The tertiary domestication process of *korati*, *Setaria pumila* (Poaceae), through the mimicry of other grain crops in the Indian subcontinent

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In South India, one domesticated type of *Setaria pumila* is always cultivated in mixed stands along with *Panicum sumatrense*, whilst the others, and the weed types, are grown mostly with *Paspalum scrobiculatum*, sometimes *Eleusine coracana*, and upland rice, *Oryza sativa*, in diverse agro-ecological niches in East India. Several botanical traits of *S. pumila* indicate that the domesticated type is descended from the weed types, and that these traits have become evolutionarily synchronized with those of *P. sumatrense* and/or other cereals. This domestication process has been promoted morphologically through mimicry of other cereal species and acts as a means of adapting to the arid climate. Moreover, the pigmentation of leaves and leaf sheaths in terms of anthocyanin composition (HPLC analysis) is involved in mimicry among the other grain crops and the closely-related weeds. The weed and companion weed types are used as fodder for cattle and as an insurance crop for farmers in times of famine. The grains of *S. pumila* are always mixed with other millet grains, used to make six traditional foods as a supplementary ingredient, and in turn related to diversification of the basic agricultural complex. This tertiary domestication of *S. pumila* has proceeded from inter- and intraspecific mimicry by natural hybridization and artificial selection in sympatric fields.

Key words: adaptation, hybridization, millet, mimic companion weed, tertiary crop

Introduction

Human beings had domesticated more than 30 grass species, as cereal crops, in several parts of the world, possibly as long as 12,000 years ago. However, several species are threatened and in spite of their potential food value in their native habitats, have disappeared or have hardly been cultivated. This is because the three major crops of wheat, rice, and maize have rapidly increased in their yield and production, due to huge technical innovation in crop improvement programs. The other grain crops, that is to say, millets, have decreased gradually during the previous century, resulting in genetic erosion of their local varieties. Today, we need recognize their value as exploitable and underutilized genetic resources, based on their adaptability to stress-prone environments. These species of millet are mostly C_4 plants, which are early to mature, and can be cultivated under conditions of severe drought and harsh sunlight.

Local farmers continue to cultivate a few useful varieties of millet even today. These indigenous varieties are excellent materials for investigating crop evolution, particularly the origin and dispersal route of domesticated plants. In the Indian subcontinent, a few small millets are still undergoing the domestication process (Kimata et al. 2000; Singh and Arora 1972). While crop evolution can be reconstructed mostly from botanical data, the aspects of geographical origin and dispersal will become clear from the information on the basic agricultural complex offered by local farmers.

Vavilov (1926) showed the domestication process from weeds associated with wheat to the secondary crops in two genera, *Avena* and *Secale*. For example, *Secale cereale* L. had built up strong resistance to cold in high altitude and/or latitude areas, and, subsequently, this species had been able to grow under more severe

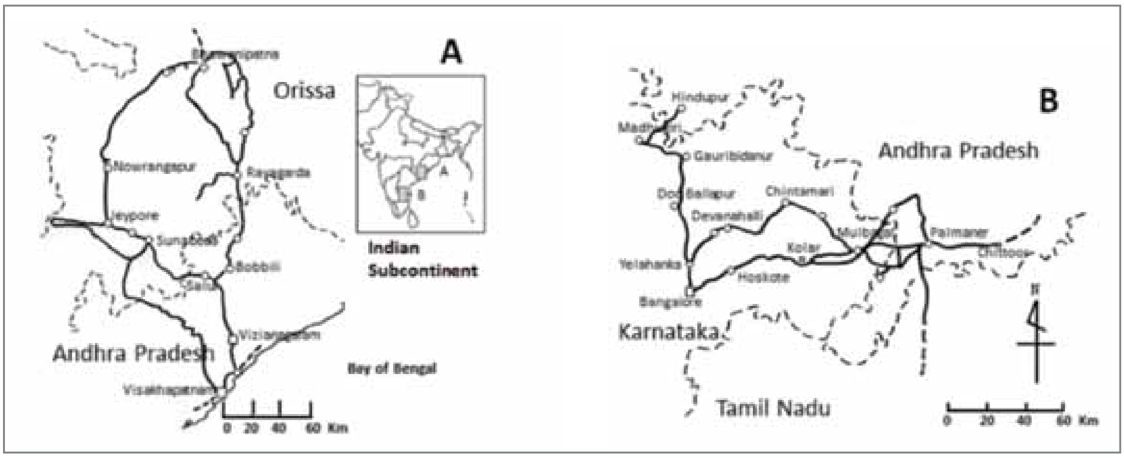

Fig. 1. Field survey: a) in Karnataka and Andhra Pradesh (1985, 1996, 2001); and b) Orissa (1987, 2001), Indian subcontinent.

conditions than those under which wheat can grow. Kobayashi (1987, 1989) proposed an integrating model of the domestication process of Indian millets as secondary crops from mimic companion weeds associated with *Oryza sativa* L.

O. sativa had been spread first from wetlands to uplands, secondarily, in the Indian subcontinent. Their ancestral weedy plants had invaded paddy and upland rice fields in turn. These are *Panicum sumatrense* Roth. (little millet), *Paspalum scrobiculatum* L. (kodo millet), *Echinochloa frumentacea* Link (Indian barnyard millet), *Brachiaria ramosa* (L.) Stapf. (*korne*), *Digitaria crusiata* (Nees) A. Camus (*raishan*), and *Setaria pumila* (Poir.) Roem. & Schult. (*korati*; syn. *S. glauca* (L.) P. Beauv.) (Chandra and Koppar 1990; de Wet et al. 1983a, b, c). *Pas. scrobiculatum, P. sumatrense*, and *E. furumentacea* were subsequently domesticated by local farmers as secondary crops to upland rice, because these had put up stronger resistance to drought than upland rice in Eastern India. In this region, several species of millet were domesticated.

Recently, archeological studies have shown very useful data in the Indian subcontinent. The material of millet grasses came from archaeological levels, Phases II (2300–1800 cal. BC) and Phases III (1800–1200 cal. BC) in the Southern Neolithic chronology. They have been identified as being primarily from two species, browntop millet, *Brachiaria ramosa*, and bristly foxtail millet-grass, *Setaria vercillata*. Yellow foxtail millet, *Setaria pumila* was present in limited quantities, possibly gathered from the wild (Fuller et al. 2001). The first occurrence of cereals in the Harrappan Civilization had been wheat, barley, and oats in the Early phase (before 2600 BC); *Eleusine sp.* (problematic, *E. coracana*), *Setaria* sp., and *Panicum* sp. in the Mature phase (2600–2000 BC); and *Paspalum* sp., *Echinochloa* sp., *Sorghum* sp., and *Pennisetum* sp. in the Late phase (more recent than 2000 BC) (Fuller and Madella 2000; Weber 1992).

S. pumila is a weedy annual growing 30-60 cm tall. The inflorescence is cylindrical, densely flowered, spike-like raceme, 2.5-10cm long, usually yellow, or more rarely purplish or pale green. It is a fairly common grass, especially in cultivated lands, along the roadsides, and in cleared forests, up to altitudes of 700 m. The spikelet (c.a. 3-mm long) is pale green or brownish-green. Cattle are fond of it (Achariyar 1921; Singh 1988). *S. pumila* is a multiple polyploid species (2n=18, 36, 72) and an unknown genome constitution (almost genome D) (Zhao et al. 2013). The present paper is concerned with the tertiary domestication process of *S. pumila* through the mimicry that is related ecologically and genetically to the relative weeds and several grain crops in the Indian subcontinent.

Materials and Methods

Many local varieties and relative weeds of *Setaria pumila* (Poir.) Roem. & Schult.(*syn. S.glauca* (L.) P.

Table 1. Materials used of *Setaria pumila*

Collection no.	Sample no. & Status	Main crop and remarks	Locality
85-10-31-3-12	1Ds-A	*Panicum sumatrense*	Duggamvapalli, Andhra Pradesh
k87-9-28-9-4	2Ws-M	*P. sumatrense*	Kumbharoshi (800m), Maharashtra
k87-9-28-9-6	3Ws-M	*P. sumatrense*	
k87-10-1-7-8	4Ds-M	*P. sumatrense*	16km from Lanja (200m), Maharashtra
k87-10-3-3-1	5W-M	none	Gabi (650m), Maharashtra
k87-10-3-5-7	6Ws-M	*P. sumatrense*	Nadagao village (541m), Maharashtra
k87-10-4-6-7	7W-M	*Oryza sativa*	8km W from Kolhapur (600m), Maharashtra
k87-10-5-10-5	8W-M	*Setaria italica*	Udtare village (652m), Maharashtra
k87-10-5-10-6	9W-M	*S. italica*	Udtare village (653m), Maharashtra
k87-10-9-1-1	10Ms-O	*P. sumatrense*	Sunabeda (895m), Orissa
k87-10-9-1-6	11Ms-O	*P. sumatrense*	
k87-10-9-1-7	12Ms-O	*P. sumatrense*	
k87-10-9-1-8	13Ms-O	*P. sumatrense*	
k87-10-9-2-2	14Wsk-O	*P. sumatrense* mixed	Kundali village (875m), Orissa
k87-10-9-5-6	15Ws-O	*P. sumatrense*	Potang (895m), Orissa
k87-10-10-2-1	16W-O	none	7km from Sunabeda (900m), Orissa
k87-10-10-5-5b	17Ws-O	*P. sumatrense*	2km of Boipariguriha (608m), Orissa
k87-10-10-5-6b	18Ws-O	*P. sumatrense*	
k87-10-10-5-10d	19Ws-O	*P. sumatrense*	
k87-10-10-5-13A	20Ws-O	*P. sumatrense*	
k87-10-10-5-13B	21Ws-O	*P. sumatrense*	
k87-10-10-5-14e	22Ds-O	*P. sumatrense*	
K87-10-10-5-16A	23Ds-O	*P. sumatrense*	
k87-10-10-5-16B	24Ds-O	*P. sumatrense*	
k87-10-10-6-8	25Ws-O	*P. sumatrense*	Beragaon, 12km of Koraput (605m), Orissa
k87-10-11-2-2	26Mk-O	*Pas. scrobiculatum*	Anchalguda village, 20km of Kolaput (870m), Orissa
k87-10-11-2-3	27Dk-O	*Pas. scrobiculatum*	
k87-10-11-2-5	28Mk-O	*Pas. scrobiculatum*	
k87-10-11-6-7	29Ws-O	*P. sumatrense*	Damaniganda village (728m), Orissa
k87-10-11-6-8	30Ms-O	*P. sumatrense*	
k87-10-12-2-3	31W-O	none	Sagada village (240m), Orissa
k87-10-12-2-7	32W-O	none	
k87-10-12-5-4	33Ws-O	*P. sumatrense*	47km NW of Bhawanapatna (690m), Orissa
k87-10-12-5-5	34Ws-O	*P. sumatrense*	
k87-10-12-5-7	35Ms-O	*P. sumatrense*	
k87-10-12-5-8	36Ws-O	*P. sumatrense*	
k87-10-12-6-2	37Wp-O	*Oryza sativa* mixed	Balsora village (690m), Orissa
k87-10-12-6-3	38Mp-O	*O. sativa* mixed with	
k87-10-12-6-4	39Mp-O	*O. sativa* mixed with	
k87-10-12-7-4	40Ws-O	*P. sumatrense*	Duliguda village,11km of Gopalpur(922m), Orissa
k87-10-12-7-5	41Ws-O	*P. sumatrense*	
k87-10-12-8-4	42Ws-O	*P. sumatrense*	Dakuta (937m), Orissa
k87-10-13-4-14	43Wk-O	*Pas. scrobiculatum*	Puda Pali village (269m), Orissa
k87-10-13-5-6	44Mk-O	*Pas. scrobiculatum*	12km of Kharhiar (272m), Orissa
k87-10-13-5-11	45Mk-O	*Pas. scrobiculatum*	
k87-10-14-2-1	46W-O	none	Mandiapadar village (139m), Orissa
k87-10-14-2-3	47W-O	none	
k87-10-14-2-4	48W-O	none	
k87-10-14-4-3	49Mk-O	*Pas. scrobiculatum*	Budhitadar village (146m), Orissa
k87-10-15-1-6	50Mk-O	*Pas. scrobiculatum*	Ramisarda Tilemal (149m), Orissa
k87-10-16-2-3	51Ms-O	*P. sumatrense*	Kolarapaju village (766m), Orissa
k87-10-16-2-4	52Ms-O	*P. sumatrense*	
k87-10-16-3-4	53Mk-O	*Pas. scrobiculatum*	Bekarakhol village, 30km of Phulabani (522m), Orissa
k87-10-16-5-4	54Ms-O	*P. sumatrense* mixed w	4km from Tikaball (569m), Orissa
k87-11-7-0-26	55W-W	none	Kalimpong, West Bengal
96-11-5-1a-2	56Dk-K	*Domesticated type, a*	Kalidevapura, Karnataka
96-11-5-2b-6	57Ds-K	*A few mixed with P. sumatrense*	Madhagiri, Karnataka
96-11-5-7-2	58Dk-K	*A little shattering.*	
97-4-12-2-2	59Ds-A	*P. sumatrense*	Jalaripalli, Andhara Pradesh
97-4-12-2-3	60Ds-A	*P. sumatrense*	

Sample number and status: W, weed type; M, mimic weedy medium type; D, domesticated type. Main crop: s, *samai* (*Panicum sumatrense*); k, *kodo* (*Paspalum scrobiculatum*); p, paddy (*Oryza sativa*); o, other species. Locality: A, Andhara Pradesh; K, Karnataka; M, Maharashtra; O, Orissa; P, Pakistan; T, Tamil Nadu; U, Uttar Pradesh; W, West Bengal.

Table 2. Materials used of *Setaria pumila* and other millet species

Collection no.	Cultivation no.	Species	Status	Locality	Remarks observed in the field
96-11-5-1a-1	ps1	*Paspalum scrobiculatum*	domest	**Site 5**, Kalidevapura village, Tumkur, Karnataka	main crop
96-11-5-1a-2	st56	*Setaria pumila*	medium		a few mixed
96-11-5-2b-1	pn1	*Panicum sumatrense*	domest	**Site 6**, Kalidevapura village, Tumkur, Karnataka	main crop
96-11-5-2b-4	br1	*Brachiaria ramosa*	domest		a few mixed with weedy type
96-11-5-2b-6	st57	*Setaria pumila*	medium		a few mixed
96-11-5-7-1	ps2	*Paspalum scrobiculatum*	domest	**Site 7**, Kodigenahalli Hogli, Tumkur, Karnataka	pure stand
96-11-5-7-2	st58	*Setaria pumila*	domest		1 plant mixed, small seed, compact spike
97-4-12-2-1	pn2	*Panicum sumatrense*	domest	**Site 8**, Jalaripali village, Andhra Pradesh	mixed croping
97-4-12-2-2	st59	*Setaria pumila*	domest		mixed croping, brown seed
97-4-12-2-3	st60	*Setaria pumila*	domest		mixed croping, black seed
01-10-8-1-1	pn3	*Panicum sumatrense*	domest	Thatigul village, Kolar, Andhra Pradesh	main crop, purple disperse spike, yellow seed
01-10-8-1-2	pn4	*Panicum sumatrense*	domest		green disperse spike, gray-yellow seed
01-10-8-1-3	pn5	*Panicum sumatrense*	domest		green dense spike, gray-yellow seed
01-10-8-1-4	pn6	*Panicum sumatrense*	domest		purple dense spike, yellow seed
01-10-8-1-5	st61	*Setaria pumila*	domest		a few mixed, black seed
01-10-8-1-6	st62	*Setaria pumila*	weed		shatering black seed
01-10-8-2-1	pn7	*Panicum sumatrense*	domest	**Site 1**, Illur village, Chittoor, Andhra Pradesh	main crop, green disperse spike, gray-yellow seed
01-10-8-2-3	pn8	*Panicum sumatrense*	medium		green disperse spike, dark gray-yellow seed
01-10-8-2-5	st63	*Setaria pumila*	weed		short spike
01-10-8-2-6	st64	*Setaria pumila*	medium		a few mixed, pale green dense spike, pale green seed
01-10-8-2-7	st65	*Setaria pumila*	medium		a few mixed, purple spike, black seed
01-10-8-2-8	st66	*Setaria pumila*	domest		
01-10-9-2-1	pn9	*Panicum sumatrense*	domest	Dombarpally village, Palmaner, Andhara Pradsh	main crop, gray-yellow seed
01-10-9-2-4	st67	*Setaria pumila*	domest		many mixed, black seed
01-10-9-3b-3	st68	*Setaria pumila*	domest	Punganuru, Andhra Pradesh	mixed with *P. sumatrense*, pale green seed
01-10-9-3b-4	br2	*Brachiaria ramosa*	domest		dense spike
01-10-9-3b-5	br3	*Brachiaria ramosa*	medium		disperse spike
01-10-10-1	ps3	*Paspalum scrobiculatum*	domest	Jalaripali village, Andhra Pradesh	
01-10-17-1	st69	*Setaria pumila*	weed	**Site 3**, Kundi village, Orissa	mixed with *E. coracana*, small black seed
01-10-17-2c	ors	*Oryza sativa*	domest		upland rice
01-10-17-4	sw	*Setaria sp.*	weed		
01-10-18-1-1	orr	*Oryza rufipogon*	weed	Bhawanipatna, Orissa	growing near rice paddy
01-10-18-1-2	psw	*Paspalum sp.*	weed		growing near rice paddy
01-10-19-2a-1	pn10	*Panicum sumatrense*	domest	West Polehorebrdle village, Bhawanipatna, Orissa	mixed with *E. coracana*, pale brown seed
01-10-19-2a-2	el	*Eleusine coracana*	domest		small seed
01-10-19-2a-3	st70	*Setaria pumila*	weed		small seed
01-10-19-2b-1	ps4	*Paspalum scrobiculatum*	domest	West Polehorebrdle village, Bhawanipatna, Orissa	mixed with upland rice field
01-10-19-2b-2	ef	*Echinochloa frumentacea*	domest		mixed with upland rice field

medium = associated mimicry weedy type

Beauv.) have been collected through field surveys of the Indian subcontinent since 1983. Concentrated field surveys were conducted in Karnataka, Andhra Pradesh, and Orissa in 1985, 1987, 1996, and 2001 as illustrated in Fig. 1. At the same time, the component species of millets and weeds were examined in five plots (1 m^2) each in four typical cropping fields (sites) through the quadrat method. Voucher herbarium specimens and grain samples were collected along the entire survey route and deposited at Tokyo Gakugei University and the University of Agricultural Sciences (Bangalore). The information on agricultural practices, grain processing, food preparation, and vernacular names was obtained from local farmers.

As part of these accessions, 60 experimental strains were selected and grown in a glasshouse at Tokyo Gakugei University, Japan, in order to compare their morphological and ecological characters in 1998 (Table 1) and in 2002 (Table 2). These 10 grains of 60 strains were sown with a row spacing of 8 cm and seed spacing of 2 cm in a box on June 8, 1998 and June 10, 2002. Two weeks after sowing, germinated plants were transplanted with row spacing of 30 cm and plant spacing of 15 cm in the glasshouse. Chemical fertilizer [N:P:K = 8:8:5 (g)] was supplied at 100 g per m^2. Five plants of *S. pumila*, each of the weed type, companion weed, and mimic companion weed types associated with *Pas. scrobiculatum* and other cereals, and the domesticated type mixed with *P. sumatrense* were measured for number of tillers, plant height, spike length, duration to flowering, length and width of the flag leaf, last internode diameter, number of leaves on the main culm, and number of seeds germinated every 10 days after sowing. Plant color was assessed using the PANTONE Formula Guide. These data were analyzed statistically using IBM SPSS (version 21).

Additionally, the anthocyanin composition included in the leaf and leaf sheath was analyzed by high-performance liquid chromatography (HPLC) in 2013. The materials were *S. pumila* (5 accessions) from site 5 to 8, *P. sumatrense* (8), *Pas. scrobiculatum* (2), *B. ramosa* (1), and *S. viridis* (2, fresh and dried). In order to identify the retention time (Rt) and measure the content of each anthocyanin, natural pigments of cyanidin, pelargonidin, and malvidin were used. The experimental method was as follows. The extraction of anthocyanins from leaf and leaf sheath was performed according to the method reported by Mochioka et al. (1995), Tsuda et al. (2009), and Chiba et al. (2010), with some modifications. Briefly, liquid nitrogen was added to leaves (300 mg) that had been stored at -30°C and these were then ground using a ceramic mortar. The ground material was placed into a safety-rock tube (1.5 ml), followed by the addition of 300 μl 50% methanol containing 5% acetic acid. The tube was allowed to stand in a sonicater for 30 min, and the solution was filtered through a membrane filter (UST-5, ADVANTC). The solution of standard pigments was also filtered through the same membrane filter. After the treatment, the solution was placed in a refrigerator at 6°C overnight. The anthocyanin composition of the leaves was identified by the retention time and the content was calculated using a calibration curve. The HPLC system employed to analyze the anthocyanins was a Shimazu system CLASS-M10A equipped with a Shimazu chromatography data station, including pump, column oven, and absorbance detector SPD-10A for monitoring at 520 nm. For the column, Shin-Pak (4.6 × 250 mm, PREP-ODS(H)KIT; Shimazu) was used at 40°C. Linear gradient elution was performed with solution A (1.5% phosphoric acid) and solution B (1.5% phosphoric acid, 20% acetic acid, and 25% acetonitrile) delivered at a flow rate of 1.0 ml/min as follows: 80% of solution A initially; 15% A for the next 40 min; 80% A for a further 5 min; and finally 80% A for 15 min (P.MAX; 200 kgf/cm^2: P.MIN; 10 kgf/cm^2). The injection volume for the extract was 20 μl.

Results

Morphological and ecological diversity of *S. pumila*

S. pumila is still cultivated through mixed cropping mostly with *P. sumatrense* in restricted regions in peninsular India as shown in Fig. 2e and 2f. The weed types and mimic companion weed types are also used

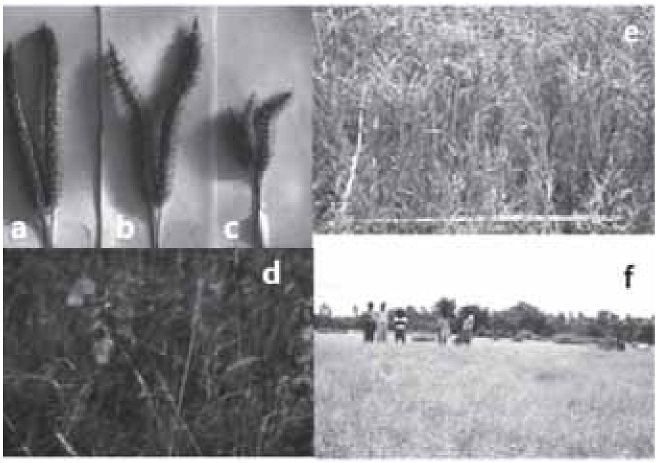

Fig. 2. Panicle types of *Setaria pumila*: a) and b) domesticated types (Dk) mixed with *Paspalum scrobiculatum*; c) domesticated type (Ds) mixed with *Panicum sumatrense* and d) weed type shattering seed grains; e) and f) cropping fields mixed with *Panicum sumatrense* and *Setaria pumila*: at Illur village near Chittoor in Andhra Pradesh.

together as a wild grain for food and forage today. Their spikes show a large variability, for example, in the domesticated type (Dk) mixed with *Pas. scrobiculatum* (Fig. 2a and 2b), another domesticated type (Ds) mixed with *P. sumatrense* (Fig. 2c), and the weed type (W) of *S. pumila* (Fig. 2d)

The morphological characteristics and the duration to flowering of *S. pumila* (60 accessions) were compared among weed, companion weed, mimic companion weed, and domesticated type mixed with the other crops (Fig. 3). The plant height of weed types had a large range (from 81 to 220 cm), and the height of the mimic companion weed and domesticated types were within the same range. The number of tillers showed very broad variability from 10 to over 60, but mostly 10 to 25, irrespective of the type. The spike length was 5 to 14 cm in weed types, and a little longer (9 cm to over 16 cm) in mimic companion weed and domesticated types. The duration to flowering of many strains was ranged from 121 to 140 days, but that of domesticated types showed a bimodal pattern, 81 to 100 days and 121 to 150 days. The ratio of length/width of the flag leaf had a modest range from 21 to 31, but that of domesticated types had a broad variation of range from 21 to 70. The last internode diameter was 1.1 to 2.5 mm in weed

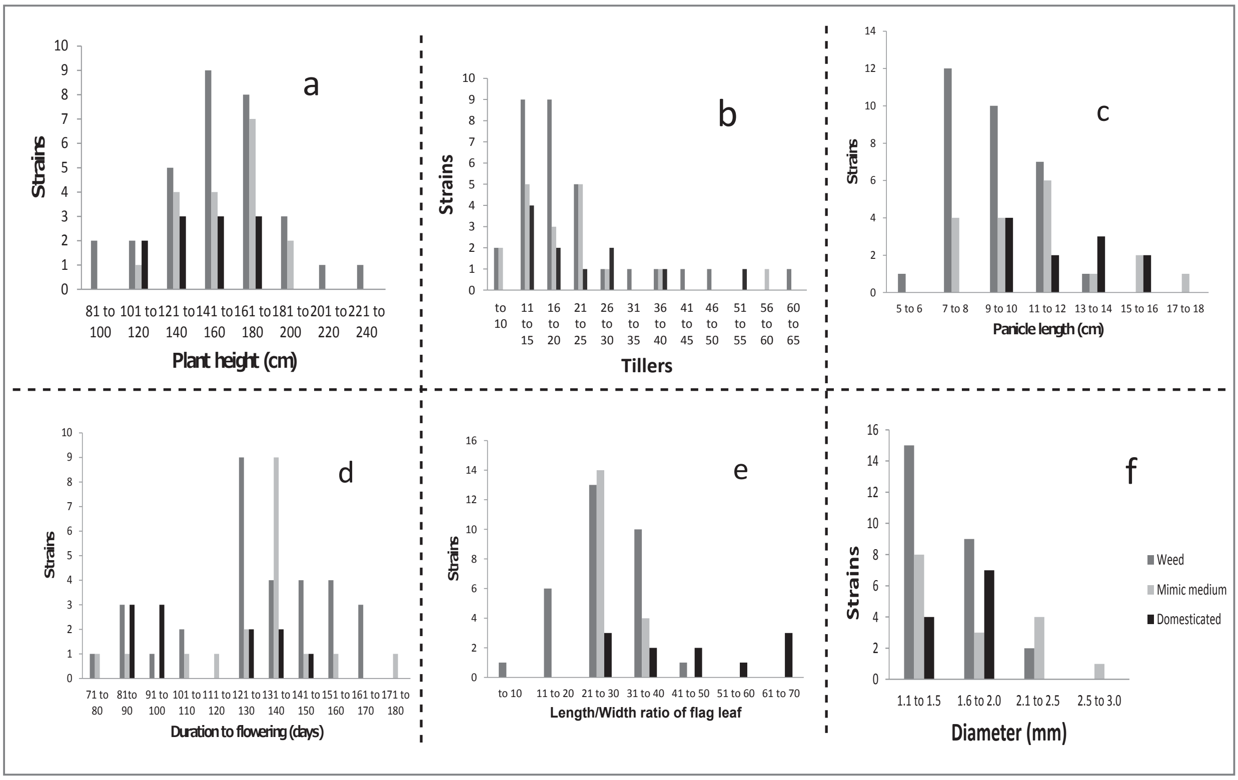

Fig. 3. Morphological and ecological characters of 60 accessions in *Setaria pumila*: a) plant height (cm), b) number of titters, c) spike length (cm), d) duration of flowering (days), e) length/width ratio of flag leaf, and f) last internode diameter (mm).

Table 3. Component species per m² of four millet fields (sites)

Site no.	Locality	*Panicum sumatrense*	*Setaria pumila*	*Eleusine coracana*	*Brachiaria* sp.	*Digitaria* sp.	Total
Site 1	01-10-8-2 (805m alt.) Cittoor, Andhra Pradesh						
	No. of plants	163.5	90.75	0	22	0.25	276.5
	s.d.	76.43	36.48		24.99	0.5	75.54
	Range	78–264	55–141		0–56	0–1	191–355
	%	59.13	32.82	0	7.96	0.09	100
	Highest plant (cm)	77.38	66.88				
	s.d.	7.63	10.17				
Site 2	01-10-9-1 (690m alt.), Cittoor, Andhra Pradesh						
	No. of plants	105.5	312.25	0	0.75	1	419.5
	s.d.	68.83	60.91		0.5	0.82	68.98
	Range	30–172	240–368		0–1	0–2	352–517
	%	25.15	74.43	0	0.18	0.24	100
	Highest plant (cm)	76	68.25				
	s.d.	24.07	15.37				
Site 3	01-10-17-1 (855m alt.) Kundli, Orissa						
	No. of plants	1.75	1.5	42.25	0	0.25	45.75
	s.d.	0.96	1	6.85		0.5	26.12
	Range	1–3	1–3	34–50		0–1	46–101
	%	3.83	3.28	92.35	0	0.55	100
Site 4	01-10-19-3 (375m alt.), West Polehorebrdle, Orissa						
	No. of plants	40.5	2	0	0	0	45.75
	s.d.	8.23	1.63				20.59
	Range	30–50	0–4				40–86
	%	95.29	4.71	0	0	0	100

types, 1.1 to 3.0 mm in mimic companion weed types, and 1.1 to 2.0 mm in domesticated types.

Species component of millet and weed grown in four cropping fields (sites)

The species components (per m²) of four millet cultivation fields are shown in Table 3. At site 1, the percentages of *P. sumatrense*, *S. pumila*, and weed

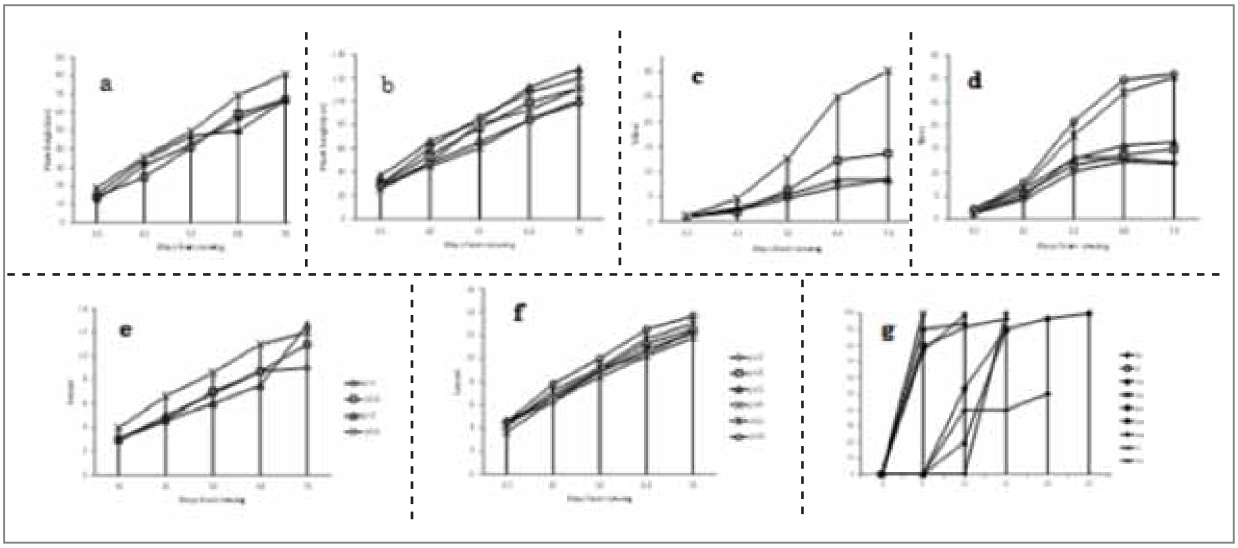

Fig. 4. Early growing patterns of seedling in *S. pumila, P. sumatrense* and *Pas. scrobiculatum*: a/b) plant height, c/d) number of tillers and e/f) number of leaves on main culm and g) seed germination.

Brachiaria sp. were 59%, 33%, and 8%, respectively, while at the nearby site 2, the percentages were 25%, 74%, and trace. At site 3, a single stand of *E. coracana*, and at site 4, a single stand of *P. sumatrense*, the weed type of *S. pumila*, had invaded significantly, but represented only 3–5% into both cropping fields.

Comparison of early growing among companion weeds and crops

With respect to early growing during the period of 30 to 74 days after sowing, five characteristics were compared among *S. pumila* (2, Mk-st56 and Dk-st58) and *Pas. scrobiculatum* (2, Dk-ps1 and Dk-ps2) collected at site 5 and 7 in Tumkur, Karnataka, and *S. pumila* (Ds-st61 and Ws-st62) and *P. sumatrense* (D-pn3 to D-pn6) in Kolar, Andhra Pradesh (Table 2). The early growth of the number of tillers of *S. pumila* (st58 from Karnataka) showed a marked increase, but there was little difference in the plant height or the number of leaves among the four strains (Fig. 4a-c-e). For the strains from Andhra Pradesh, the number of tillers of *S. pumila* (st61, st62) was also markedly increased 52 days after sowing, while there were little difference in plant height or the number of leaves among the six strains (Fig. 4b-d-f). On the other hand, the seed germination rates of *O. sativa*, *B. ramosa*, *E. coracana*, *P. sumatrense* and *S. pumila* were approximately 80% by 5 days, and nearly 100% by 10 days, but the germination of *Pas. scrobiculatum* (D-pas, W-pasw), *Setaria* sp. (W-stw) and *O. rufipogon* (ancestral W-orr) was only starting at 5 days and the rates were still increasing at 15 days (Fig. 4g).

Mimicry in the color of leaf and leaf sheath

The leaf and leaf sheath colors of *S. pumila* were the same green (370c~371c) and reddish purple (206c~209c), respectively, using the PANTONE Formula Guide, as a mixed cropping *Pas. scrobiculatum* at site 5 in Karnataka (Table 4 and 5). The colors of *S. pumila* were also the same green (363c~371c) and reddish purple (206c~208c), respectively, at site 7 in Karnataka. At site 6 in Karnataka, the colors of *S. pumila* and *P. sumatrense* were the same green (369c~377c) and reddish purple (206c~207c), except for the leaf sheath color of *B. ramosa* (green, 374c). Among the control strains used, the colors of *P. sumatrense* ranged from green (366c-370c) to pink (203c), but those of *S. viridis* ranged from green (leaf 371c) to purple (leaf sheath 209c-5195c).

The anthocyanin composition of leaf and leaf sheaths was analyzed by HPLC as illustrated in Fig. 5. The leaf sheath of *S. pumila* showed many peaks (present on each one) on Rt (Fig. 5a), while those of *P. sumatrense* (Fig. 5b) and *Pas. scrobiculatum* (Fig. 5c) indicated only

Table 4. Comparison of leaf and leaf sheath color based on PANTONE formula guide in four millet cultivation fields (sites)

Site no.	Collection no.	Cultivation no.	Species	Leaf developed	Leaf undeveloped	Under part of leaf sheath
Site 5	96-11-5-1a-1	ps1	*Paspalum scrobiculatum*	371c-green	370c-green	208/209c-reddish purple
	96-11-5-1a-2	st56	*Setaria pumila*	371c-green	371c-green	206c-reddish purple
Site 6	96-11-5-2b-1	pn1	*Panicum sumatrense*	377c-green	371c-green	206c-reddish purple
	96-11-5-2b-4	br1	*Brachiaria ramosa*	371c-green	377c-green	374c-green
	96-11-5-2b-6	st57	*Setaria pumila*	371c-green	369c-green	205/207c-reddish purple
Site 7	96-11-5-7-1	ps2	*Paspalum scrobiculatum*	371c-green	370c-green	208c-reddish purple
	96-11-5-7-2	st58	*Setaria pumila*	371c-green	363c-green	206/207c-reddish purple
Site 8	97-4-12-2-1	pn2	*Panicum sumatrense*	371c-green	377c-green	374c-green
	97-4-12-2-2	st59	*Setaria pumila*	371c-green	377c-green	198/202c-reddish purple
	97-4-12-2-3	st60	*Setaria pumila*	371c-green	377c-green	198/202c-reddish purple

Table 5. Samples used for HPLC

Collection no.	Site no.	Species name	Organ	Panton formula guide
96-11-5-1a-1	**Site 5**	*Paspalum scrobiculatum*	Leaf sheath	208c/209c
96-11-5-1a-1			Leaf	371c/370c
96-11-5-1a-2		*Setaria pumila*	Leaf sheath	206c
96-11-5-1a-2			Leaf	371c
96-11-5-2b-1	**Site 6**	*Panicum sumatrense*	Leaf sheath	206c
96-11-5-2b-1			Leaf	277c
96-11-5-2b-4		*Brachiaria ramosa*	Leaf sheath	374c
96-11-5-2b-4			Leaf	371c
96-11-5-2b-6		*Setaria pumila*	Leaf sheath	205c/207c
96-11-5-2b-6			Leaf	371c
96-11-5-7-1	**Site 7**	*Paspalum scrobiculatum*	Leaf sheath	208c
96-11-5-7-1			Leaf	371c
96-11-5-7-2		*Setaria pumila*	Leaf sheath	206c (207c)
96-11-5-7-2			Leaf	371c
97-4-12-2-1	**Site 8**	*Panicum sumatrense*	Leaf sheath	374c
97-4-12-2-1			Leaf	371c
97-4-12-2-2		*Setaria pumila*	Leaf sheath	198/202c
97-4-12-2-2			Leaf	371c
97-4-12-2-3		*Setaria pumila*	Leaf sheath	198/202c
97-4-12-2-3			Leaf	371c
85-10-12-6-1		*Panicum sumatrense*	Leaf sheath	366c
85-10-12-6-1			Leaf	370c
85-10-12-6-2		*Panicum sumatrense*	Leaf sheath	203c
85-10-12-6-2			Leaf	370c
85-10-12-6-4		*Panicum sumatrense*	Leaf sheath	203c
85-10-12-6-4			Leaf	370c
85-10-19-3-1		*Panicum sumatrense*	Leaf sheath	366c
85-10-19-3-1			Leaf	370c
85-10-27-3-9		*Panicum sumatrense*	Leaf sheath	366c
85-10-27-3-9			Leaf	370c
85-10-31-3-1		*Panicum sumatrense*	Leaf sheath	203c
85-10-31-3-1			Leaf	370c
12-11-19		*Setaria viridis*	Leaf sheath	209c
12-11-19			Leaf	371c
12-11-19		*Setaria viridis* (dried)	Leaf sheath	5195c
12-11-19			Leaf	5185c

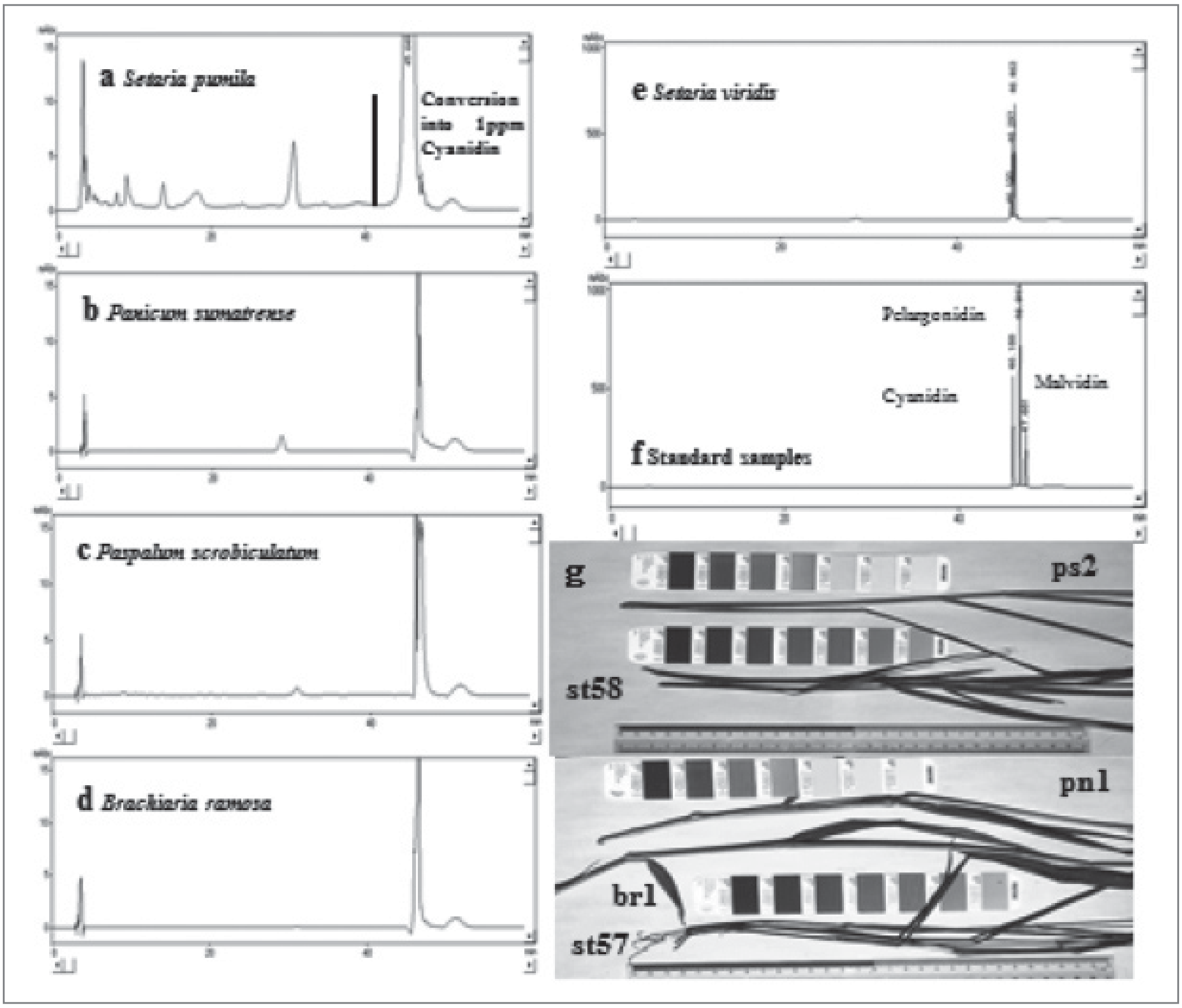

Fig. 5. HPLC analysis and comparison between plant pigmentations through PANTON Formula Guide. a) *S. pumila*, b) *P. sumatrense*, c) *Pas. scrobiculatum*, d) *B. ramosa*, e) *S. viridis*, f) standard samples and g) in a field of *Pas. scrobiculatum*: ps2, *Pas. scrobiculatum*; st58, *S. pumila*; in a field of *P. sumatrense*: pn1, *P. sumatrense*; br, *B. ramosa*; st57, *S. pumila*.

a small peak (Rt around 30) except for some occasional peaks (around 45.6). However, that of *B. ramosa* showed no peak at all around Rt (30) (Fig. 5d). Only the Rt (46.1) of anthocyanin contained in *S. viridis* (dried matter, Fig. 5e) concurred with natural cyanidin's Rt (46.2), but most of the Rts of anthocyanins contained in samples did not coincide with the Rt of three natural pigments (Fig. 5f). Therefore, cyanidin was used as an indicator of anthocyanin content. By means of cluster analysis (Ward method), these samples were categorized into two groups as illustrated in Fig. 6. Cluster I consisted of five samples (st56~st60), which were only the leaf sheath of *S. pumila*. The color was reddish purple (198/202/205/206/207c in the PANTONE Formula Guide). Sub-cluster IIa (11 samples) consisted of the green leaf of *S. pumila* (1, st57), the green leaf (1, ps1) and leaf sheath (2, ps1 and ps2) of *Pas. scrobiculatum*, which were reddish purple color (208/209c), the leaf sheath of *P. sumatrense* (3, pn1-reddish purple, green pn2 and the other), the green leaf sheath of *B. ramosa* (1, br1) and the green leaf (1) and leaf sheath (2) of *S. viridis*. Moreover, Sub-cluster IIb (20) consisted of the green leaf of *S. pumila* (4, st56, st58, st 59 and st60), *Pas. scrobiculatum* (1, ps2), *S. viridis* (1), *P. sumatrense* (8, pn1, pn2 and so on), and *B. ramosa* (1, br1).

Seed color mimicry was found in the mixed grains of *P. sumatrense* and *S. pumila* at Illur village near

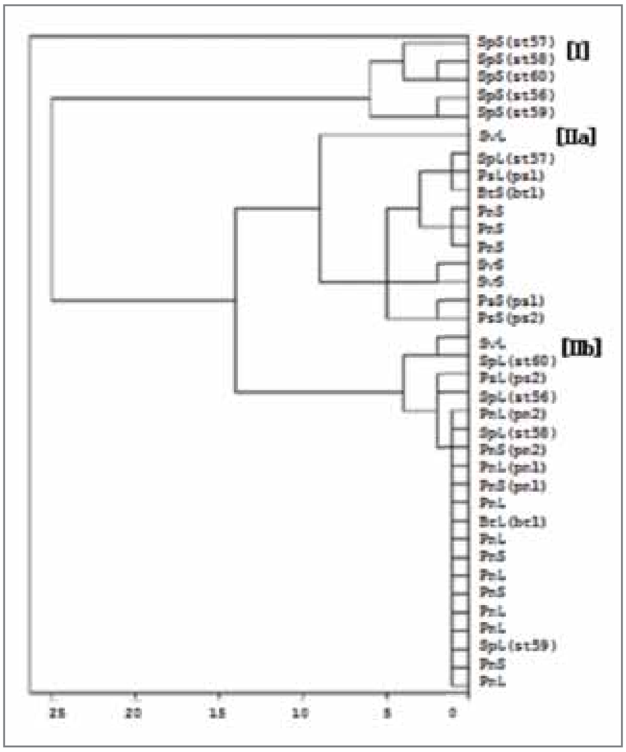

Fig. 6. Dendrogram based on the pigmentation of leaf and leaf sheath in five species.

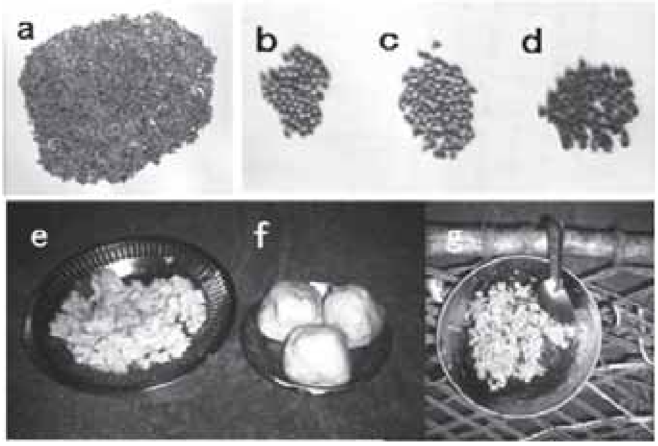

Fig. 7. Seed mimicry of *S. pumila* to *P. sumatrense* and three foods made from their mixed grains at Jalaripali village, Andhra Pradesh: a) mixed grains harvested and sold; after identified them, b) brown seeds of *P. sumatrense*, c) brown seeds and d) black seeds of *S. pumila*; e) *annamu* (boiled grain), f) *sankati* (thick flour porridge) and g) *uppitu* (semi-solid porridge).

Chittoor in Andhra Pradesh as shown in Fig. 7a. The seed grains of *P. sumatrense* (Fig. 7b) had a very similar pale brown color to the seed grains of *S. pumila* (Fig. 7c), except for their black seed grains (Fig. 7d). However, it was possible to distinguish the seed grains of *P. sumatrense* from those of *S. pumila* in detail, because of their glossy lemmata. Here, villagers have made three foods from *S. pumila*. They are boiled grain, *annamu* (Fig. 7e), flour porridge, *sankati* (Fig. 7f) and semi-solid porridge, *uppitu* (Fig. 7g).

Discussion

The domestication process of *S. pumila* may have passed through four steps as illustrated in Fig. 8. The first step was a weed that had grown along roadsides and other unstable habitats and then invaded upland rice fields. The second step was an evolutionary process to obtain an agro-ecological niche out of weed status, using for fodder, in order to get the companion weed status growing in upland rice and some millet fields. The third step was a process of advancing from the mimic companion weed status to a semi-domesticated and insurance crop, used in case of famine, under mixed cropping with *Pas. scrobiculatum*, *E. coracana*, and *P. sumatrense*. The weed types after their invasion into upland rice and millet fields obtained mimicry associated with a particular crop, and made a close weed-crop complex under the severe weed control measures practiced by farmers. In the fourth and final step, the mimic companion weed forms were used not only a fodder source for cattle, but also as a supplementary grain to the main cereal species. In the case of *S. pumila*, too strict weeding was avoided as a means of crop insurance in years of extreme drought in the Deccan Plateau. This possibly led to *S. pumila* growing taller with larger spikes and large seeds, accompanied with less shattering, and gradually progressing toward domestication. Actually, *S. pumila* has obtained mimic traits such as long leaf, a few tillers, and tall plant height in the field of *P. sumatrense* as shown in Figs. 3 and 4. The morphological and ecological characteristics of the 60 strains in *S. pumila* were very variable, as shown in Fig. 3. The plant height (cm) indicated a trend toward higher tallness in the domesticated type than in the weed type (Fig. 3a). The number of tillers showed a slight reduction in the domesticated type compared to the weed type, excluding two exceptional strains (Fig.

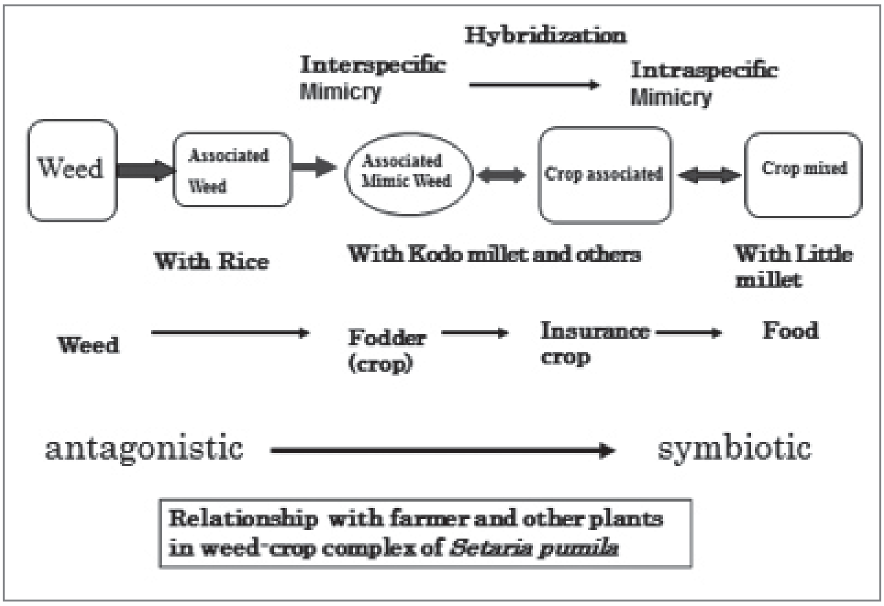

Fig. 8. Domestication process of *Setaria pumila*

3b). The spike length (cm) clearly increased more in the domesticated type than in the weed type (Fig. 3c). The duration to flowering (days) showed a bimodal pattern: early and late (Fig. 3d). The early domesticated type was similar to the weed type, while the late domesticated type was similar to the mimic companion weed type mixed with other cereals. The length/width ratio of the flag leaf showed two trends, wide and slender, as shown in Fig. 3e. The last internode diameter (mm) was clearly thick in the domesticated type than in the weed type (Fig. 3f). The domesticated type mixed with *P. sumatrense* had a higher ratio than the others, which had a medium ratio. It was morphologically clear that spikes had become longer and the last internode diameter had become bolder in the domestication process.

It was very difficult for farmers to detect the difference among mimic companion weeds and domesticated types in sympatric fields during the early growing stage, because at this stage they resemble each other very closely. The seed germination of domesticated and annual plants was usually rapid and uniform, but that of *Pas. scrobiculatum*, perennial plants, and weeds was a little late and irregular.

At the same time, *S. pumila* diversified their traits in totality through hybridization among the types under natural and artificial selection in the severely arid environment. The mimic companion weeds were harvested together with other millet, and sown involuntarily again next season. In the third step, farmers changed their hostile weed control into a friendly one. Recently, during the fourth step, this situation was followed by mixed cropping. *S. pumila* is termed a tertiary crop in relation to its associated plants, which were secondary crops, such as *P. sumatrense*, *Pas. scrobiculatum*, with respect to rice.

The species ratio composition of each mixed cropping field was variable annually, according to some field conditions such as summer precipitation (Table 3). At the early growing stage, it was very difficult to recognize morphological differences from each other in a set of plant species collected from the same cropping field. The inter- and intra-specific mimic variation had occurred not only in the morphological characteristics e.g., plant height, leaf size and number of tillers, but also in the ecological ones, e.g., seed germination pattern, seed color, and leaf sheath color. It was clear that the leaf sheath color of *P. sumatrense* and *Pas. scrobiculatum* (Sub-cluster IIa) was mimicry related to that of *S. pumila* (Cluster I) as shown in Fig. 5. The glume color of *S. pumila* was also mimicry of the glume of *P. sumatrense*. The domestication process and dispersal of *S. pumila* has developed by means of elaborate mimicry in several characteristics of other crops to itself as shown in Fig. 5, 6, and 7.

Pas. scrobiculatum is perennial and the seed germination, tillering, and plant height elongation are usually slow. Therefore, *Pas. scrobiculatum* was distinguished relative to the other species. Among others, because these characters were synchronized to each other, it was very difficult to distinguish them, especially plant height and the number of leaves on main culm in the early growing stage. The domesticated type of *S. pumila* was adjustable to its associated mimic species in morphological characters, ecological traits in early growth, and plant pigmentation as shown in Table 4 and Fig. 6. It indicated mimicry of leaf and leaf sheath by way of color among *S. pumila*, *Pas. Scrobiculatum*, and *P. sumatrense*, but not *B. ramosa*. However, both leaf color of *S. pumila* (Ds) and *P. sumatrense* were the same green (371c~377c), while the leaf sheath color of *S. pumila* was reddish purple (198c~202c) in spite of a finding of green color (206c~207c) in *P. sumatrense* at site 8 in Andhra Pradesh. This case did not indicate mimicry of leaf sheath color.

Moreover, *S. pumila* has been domesticated as a tertiary crop, by way of the other millet species, to upland rice, because it has built up the strongest resistance to frequent droughts in the Deccan Plateau. The domesticated type of *S. pumila* is commonly cultivated in mixed stands mostly along with *P. sumatrense* in South India today, while the semi-domesticated crop and mimic companion weed types are not only grown with *P. sumatrense*, but also mainly with *Pas. scrobiculatum*, *E. coracana*, and upland rice in diverse agro-ecological niches in Orissa. The weed type of *S. pumila* grows widely through the India subcontinent and tropical and temperate Northern Hemisphere. The various types have adapted to arid conditions and agro-ecological niches in the Deccan Plateau during their distribution from eastern humid areas to southern dry areas in the Indian subcontinent (Sehgal et al. 1992).

The domesticated types of *S. pumila* have broadly promoted the biocultural diversity through the mimicry of other grain crops. The domesticated type of *S. pumila* was always sown, harvested, and consumed together with *P. sumatrense* as shown in Table 7e, 7f, and 7g. Farmers called the mixed grains *tela samuru* (meaning white little millet) in Telugu. The proportion of grains purchased at a local market was 70% of *P. sumatrense* and 27% of *S. pumila*, respectively, in 1997 (Kimata et al. 2000). These authors are of the opinion that in severe drought, *S. pumila* provides a reasonable harvest while *P. sumatrense* might fail completely. This situation recommends the domestication process of secondary crops in other cereal fields against an arid climate. These crops are used to make six traditional foods in total, including *ganji* (thin flour porridge), *kheer* (sweet gruel) and *roti* (unleavened bread), as a supplementary ingredient (Kimata and Sakamoto 1992, Kimata et al. 2000).

Furthermore, this domestication process is supported by the linguistic recognition of their various vernacular names by farmers. For example, a mimic companion weed or semi-domesticated plant occurring with *Pas. scrobiculatum* was called *varagu korali*, meaning just the same as kodo millet, while a mimic companion weed or semi-domesticated plant occurring with *P. sumatrense* was called *samuru korali*, meaning also just the same as little millet. The domesticated type was called various vernacular names (e.g., *korati, kora samuru, korin*) in each local language and region. The linguistic differentiation shows a close relationship to the domestication process (Chandra and Koppar 1990; Kawase 1987; Kimata et al. 2000, Kimata 2015c; Kobayashi 1987, 1989).

B. ramosa is cultivated under pure single cropping as a sole tertiary crop, while *S. pumila* is still cultivated under mixed cropping with *P. sumatrense* as a minor domesticated plant. *B. ramosa* tolerates drought better than *S. pumila*, and has been undergoing a specializing adaptation to arid regions, and has nearly attained the tertiary domesticated phase. On the other hand, the landraces of *S. pumila* have adapted to drier fields in South India than in Orissa. *S. pumila* was almost always grown with *P. sumatrense*, but seemed to grow singly when the later fail to grow in severe droughts, as was observed in our survey of 1987. This possibly suggests that *S. pumila* could become an independent crop.

In this case of multiple polyploidy species, *S. pumila* indicated that diversity had been increased mainly by mimicry and inter- and intra-specific hybridization under natural and artificial selection. This domestication process has been promoted as a means of imparting adaptation to an arid climate, and also produced a symbiotic relationship among weeds, other crops, and farmers, while departing from an antagonistic one. The domestication process of *S. pumila* indicates the importance of weed-crop complexes and biocultural diversity as a plant-man symbiosis. The diversity of *S. pumila* and its relatives should be conserved both in *situ* and *ex situ*, especially in on-farm conservation sites.

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