Domestication and dispersal of *Panicum miliaceum* L. (Poaceae) in Eurasia

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Common millet (*Panicum miliaceum* L.) was the most important grain crop in Eurasian civilization for several thousand years starting from the Neolithic era. It is still cultivated and has various uses around the world. Although it is one of the oldest domesticated plants in Eurasia, the ancestral plant and place of origin have yet to be definitively determined. A series of studies was started based on the plant's morphological and ecological characteristics, followed by studies of its genetic characteristics and secondary compounds, to elucidate its domestication process and dispersal in Eurasia. Accessions (650 local varieties obtained from local famers) and herbarium specimens collected by field surveys were used for observations and experiments on morphological and ecological characteristics, crossability, amplified fragment length polymorphism of total DNA, phenol and iodine color reactions of seeds, fatty acid component in seeds, traditional food styles, and archeolinguistic data. The botanical origin, domestication process, and geographical dispersal of common millet are discussed and then integrated through the characteristics mentioned above. In conclusion, common millet was domesticated from a wild population of *P. miliaceum* subsp. *ruderale* in Central Asia, specifically from the Aral Sea to the Southwest Tien Shan Mountains. Since the Neolithic era, the millet has been dispersed eastward to China, westward to Europe, southward to the Indian subcontinent, and northward to Siberia by nomadic groups.

Key words: common millet, dispersal, domestication, Eurasia, local varieties, original place

Introduction

The genus *Panicum* (Poaceae) consists of about 500 species. These species have been used for wild grains, fodder, and medicine up to the present day, while three cereals, *P. miliaceum* L. (common millet), *P. sumatrense* Roth. (*samai*, little millet), and *P. sonorum* Beal. (*saui*, panic grass), were domesticated in different places and times. These are C_4 plants endowed with strong drought resistance, early maturation, and high nutrient content. *P. sumatrense* was domesticated from an ancestral species, *P. sumatrense* subsp. *psilopodium*, after around 2200 BC on the Indian subcontinent (Weber 1992), while *P. sonorum* was domesticated from *P. hirticaule* around 600 BC in northwestern Mexico (Nabhan and de Wet 1984). On the other hand, common millet was the most important grain crop that supported civilization around Eurasia over several thousand years starting in the Neolithic era. It is still cultivated and has various uses around the world. Although it is one of the oldest domesticated plants in Eurasia, the ancestral plant and place of origin have yet to be definitively determined. The place of origin of common millet has been discussed for many years (e.g., Bellwood 2005, Church 1886, de Candolle 1886, Gerard 1597, Harlan 1995, Jones 2004, Kimata 2009, Sakamoto 1987, Vavilov 1926). Vavilov (1926) proposed that the original place was North China, and Chun et al. (2004) suggested that common millet was domesticated in the southern part of the middle reaches of the Yellow River 8000–7000 years BP.

Harlan (1975) suggested that the two homelands were North China and eastern Europe. Nesbitt (2005) also suggested that it might have been domesticated independently in each area. Although both the wild ancestor and the place of domestication of common millet are unknown, it first appeared as a crop in both Transcaucasia and China about 6000 BC. Zohary and Hopf (2000) suggested that common millet may have originated somewhere between the Caspian Sea and Xinjiang. The earliest sites bearing remains of common millet are in China and Europe from the seventh millennium BC, on opposite sides of the Eurasian continent (Jonse 2004). Furthermore, Sakamoto (1987) indicated that the area was located within Central Asia and the northwestern parts of the Indian subcontinent. Common millet was found from the sixth millennium BC at Tepe Gaz Tavilla in southeastern Iran (Meadow 1986). One explanation for its domestication could be an altered climate earlier in the Holocene. Meadow (1986) suggested that it might have been grown using floodwater runoff to supplement rainfall in this dry area as a spring or autumn crop. Similarly, farmers grow common millet in the Ganga area after winter floods. Detailed local analyses of this kind should underpin future considerations of common millet's origin in Central Asia to determine its possible routes of spread through this critical but underexplored area (Hunt and Jones 2006). However, the ancestor and original place of common millet have yet to be clearly determined.

Bellwood (2005) summarized recent thinking on the origin and spread of common millet based on recent archaeological contributions as follows. Common millet perhaps originated in Central Asia (Sakamoto 1987, Zohary and Hopf 2000). Neolithic settlers may have migrated from there to Afghanistan, the Russian steppes, or even western China. Common millet has been reported widely in the Neolithic cultures of Europe and the Eurasian steppes, but it would appear that the oldest known dates of cultivation are from North China from about 6500 BC onward. The first known occurrence in southeastern Iran was in the sixth millennium BC (Meadow 1986), c. 1550 BC in northwestern Iran (Nesbitt and Summers 1988), and about 2600 BC in South Asia (Fuller et al. 2001).

A series of studies was started on common millet's morphological and ecological characteristics, followed by studies of the secondary compounds in the grain (Kimata and Negishi 2002, Kimata et al. 2007). The present paper is concerned with the ancestor, domestication, and geographical dispersal of common millet in Eurasia. The purpose is to examine these questions using all botanical characteristics, including biocultural diversity (traditional food styles and archeolinguistic data) and genetic characteristics (crossability, F_1 hybrids and AFLP markers).

Materials and Methods

Many endemic varieties and relatives of *Panicum miliaceum* L. have been collected from all of Japan and the Eurasian continent through field surveys since 1973. Grain samples (650 accessions) were collected along the survey route and the voucher herbarium specimens were deposited at Tokyo Gakugei University (Tokyo, Japan). Information on agricultural practices, grain processing, food preparation, and vernacular names was gathered from local farmers.

Some of these accessions, 441 local varieties, were selected and grown at the greenhouse of Tokyo Gakugei University, Japan to compare their morphological and ecological characteristics starting on July 10, 1986. These local varieties included 132 from Japan, 39 from eastern Asia, 78 from the former USSR, 90 from southern Asia, 26 from western Asia, 43 from Europe, two from Africa, and one from Canada (Kimata and Negishi 2002).

Ten grains of each strain were sown in a seeding box with a row spacing of 8 cm and seed spacing of 2 cm. Two weeks after sowing, germinated plants were transplanted into the greenhouse, with 30-cm row spacing and 15 cm between plants. Chemical fertilizer (N:P:K = 8:8:5) was supplied at 100 g/m^2. Five plants of each strain were measured for traits, including the duration to flowering (days), number of leaves on the main culm, number of productive tillers, hairiness of the uppermost internode, panicle type, lemma color, pistil stigma color, and others. These morphological and ecological data were analyzed statistically using partial

Table 1. Materials used of *Panicum miliaceum* and the relative species

Area collected	Sample no.	Total
Japan	p1, p2, p30, p37, p38, p39, p60	7
Korea	p3, p4, p23,	3
China	p5, p14, p15, p19, p29, p51	6
Mongolia	p18, p20,	2
Nepal	p13, p16, p52	3
Bangladesh	p50	1
Uzbekistan	p68, p69, p70	3
Afghanistan	p6, p7,	2
India	p53, p54, p55, p56, p57, p61; (*P. sumatrense*) pm2, pw1, pw68	9
Pakistan	p58, p59,pp62, p63, p64, p65, p66, p67	8
Turkey	p17, p33, p91 (weed)	3
Greece	p36,	1
Romania	p9, p10, p24, p31, p32, p34, p35	7
Czechoslovakia	p21	1
Yugoslavia	p40	1
USRR-E	p41, p43, p46, p49	4
USSR-CA	p42, p45, p48	3
Poland	p44	1
Bulgaria	p22	1
Germany	p25, p26, p27, p28,	4
Belgium	p8	1
France	p11	1
Spain	p12	1
Canada	p47	1
USA	(*P. sonorum*) p111	1
Total		75

correlation coefficients and hierarchical cluster analysis in SPSS (ver. 21, IBM Corp).

Moreover, 70 local varieties, including six pollen testers, were selected and grown in the greenhouse from 1990 to 1995. These accessions included 21 from eastern Asia, 8 from Central Asia, 19 from southern Asia, 21 from Europe, and one weed, *P. miliaceum* subsp. *ruderale*, from Romania. The crossability among the 70 Eurasian varieties and the morphological characteristics of their F_1 hybrids were examined in the six pollen testers from France, Central Asia, India, China, Japan, and a weed.

Ten grains of each of 75 accessions were sown by the same method as above on Oct. 4, 2007 (Table 1). DNA extraction was performed on young leaf tissue ground in liquid nitrogen and incubated in 1.5-ml tubes containing 0.5 ml of buffer A for 10 min at 60 °C by using CTAB (hexadecyl-trimethyl-ammonium bromide) methods (Murray and Thompson 1980). The AFLP procedure was performed according to Applied Biosystems (2005), Bai et al. (1999), and Suyama (2001) with some modifications. Amplification reactions were performed according to the same protocol. Five primers associated with *Eco*RI (E+AAC, E+AAG, E+AGG, E+ACT, and E+ACA) were used in combination with 5 primers associated with *Mse*I (M+CAG, M+CTG, M+CTA, M+CAT, and M+CAA). Five microliters of amplification products were loaded onto a 5.75% denaturing polyacrylamide gel (LONZA) and electrophoresed in 1× TBE for 1 h. Bands were detected using the silver staining protocol described by Cho et al. (1996). The bands were detected on the gel at the finest level of sensitivity by Lane Analyzer (ATTO), the raw data were adjusted, and then the visible and reproducible bands were scored for accessions as present (1) or absent (0). The dendrogram of the AFLP markers was constructed using the neighbor-joining and UPGMA methods (Nei and Kumar 2000) with the bootstrap

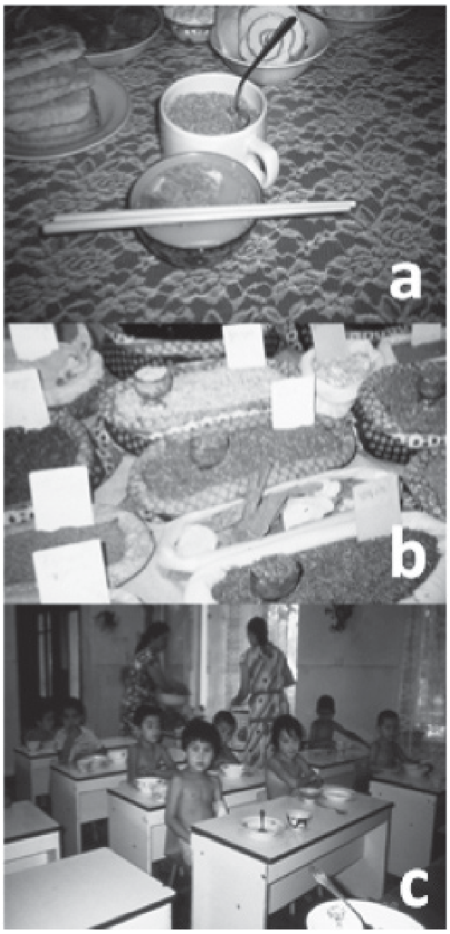

Fig. 1. Foods from common millet in Uzbekistan and Inner Mongolia. a, milk tea with roasted grains in Inner Mongolia; b, colored grains for a topping of bread in Uzbekistan; c, milk porridge for healthful lunch at a nursery school in Uzbekistan.

Table 2. Foods made from common millet around Eurasia

Locality	glutinous/non-glutinous	grain				coarse-ground flour	ground flour			drinks	
		boiled	steamed	porridge	*mochi*	porridge	dumpling	gruel	bread	non-alcohol	alcohol
Japan	non-glutinous	○		○			○	○			
	glutinous		○		○		○				○
Korea	non-glutinous	○									
	glutinous		○		○						○
China	non-glutinous	○		○					○		○
	glutinous		○		○				○		○
Taiwan	non-glutinous	○									
	glutinous		○		○		○				○
Bataan Isles	non-glutinous					○					
Halmahera	non-glutinous					○					
India	non-glutinous	○				○		○	○		
Pakistan	non-glutinous	○							○		
Afghanistan	non-glutinous					○	○		○		
Uzbekistan	non-glutinous					○			○		
Kazakhstan	non-glutinous					○					
Caucasia	non-glutinous					○				○	
Turkey	non-glutinous					○					
Ukraine	non-glutinous					○				○	
Bulgaria	non-glutinous					○				○	
Romania	non-glutinous					○			○		
Germany	non-glutinous					○					
Belgium	non-glutinous					○					
Italy	non-glutinous					○					

analysis (PAUP* ver. 4.0) and the hierarchical cluster analysis (group average method, SPSS ver. 21) on all data matrices of 75 local varieties.

Results

Food preparation and secondary compounds in grain

The Eurasian foods made from common millet are classified into four processing methods: grain, coarse-ground flour, fine flour, and drinks. Asian people cook boiled grain and porridge from the polished grains of non-glutinous varieties (Table 2). Especially, East Asians cook steamed grain and *mochi* (a kind of cake) from the polished grains of glutinous varieties and ferment alcoholic drinks from polished grains of both non-glutinous and glutinous varieties. Inner Mongolians drink daily milk tea with roasted grains (Fig. 1a). Uzbeks top *non* (a kind of bread) with colored grains (Fig. 1b) and cook milk porridge from non-glutinous varieties for lunch at a nursery school (Fig. 1c). Europeans cook milk porridge from coarse-ground flour, bread from fine flour, and ferment non-alcoholic drinks from polished grains of only non-glutinous varieties. Based on the endosperm starch in seed grains, the varieties were divided into two glutinous or non-glutinous categories. The distribution of glutinous varieties of common millet and *Setaria italica* were restricted to eastern Asia. On the contrary, the geographical distribution of phenol color reaction to seed coats in *S. italica* was very similar to that of *Oryza sativa*, but the distribution in common millet was different from the trends in *S. italica* and *O. sativa* (Sakamoto 1982, Kawase and Sakamoto 1982, Kimata and Negishi 2002).

The four types of local varieties of common millet were categorized by the composition of the minor fatty acids arachidic, behenic, and eicosapentaenoic acid. If the ancestral prototype was the weedy AE type containing arachidic and eicosapentaenoic acids, the AB type (arachidic and behenic acid) may have been bred both in Europe and Asia, while the ABE (all three fatty acids) and O (no fatty acids) types may have originated around Central Asia and then spread to both Europe and Asia (Kimata et al. 2007).

Vernacular names

The linguistic data are as follows (Table 3). Prefixes for the word for "common millet" were mainly "*ki-,*" "*che-,*" "*va-,*" or "*ba-*" in East and South Asia, but several variations were noted in China. The prefixes were widely

Table 3. Vernacular names of common millet around Eurasia

Region	Country	Modern name	Ancient name
East Asia			
	China	chi, huangmi, nianmi, shu, shuzi,	shu
	Inner Mongolia	horeibata	
	Korea	kijan	
	Japan	inakibi, kibi, kokibi	kimi, shipshi-kepp
Central Asia			
	Kazakhstan	psheno	
	Afghanistan	arzan	
South Asia			
	Pakistan		
	North	bau, cheena, chiena, olean, onu	
	South	tzetze	
	India		
	North	charai, cheena, chin, china, sawan, worga	unoo, vreelib-heda, vreehib-heda
	South	baragu, cheena, , katacuny, panibaragu, tane, variga, varagu, wari	
	Nepal	china	
	Sri Lanka	mene'ri	
West Asia			
	Arabia	dokhn, kosaeib, kosjaeib	
	Turkey	dari, kundari	
	Israel	dokhan	
Africa			
	Egypt	dokhn	
Europe			
	Greece		kegchros
	Hungary	ko″les	
	Russia	proso	
	Poland	proso	
	Croatia	proso	
	Lithuania		sora
	Netherlands	gierst	
	Germany	rispen hirse	
	Italy	milium	miglio
	Spain	mijo commun	
	France	millet commun	
	United Kingdom	common millet	mill

cf. Kawase 1991, Sakamoto 1986, and many dictionaries.

diverse in Central Asia and the mountainous area of Pakistan. It was mainly "*d-*" in western Asia and Egypt. There were also many European prefixes, including "*mi-*" and "*proso*." Because the vernacular names of common millet were remarkably diverse around all Eurasia, this indicates that the crop was domesticated and/or broadly dispersed starting in a very ancient period. However, common millet is called "*cheena,*" "*chiena,*" or "*chin*" in the Indian subcontinent. Based on the Farming/Language Dispersal Hypothesis (Bellwood and Renfrew 2002), these vernacular names might be derived from China and "Qin" (an ancient Chinese Empire), indicating that common millet was dispersed from China to the Indian subcontinent through a route via Nepal.

Taxonomy

Fig. 2 shows typical panicle types such as sparse (a1), compact (a2), and dense (a3). It also shows a domestic type (b1) and an escaped weed (b2) in Pakistan; a domestic type (b3) and a weed, ssp. *ruderale* (b4), in Romania; a domestic type (b5) and a weed, subsp. *agricolum* (b6), in Uzbekistan; a crop-like weedy biotype, subsp. *miliaceum* (c1); and an F_1 hybrid between a domestic type and the subsp. *ruderale* in Pakistan (c2), with both sparse and shattering panicles. It also shows a weed, subsp. *ruderale*, in Inner Mongolia (d, taken in 2004) and European common millet (e1 and

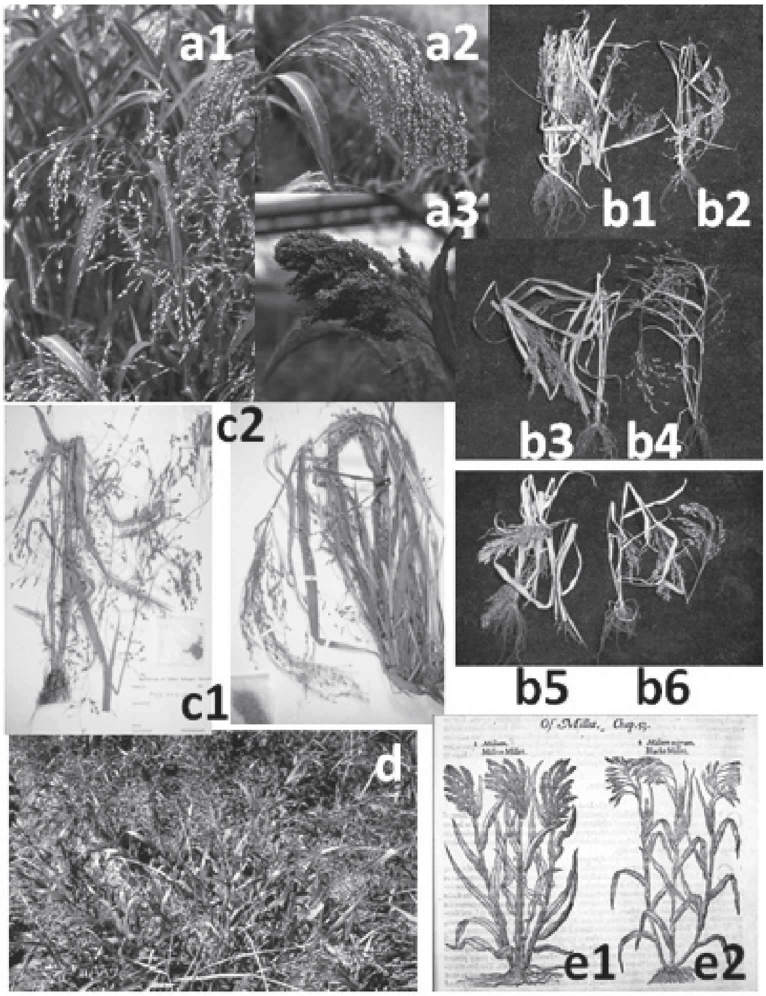

Fig. 2. Morphology of common millet, *Panicum miliaceum*. Types of panicle: a1, sparse; a2, compact; a3, dense. Domestic type and mimic weed in Central Asia: b1, b3 and b5, subsp. *miliaceum*; b2, an escaped weed; b4 and d, subsp. *ruderale*; and b6, subsp. *agricolum*. c1, a crop-like weedy biotype in Pakistan and c2, a F_1 hybrid between subsp. *miliaceum* and subsp. *ruderale*. e1 and e2, European common millet in 17th century (Gerarde 1597).

e2) illustrated in a book (Gerarde 1597). The panicles of common millet can be divided into five types: sparse, compact, dense, and two intermediate types (relatively sparse or dense). Common millet is generally a densely piliferous plant, but the hairiness of the uppermost internode is very variable. This trait can be divided into four types: glabrous, sparsely, moderately and densely piliferous.

Lyssov (1968, 1975) classified *P. miliaceum* L. into five groups based on the panicle types as follows. The panicle of race *miliaceum* was similar to that of a wild species. *Race patentissimum* had long, slender, and sparse panicles (a1), but it was very difficult to divide these two races, which were distributed from Eastern Europe to Japan. *Race contractum* had a droopy, compact panicle (a2). Race compactum had a cylindrical, erect panicle. *Race ovatum* had an oval, dense panicle (a3). Because these morphological characteristics did not clearly show a geographical cline, this classification was not indicative of their taxonomical characteristics. The taxonomy of common millet needs to identify intra-specific differentiation through a matrix of various characteristics. Scholz and Mikoláš (1991) classified *P. miliaceum* into three subspecies: *miliaceum*, *ruderale*, and *agricolum*. Subsp. *miliaceum* consisted of the cultivar form (b1, b3, b5) and crop-like weedy biotype (c1) in Pakistan, and also in Austria, Slovakia, and Canada, respectively. Subsp. *ruderale* (b4, d) was an

Table 4. Partial correlation coefficients of 14 characteristics

	Days for flowering	No. of tillers	Plant height	No. of leaves on main culm	Length of flag leaf	Width of flag leaf	FL/FW	Panicle length	Diameter of uppermost internode	Panicle type	Lemma color	Stigma color	Hairiness of uppermost internode	Shattering
DF	1.000	0.005	0.835**	0.916**	0.501**	0.503**	-0.032	-0.400**	0.569**	0.363**	-0.055	0.131	0.078	0.027
TN	0.005	1.000	-0.203	-0.173	-0.216	-0.347**	0.297*	-0.259	-0.375**	-0.157	-0.118	-0.045	-0.008	-0.048
PH	0.835**	-0.203	1.000	0.907**	0.746**	0.736**	-0.095	-0.024	0.804**	0.543**	-0.030	0.015	0.036	0.057
LN	0.916**	-0.173	0.907**	1.000	0.594**	0.640**	-0.145	-0.310*	0.713**	0.372**	-0.009	0.172	0.056	0.066
FL	0.501**	-0.216	0.746**	0.594**	1.000	0.787**	0.164	0.179	0.726**	0.382**	0.039	-0.012	0.049	0.221
FW	0.503**	-0.347**	0.736**	0.640**	0.787**	1.000	-0.451**	0.170	0.814**	0.515**	-0.127	-0.123	-0.104	0.186
FL/FW	-0.032	0.297*	-0.095	-0.145	0.164	-0.451**	1.000	-0.052	-0.254	-0.226	0.217	0.123	0.202	0.002
PL	-0.400**	-0.259	-0.024	-0.310	0.179	0.170	-0.052	1.000	0.169	0.235	0.061	-0.240	0.052	-0.116
DI	0.569**	-0.375**	0.804**	0.713**	0.726**	0.814**	-0.254	0.169	1.000	0.548**	-0.033	-0.079	0.082	0.081
PT	0.363**	-0.157	0.543**	0.372**	0.382**	0.515**	-0.226	0.235	0.548**	1.000	-0.043	-0.335	-0.128	-0.142
LC	-0.055	-0.118	-0.030	-0.009	0.039	-0.127	0.217	0.061	-0.033	-0.043	1.000	0.358	0.102	0.043
SC	0.131	-0.045	0.015	0.172	-0.012	-0.123	0.123	-0.240	-0.079	-0.335*	0.358**	1.000	0.124	-0.011
HI	0.078	-0.008	0.036	0.056	0.049	-0.104	0.202	0.052	0.082	-0.128	0.102	0.124	1.000	0.053
SH	0.027	-0.048	0.057	0.066	0.221	0.186	0.002	-0.116	0.081	-0.142	0.043	-0.011	0.053	1.000

Controlled value is Iodine color reaction; * 5%, ** under 1% level significance.

escaped weed from subsp. *miliaceum* (b2) around the world, with the small grains shattering easily on its sparse panicle. Subsp. *agricolum* (b6) was a mutant race with characteristics intermediate between the domestic form and subsp. *ruderale*. This subspecies grew in maize fields because of its strong tolerance to herbicide. The two types of European common millet in the sixteenth century might have been the races *ovatum* (e1) and *patentissimum* (e2). A F_1 hybrid (c2) between subsp. *miliaceum* and subsp. *ruderale* was grown.

Morphological characteristics

The heading of common millet often occurred irregularly. Because the panicle flowered inside the leaf sheath 4–5 days before heading, the duration (days) to flowering from sowing was observed instead of the heading date. Generally, the duration of local varieties from high latitude areas was brief, but the number of days was remarkably variable. The varieties from China, Mongolia, the former USSR, Europe, and Japan (Hokkaido) flowered very early, by 40 days after sowing, while those from India and southern Japan flowered late, a third of them by 80 days. The varieties from China, Mongolia, the former USSR, Europe, and Japan (Hokkaido) had fewer leaves (5–10) on the main culm than those of southern and western Asia, Korea, and southern Japan (11 to 16). All of the varieties from Japan, Korea, and Nepal had only a few productive tillers (1 to 3), while the varieties from southern and western Asia, the former USSR (including Central Asia), and Europe indicated very broad variation (1–6), up to an extreme of 9 in 6.9% of samples from India.

Common millet is a densely piliferous plant. The hairiness of the uppermost internode was divided into four types: glabrous, sparse, moderate, and dense. Most varieties were glabrous or sparse, while the others were dense in Hokkaido (40.0%), western Asia (26.1%), and Europe (20.0%). The panicle was divided into five types: sparse, dense, compact, and intermediate values (Kimata unpublished). Most of the local varieties from Japan (Hokkaido), China, India, western Asia, the former USSR, and Europe were the sparse type, while the remaining varieties from Japan, Korea, and Nepal were the dense type. Only a few varieties from western Asia, the former USSR, and Europe were the compact type.

The lemma color on mature plants was classified into six colors: dark brown, brown, pale brown, ivory, orange, and grayish-green. The varieties from the former USSR and Europe showed large variations in color. Most grains from Japan (Hokkaido) and China were dark brown, but others from southern Japan were brown, pale brown, or ivory. In India, the grain color included grayish-green (45.6%) in addition to pale brown and ivory. The stigma color of the pistil in the mature stage was one of three colors: white, faint purple, or purplish-red. About 70% of the varieties showed the former two colors. Especially, in southern Japan and Nepal all the varieties had white stigmas except one. However, in Japan (Hokkaido), India, and western Asia over 73% of varieties had purplish-red stigmas. In Europe 28% of the varieties had

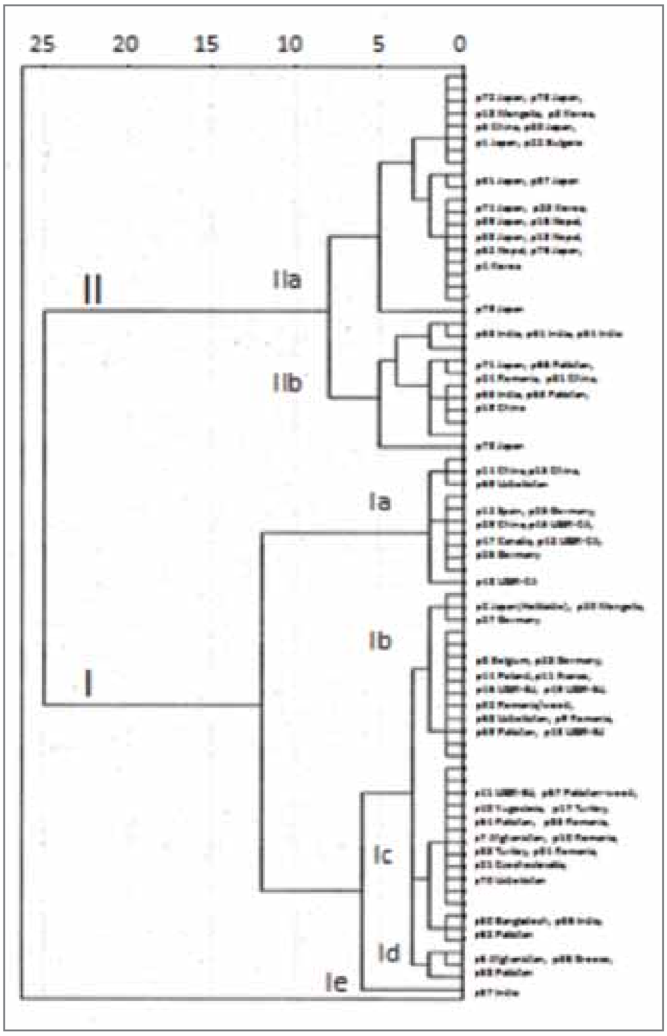

Fig 3. Cluster analysis of morphological characteristics.

purplish-red stigmas (Kimata unpublished).

The partial correlation coefficients of 14 characteristics are shown in Table 4. The coefficients greater than 0.6 under a 1% significance level were PH (plant height) and LN (number of leaves on the main culm) to days for flowering (DF). The DF, LN, FL and FW (length and width of flag leaf), and diameter of uppermost internode (DI) to PH; DF, PH, FW and DI to LN; PH, LN, and FL and FW to each other; DI to FL & FW; and PH, LN, FL, and FW to DI. The others, namely, number of tillers (TN), panicle length (PL), panicle type (PT), lemma color (LC), stigma color (SC), hairiness of uppermost internode (PI), and SH (shuttering) were not highly statistical significant. Therefore, the domestic varieties with late maturity are tall with many leaves, a large flag leaf that maintains effective photosynthesis during the growing season, and a bold culm that holds a heavy panicle.

The hierarchical cluster analysis of eight morphological characteristics and earliness is illustrated in Fig. 3 (by using the group average method of SPSS). The 75 local varieties were divided into two major clusters, I, with five sub-clusters, and II, with two sub-clusters. Sub-cluster Ia consisted of 11 varieties, from Central Asia (3 varieties, former USSR), Uzbekistan (1), China (3), Spain (1), Germany (2), and Canada (1); Ib came mostly from Western Europe, including a few from Japan (Hokkaido), Mongolia, Uzbekistan, and Pakistan; Ic came mostly from Eastern Europe, including a few from Uzbekistan and the Indian subcontinent; Id consisted of three varieties from Afghanistan, Greece, and Pakistan; and Ie consisted of only one variety from India. Sub-cluster IIa consisted of 20 varieties, mostly from East Asia but including a few from Nepal (3) and Bulgaria (1). IIb consisted of 11 varieties, mostly from the Indian subcontinent and also a few from China (2), Japan (2), and Romania (1). The distribution of morphological characteristics generally showed two geographical trends, from Central and South Asia toward Europe via Asia Minor, and from China toward India via Nepal (to the south) and Japan via Korea (to the far east).

Crossability among Eurasian varieties and morphological characteristics of F_1 hybrids

The crossability among six testers was estimated by their fructification rates. The florets (range 5 to 50, average 17.0) on panicles (1 to 3, average 1.2) were artificially crossed with the tester pollen of each variety, yielding an average fructification rate of 4.8%. Crossing tests were conducted between 351 combinations yielding 117 F_1 hybrids fructified. The artificial cross pollination of common millet was technically very difficult because the quite irregular flowering happened often before heading, and anther dehiscence was very sensitive to daily weather conditions and it did not open entirely under wet conditions on rainy days. Because of this, the observed crossability was relatively low, ranging from 0

Table 5. Crossability (%) among local varieties

Ovum Locality	No. of varieties	Pollen France	p32 Weed	Central Asia	India	p51 China	p60 Japan
East Asia	21	23.5	16.7	58.8	33.3	45.0	45.0
Central Asia	8	16.7	28.6	20.0	0	0	37.6
South Asia	19	26.7	35.7	29.4	30.8	25.0	38.9
Europe	20	41.2	16.7	17.6	17.6	45.0	21.1
Canada	1	0	0	+	0	0	0
Weed type (Romania)	1	0	0	0	0	0	0
Total combinations	70	56	58	47	65	67	58

+, with another variety.

to 63.9%. The F_1 hybrid was obtained from 18 varieties. The crossability of ovum parents was lower than that of pollen parents among the testers. One to 15 seed grains were obtained from each variety, and the germination ratio was observed in only 105 strains of the F_1 hybrid. Most seeds germinated well, while the others did not germinate or necrotized immediately after germination. All of the F_1 hybrid plants had good pollen fertility of over 78%.

Crossability among varieties was summarized to each country and region as shown in Table 5. The French tester had the largest number of sound F_1 plants (41.2%) with European varieties. The Central Asian tester made F_1 plants (58.8%) only with East Asian varieties. The Indian tester made F_1 plants with East Asian (33.3%) and South Asian (30.8%) varieties. The Chinese tester (p51) made F_1 plants with East Asian (45.0%) and European (45.0%) varieties. The Japanese tester (p60) made F_1 plants with East Asian (45.0%), Central Asian (37.6%), and South Asian (38.9%) varieties. The weed tester (p32, subsp. *ruderale* from Romania) made F_1 plants with South Asian (35.7%) and Central Asian (28.6%) varieties. The pollens of subsp. *ruderale* could artificially fertilize the ovum of domestic varieties, but the counter practices could not at all. Notably, a domestic variety with sparse and shattering panicles (PC57-2 from Hokkaido, Japan) made F_1 hybrids with the testers from Central Asia, India, and Japan, but not with the others.

The French tester made the largest number of fertile F_1 hybrids with European varieties, the Central Asian tester with East Asian varieties, the Indian tester with East and South Asian varieties, and the Chinese tester with East Asian and European varieties (Table. 5). Central Asian varieties were infertile when crossed with Indian or Chinese ovum parents similar to that when crossed with a weed (p32). These data suggested that common millet was dispersed from Central Asia to China and Europe, respectively , and then dispersed indirectly to South Asia and East Asia. The weed (p32) was not a crop-like weedy biotype because it was isolated reproductively and made no fertile hybrids as an ovum parent, notwithstanding the assured fructification among varieties from all regions in the reverse as a pollen parent. However, it might still be possible that subsp. *ruderale* was an ancestor, since it made fertile F_1 hybrids between the other varieties.

The panicle type of F_1 hybrids with the sparse-panicled Indian tester was also sparse. The F_1 hybrids with the dense-panicled Japanese tester (p60) had sparse panicles when combined with sparse varieties and dense panicles when combined with dense varieties. The F_1 hybrids between varieties with middle-type panicles generally also had middle-type panicles.

Common millet is generally a densely piliferous plant, but the hairiness of the uppermost internode was highly variable. This trait was divided into four types: glabrous, sparsely, moderately and densely piliferous. The F_1 hybrids between the moderate varieties (e.g., p9, p11, and p56) had a moderate internode except for p8 (glabrous). The F_1 hybrids with the glabrous testers from Central Asia, China, and Japan mostly had a glabrous internode except for a few in p2, p53, and p9 (moderately). The F_1 hybrids between the moderate or dense varieties and the dense Indian tester were varied widely between glabrous, sparsely and moderately, while an F_1 hybrid between a glabrous variety from Japan (Hokkaido) and the moderate Indian tester had a

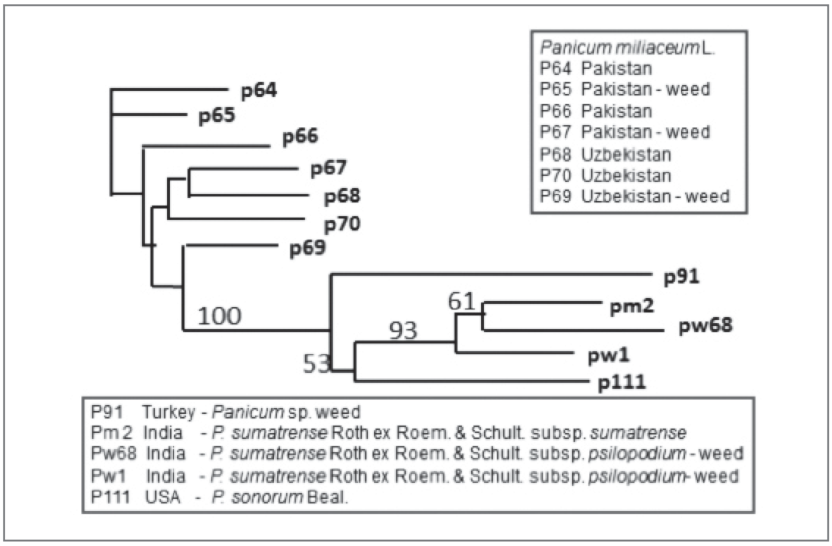

Fig. 4. Interspecific dendrogram of three domestic species in genus *Panicum* by AFLP markers.

glabrous internode.

AFLP markers

The AFLP markers of 75 local varieties were analyzed by PAUP* ver. 4.0 and SPSS ver. 21, including neighboring joint and UPGMA methods with the bootstrap test. The interspecific differentiation of *Panicum miliaceum*, *P. sumatrense*, *P. sonorum*, and their relatives is illustrated in Fig. 4 (neighboring joint tree, PAUP*). Clear interspecific differentiation among there species of *Panicum* were noted, including the domestic and weed types of *P. miliaceum* from Pakistan and Uzbekistan, and the other species, *P. sumatrense* and *P. sonorum* in the bootstrap test. However, the phylogenic differentiation of common millet was not as clear among varieties based on the bootstrap test (200 replicates), as shown in Fig. 5 (UPGMA tree, PAUP*), although there was a geographical trend in the dendrogram.

The 75 varieties were divided into two major clusters: I with six sub-clusters and II with three sub-clusters. Sub-cluster Ia consisted of five varieties from Germany, Romania (subsp. *ruderale*), China, and Japan (2 varieties). Ib consisted of seven varieties from Turkey, Greece, Romania (2), and Japan (3). Ic1 consisted of six varieties from Yugoslavia, the European portion of the former USSR (USSR-EU, 2), the Central Asian portion of the former USSR (USSR-CA, 2) and Poland. Ic2 consisted of ten varieties from Canada, USSR-EU (2), USSR-CA, China, Nepal, Bangladesh, and India (3). Ic3 consisted of eight varieties from India (3), Pakistan (4), and Japan. Id consisted of seven varieties, including Pakistan (4, with two weed types) and Uzbekistan (3, with one weed type). Sub-cluster IIa consisted of only two varieties from Afghanistan. IIb consisted of 9 varieties from China (2), Nepal (2), Romania (2), France, Spain, and Belgium. IIc consisted of 11 varieties from China, Korea, Mongolia (2), Turkey, Bulgaria, Romania, Czechoslovakia, and Germany (3). The distribution of AFLP markers generally showed two geographical trends, from Afghanistan and Mongolia toward Europe and Nepal via China (to the west and east), and from Uzbekistan and Pakistan toward India and Eastern Europe via USSR-CA/EU (to the south and west).

On one hand, based on the hierarchical cluster analysis (group average method, SPSS), only two clusters were detected among 51 varieties. Cluster I consisted of five varieties, including three weed types from Pakistan and Uzbekistan, while Cluster II consisted of 46 varieties from the other regions.

Discussion

The botanical origin, domestication, and

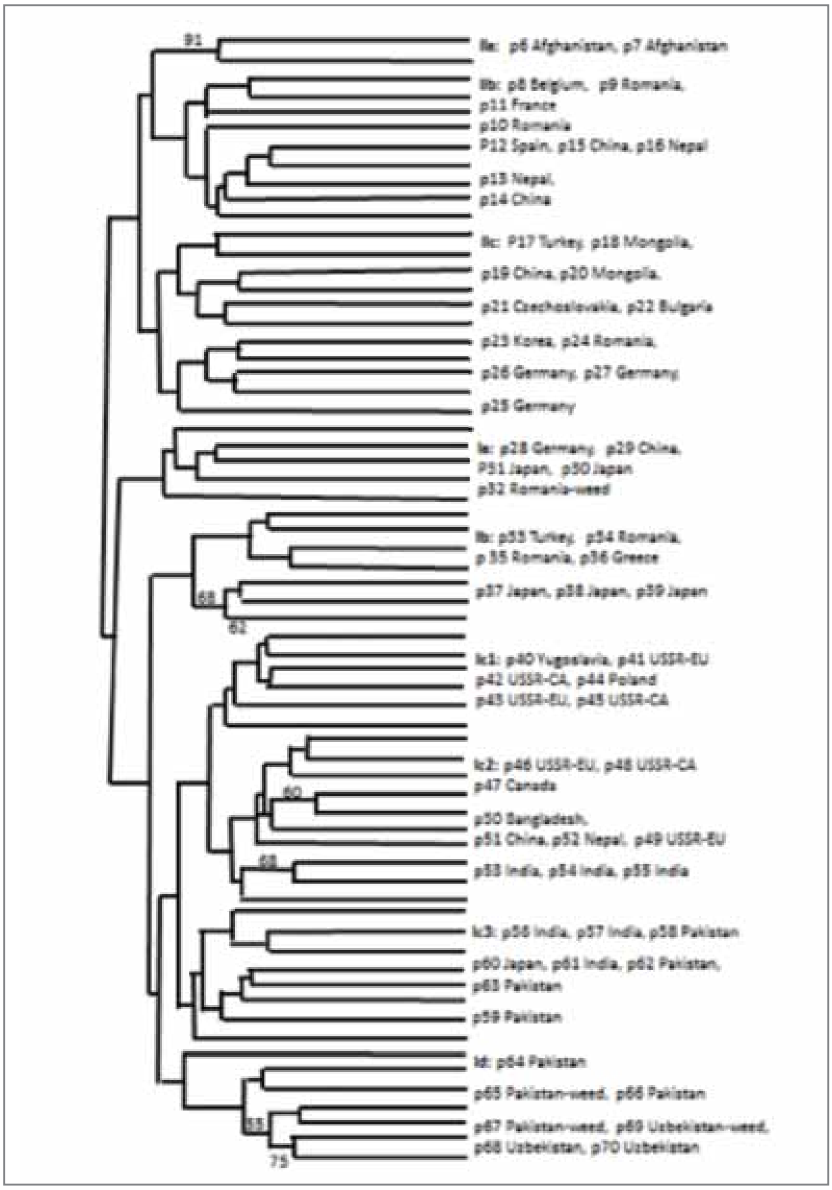

Fig. 5. Intraspecific dendrogram of common millet by AFLP markers.

geographical dispersal of common millet were discussed and then integrated through the results mentioned above. The following working hypothesis might well-explain the place of origin and dispersal of common millet with respect to recent archaeological contributions (e.g., Fuller et al. 2001, Hunt and Jones 2006, Jones 2004, Nesbitt 2005). This hypothesis is supported by the crossability among varieties in Eurasia and the geographical variation of several genetic characteristics (Table 5), although this needs further detailed study, especially the phylogeny of common millet and its close relatives. The early domestication process began in Central Asia and then progressed with a continuous process of dispersal toward China. The domestic type then dispersed from Central Asia to South Asia, directly to Europe, and indirectly to southeastern Europe via West Asia. On the other hand, this grain crop might have dispersed from China to Japan and Southeast Asia. The ancient farmers who had cultivated barley and wheat in the Near East area had not necessarily accepted common millet. However, the nomads who had moved around the Eurasian steppe had gladly accepted the millet as the food source, the same as the present day Mongolian herdsmen, because of its early maturation within the short summer season and its value as fodder for the livestock. They dispersed

Fig. 6. Dispersal routes of common millet through the Eurasia. I, original place and II, secondary dispersal center; solid lines, ancient routes from Middle Asia; dotted lines, the 13th century AD routes from China.

common millet from Central Asia to China and Europe. Common millet might have dispersed faster to Europe in an east/west direction at similar latitudes than to southeastern Europe in a south/north direction across different latitudes. It matured early in summer, but barley and wheat grew slowly in winter.

The traditional varieties cultivated by the Ainu people in Hokkaido, Japan are similar to the varieties from North China and Mongolia in their panicle type and the duration to flowering, while the other Japanese varieties are similar to the varieties from Korea and Nepal in their panicle type and stigma color (Kimata et al. 1986). The same large variability in lemma color and panicle type was shown in varieties from both the former USSR and Europe. Indian and West Asian varieties had very large variation in many characteristics (Fig. 3 and Table 4).

As compared with the other varieties through Eurasia, the varieties around Afghanistan, Pakistan, India, and Central Asia had a large diversity of characteristics, including sparse panicles and many tillers. The geographical distribution of characteristics was useful information including the biocultural diversity, particularly foods and vernacular names (Tables 2 and 3) to reconstruct the domestication process and dispersal routes (Kimata 2015c).

An ancestral form of common millet might have had early maturation, remarkable grain shattering, sparse panicles, small grains, many tillers, pale brown lemmas, white stigmas, glabrous uppermost internodes, and non-glutinous starch. Usually, the domestic form of cereals has fewer productive tillers than wild forms. Many varieties from Central Asia and the Indian subcontinent show many of the ancestral characteristics. There is only a little information on the mimic weed type associated with the domestic type of common millet (Sakamoto 1988, Scholz and Mikolāš 1991), but several weed types with remarkable grain shattering have been found in Pakistan, Uzbekistan, and Kazakhstan (Kimata 1994, 1997). These seeds were mixed with those of the domestic type. Because the varieties around Central Asia show large variation and their related weedy subspecies still grow today, this area is appropriate to be the place where common millet had been domesticated. Moreover, the weed types were classified into two subspecies, *ruderale* and *agricolum*, and a crop-like weedy biotype escaped from the domestic type. It would seem that *ruderale* was an ancestor, while *agricolum* became a weed by hybridization between these two subspecies.

Common millet was domesticated from a wild variety of *P. miliaceum* subsp. *ruderale* in Central Asia including the northern mountains of Afghanistan and Pakistan, especially from the Aral Sea to the Southwest Tien Shan Mountains. It was dispersed both eastward to China and westward to Europe, and both southward to the Indian subcontinent (de Wet 1995) and northward to Siberia by nomadic groups since the Neolithic era (Fig. 6). Moreover, when the Mongolian army invaded Europe in the thirteenth century, they carried with them common millet (Carpine 1246). It suggests the dispersal of common millet by Mongolian that a few Chinese varieties are mingled with European varieties in the clusters of morphological characteristics (Fig. 3) and AFLP markers (Fig. 5). Additionally, the traditional varieties cultivated by the Ainu people in Japan (Hokkaido) are similar to the varieties from North China and Mongolia in their panicle type and early duration to flowering, while the other Japanese varieties are similar to the varieties from Korea and Nepal in their panicle type, stigma color, and phenol reaction of young lemmas (Kimata et al. 1986, Kimata and Negishi 2002). A northern route from North China into Hokkaido is suggested by the fact that PC57-2 (Hokkaido, Japan) made fertile hybrids among the testers from Central Asia, India, and Japan.

An ancestor of common millet may have been a wild type of *P. miliaceum* subsp. *ruderale*. The early domestication process began around Central Asia and then progressed in a continuous dispersal process toward China. Furthermore, the domestic type dispersed from Central Asia to South Asia, directly to Europe, and indirectly to southeastern Europe via West Asia.

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