barnyard, little, and kodo millets, which captured genetic variation of the entire collections. These core collections could be effectively evaluated for agronomic and grain quality traits, and for biotic and abiotic stress tolerances to enhance utilization of germplasm in these crops. ICRISAT has distributed a total of >15,000 germplasm accessions of proso (6,047), barnyard (3,932), kodo (2,582), and little millet (2,449) to 25-37 countries (Table 8.4). This includes two and six sets of core collections of proso and barnyard millets, respectively. The most efficient use of germplasm conserved is using them directly as varieties. In barnyard millet, PRJ 1 was released in India during 2003, which is a selection from ICRISAT germplasm accession IEc 542 that originated in Japan.

### 8.6.1 Development of core collection

A core collection consists of a limited set of accessions (about 10%) derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection. Core collection helps to capture the entire diversity to utilize in breeding programs. Core collections have been formed in proso (Upadhyaya et al., 2011), barnyard (Gowda et al., 2009; Upadhyaya et al., 2014), little (Gowda et al., 2008; Upadhyaya et al., 2014), little (Gowda et al., 2008; Upadhyaya et al., 2014), and kodo millets (Upadhyaya et al., 2014) (Table 8.5). At ICRISAT, the entire germplasm collection of proso millets (833 accessions) was stratified into five groups based on races and data on 20 morphoagronomic traits were used for clustering following Ward's method. About 10% (or at least one accession)

India (updated on Dec. 2014)						
Total number Germplasm samples distributed to						
	of accessions			Other		
Crop	(countries)	ICRISAT	India	countries	Total	

Table 8.4 Proso, barnyard, little, and kodo millet germplasm accessions distributed from ICRISAT Genebank, Patancheru, India (updated on Dec. 2014)

849 (30)\* Proso millet 216 3,421 2,410 (37) 6,047 Barnyard millet 749 (9) 2.483 3,932 568 881 (28) Kodo millet 665 (2) 382 1,317 883 (25) 2,582 Little millet 473 (5) 184 1,877 388 (27) 2,449 2,736 1,350 9.098 4.562 15,010 Total

\* Number of countries.

Сгор	Accessions used	Traits assessed	Accessions in core collection	References
Proso millet	833	20	106	Upadhyaya et al. (2011)
Barnyard millet	729	24	50	Gowda et al. (2009)
	736	21	89	Upadhyaya et al. (2014)
Little millet	895	21	55	Gowda et al. (2008)
	460	20	56	Upadhyaya et al. (2014)
Kodo millet	656	20	75	Upadhyaya et al. (2014)

Table 8.5 Core collections in proso, barnyard, kodo, and little millets

was randomly selected from each of 101 clusters to constitute a core collection of 106 accessions (Upadhyaya et al., 2011). Similarly, Upadhyaya et al. (2014) formed core collections in barnyard (89 accessions), little (56 accessions), and kodo millets (75 accessions), representing 11–12% of the entire collection of these crops conserved at ICRISAT Genebank (Table 8.5). These core collections are thus ideal genetic resources for identifying new sources of variation for use in crop improvement and for genomic studies.

### 8.7 Limitations in germplasm use

A significant number of germplasm accessions have been conserved in proso, barnyard, little, and kodo millets (Table 8.1); however, precise characterization and evaluation of these conserved genetic resources for traits of economic importance is very limited. Large holdings in genebanks and nonavailability of precise evaluation data on traits of economic importance limit the use of germplasm in these crops as well like in major crops (Upadhyaya et al., 2009). Forming subsets like core and mini core collections are the best entry point to search a genetic variability of agronomic and nutritional traits, biotic and abiotic stresses tolerance, and their use in breeding programs.

### 8.8 Germplasm enhancement through wide crosses

Floral morphology and anthesis behavior of proso, barnyard, little, and kodo millets make them very difficult crops for hybridization. However, emasculation and crossing techniques have been suggested in proso millet (Nelson, 1984) and other small grass florets (Richardson, 1958). To-date there is little or no effort made on interspecific hybridization in proso, barnyard, kodo, and little millets. In little millet, Hiremath et al. (1990) made interspecific hybridization between *P. sumatrense* (cultivated) with

*Panicum psilopodium* (Wild) and crossability of 23–25% was reported. Hybrids resembled female parent *P. sumatrense* with regard to nonshattering spikelets, intermediate between both the parents with respect to several quantitative characters like height, thickness of stem, leaf width, and spikelet number. Hybrids were highly fertile with 84% seed set and regular bivalent formation in the hybrids strongly suggests that the genomes of *P. sumatrense* and *P. psilopodium* are basically similar and are fully homologous (Hiremath et al., 1990). In the case of barnyard millet, Sood et al. (2014) reported interspecific hybridization between the two cultivated species of barnyard millet, PRJ 1 (*Echinocloa esculenta*) and ER 72 (*Echinocloa frumentacea*). The hybrid of the cross was vigorous with more tillers, high culm branching, and was free from grain smut disease but failed to set seed due to sterility. These studies open up vast avenues for introgression of desirable traits and exploitation of genetic variability for broadening the genetic base of the cultivars.

### 8.9 Integration of genomic and genetic resources in crop improvement

The foremost challenge for the molecular characterization of proso, barnyard, little, and kodo millets is the availability of very limited genomic resources like DNA markers, lack of genetic/linkage maps, and genome sequences. However, genomic resources of closely related species like foxtail millet where two reference genome sequences are available (Bennetzen et al., 2012; Zhang et al., 2012) can be utilized toward enriching genomic resources in these crops. DNA markers, such as simple sequence repeat (SSR), expressed sequence tag-simple sequence repeat (EST-SSR), ILP (intron length polymorphic), and microRNA-based molecular markers developed using foxtail millet genome sequence information showed >85% of cross-genera transferability among millets including proso, barnyard, little, and kodo millets, as well as nonmillet species (Kumari et al., 2013; Muthamilarasan et al., 2014; Pandey et al., 2013; Yadav et al., 2014). Using the genomic data of switchgrass, Rajput et al. (2014) developed SSR markers for proso millet, which showed that 62% of the switchgrass SSR markers were transferable to proso millet.

Developments in sequencing technologies have made it possible to analyze large amounts of germplasm against low production cost. It enables to screen genebank collections more efficiently for DNA sequence variation, which will be useful for mining sequence variation associated with economically important traits through genomewide association studies (GWAS). Most recently, Wallace et al. (2015) genotyped the barnyard millet core collection (Upadhyaya et al., 2014) using GBS approach (Elshire et al. (2011) and identified several thousand single-nucleotide polymorphisms (SNPs), and studied the patterns of population structure and phylogenetic relationships among the accessions. The procedure used to identify SNPs following GBS approach in barnyard millet can also be applied easily and rapidly to characterize germplasm collections of other crops as well (Wallace et al., 2015). The GBS approach can play a major role in the crop species like proso, barnyard, little, and kodo millets for which genome sequences are not available.

### 8.10 Conclusions

Proso, barnyard, little, and kodo millets are nutritious, grown under marginal lands of arid and semiarid regions. Globally, significant numbers of germplasm accessions of these crops are being conserved in genebanks, and reported to have substantial variation for economically important traits. Very limited reports on trait donors for various agronomic and nutritional traits, and biotic and abiotic stress tolerance traits have been reported, and germplasm subsets like core collections in these crops are available for exploitation in crop improvement. Limited availability of genomic resources in proso, barnyard, little, and kodo millets is the major challenge in these crops; however, this could be overcome through use of genomic resources available in taxonomically closest species and high-throughput genotyping technologies. However, these crops continue to be of low priority with limited funding for research and development. Assessing genetic variability of germplasm, use of genetic and genomic resources for breeding high-yielding cultivars, developing crop production and processing technologies, value addition for improving consumption, public-private partnerships, and policy recommendations are needed to upscale these crops as remunerative to farmers.

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