Biodiversity and Conservation **1**, 170–178 (1992)

Bimodal phenotypic structure of two wild pearl millet samples collected in an agricultural area

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Received 24 October 1991; revised and accepted 28 January 1992

On the basis of botanical and ecological characters, pearl millet species have been separated into three subspecies: *Pennisetum americanum subsp. americanum*, cultivated form, *P. americanum subsp. monodii*, wild form and *P. americanum subsp. stenostachyum*, weedy form. It has been

stenostachyum form has a morphology intermediate between those of the other two subspecies.

In the Sahelian agricultural area, wild pearl millet populations often appear as a mixture of monodii and stenostachyum forms. It was therefore decided to collect separately bulk samples of seeds from monodii plants and from stenostachyum plants. Tostain (1991) has observed on such samples that in each part of the Sahel, from Mauritania to Sudan, monodii seeds from agricultural areas have an enzyme composition very close to that of monodii seeds from the pastoral area, but significantly different from that of local cultivated millets.

Two monodii samples collected in agricultural areas, one in Senegal, the other in Niger, have been analysed for botanical and enzyme characters in order to test Brunken's typology, to estimate the hybridization rate with cultivated millets, and to understand how monodii plants maintain their genetic identity when growing among cultivated millets.

Materials and methods

The Senegalese monodii sample was collected in 1987 near Saint-Louis in a hybrid swarm bordering gardens. The monodii sample from Niger was collected in 1985 in the neighbourhood of Keïta, in a hybrid swarm stretching several hundred metres along a streambed. The seeds were sown at Sadoré, Icrisat Sahelian Centre in Niger in March 1990, to obtain and observe about 60 plants per sample. These plants were observed for the heading date (CY) and the following botanical characters:

LT main stem height,
VG foliar limb pubescence (presence: 1, absence: 0),
LF length of the third limb from the flag,
lf breadth of the third limb,
LC main spike length,
DR main spike rachis diameter,
LPI length of the involucre stalk,
LS length of the involucral bristles,
AR length of involucral terminal bristle,
LGL lower floret lemma length,
NE number of spikelets per involucre,
LG seed length,
DG seed breadth,
VI seed endosperm texture, and
OS involucre bristle structure: glabrous, ciliate, plumose, cotton-wool.

Individual plant genotypes were determined for the six following loci: Esterase, Est A; Alcohol dehydrogenase, Adh A; Phospho-glucomutase, Pgm A; Glutamate oxalo-acetate transaminase, Got A; Phospho-gluconate dehydrogenase, Pgd A; Catalase, Cat A; according to the methods described by Tostain *et al.* (1987).

Monodii controls were chosen in the Niger pastoral area: one sample from Gourma plateau, five from Azaouak valley and one from Tiguidit cliff. Hybrid controls were obtained by crossing these monodii controls with local cultivated varieties from Niger.

The groupings within each sample were investigated by principal component analysis and discriminant analysis (STAT-ITCF software, Institut Technique des Céréales et des Fourages, Paris, France). The enzyme variables used were allele frequencies. Principal components were computed on the correlation matrix for morphological characters and the covariance matrix for enzyme frequencies. Joint analysis of morphological and enzyme characters was performed after standardization such that the first principal components of each set had the same variance.

For each identified group, the following statistics were computed: The fixation index of a locus F_i

$$F_i = 1 - h_i/P_i$$

where h_i represents the observed number of heterozygous plants and P_i the expected number under panmixia.

The hypothesis ($F_i = 0$) was checked by the chi-square test with 1 d.f.

$$\chi^2 = F_i^2 N \text{ (Hedrick, 1985)}$$

where N is the total number of individuals in the group.

The multilocus fixation index F was estimated by maximum likelihood, as recommended by Weir and Cockerham (1984)

$$F = 1 - \Sigma_i h_i/\Sigma_i P_i$$

The hypothesis ($F = 0$) was checked using a 1 d.f. chi-square test, comparing the total number of observed heterozygotes to the total number expected under panmixia:

$$\chi^2 = F^2 NLP_o/(NL - P_o)$$

where L is the number of loci:

$$P_o = \Sigma_i P_i$$

The multilocus F of both groups was compared by computing their approximate variance (Ritland, 1983) extended to all the loci:

$$\text{Var}(F) = (1 - 2F)(1 - F)^2/NL + F(1 - F)(2 - F)/P_o$$

The F_i heterogeneity test was computed by the chi-square test with $L - 1$ d.f., by comparing the observed distribution at the six loci and the expected distribution with the same multilocus F at the six loci.

Two loci gametic disequilibrium was tested by the correlation coefficient of allelic frequencies, which corresponds to the test of the composite measure of Weir (1979). The mean gametic disequilibrium on pairs of loci was tested by the multilocus measure of Brown *et al.* (1980), i.e. the observed variance of the number of heterozygous loci in each individual compared to the variance expected by the hypothesis of random association of alleles.

Let h_j be the locus j diversity

$$h_j = 1 - \Sigma_i P_{ji}^2$$

P_{ji} being the frequency of allele i.

With random association of alleles, the variance $s^2k_{/Ho}$ is estimated by $s^2{}_{k/Ho} = \Sigma h_j - \Sigma h_j^2$. The variance of $s^2{}_{k/Ho}$ is estimated by:

$$\mathrm{Var}(s^2{}_{k/Ho}) = \{\Sigma h_j - 7\Sigma h_j^2 + 12\Sigma h_j^3 - 6\Sigma h_j^4 + 2(\Sigma h_j - \Sigma h_j^2)^2\}/N$$

where N is the number of individuals.
The multilocus gametic disequilibrium is significant if the observed $s^2{}_k$ is greater than $s_k{}^2{}_{/Ho} + 2\{\mathrm{var}(s^2{}_{k/Ho})\}^{1/2}$.

Results

Structure of the Senegalese sample

The range of variation of the first principal component computed on morhological characters has been split into discrete equal interval classes. The frequency distribution along these classes shows two groups marked by two modes (classes 1 and 4) and foliar limb pubescence (Table 1). The first group (SS) has a pubescent limb (classes 1, 2 and one plant in class 3). The second group (HH) (classes 3, 4, 5, 6) has a glabrous limb except the extreme individual of class 6. Controls show that the SS group is monodii-like and the HH group is similar to hybrids (stenostachyum-like). The foliar limb pubescence marking fits well with available general information on Senegalese millets. Bono (1973) mentions that cultivated millets usually have a glabrous limb whereas wild millets have a hairy limb. Farmers cull out hairy plants from the field to protect the crop against the invasion of wild germplasm. Moreover, it is known that glabrous limb is controlled by a dominant allele. That locus is linked to grain shedding, involucre stalk length and Got locus but not to grain size or to spike rachis size, which are other essential characters of domestication syndrome (Marchais and Tostain, 1985). It is to be noted that controls are not classified according to limb hairiness because that character is found in Niger on cultivated and wild millets as well (Table 2).

Heading in the SS group occurred about one month later than in the HH group (Table 2). Although this interval may differ from the one in the rainy season, it is possible that flowering asynchronism exerts an important effect on the isolation of the monodii type from hybrids and cultivated millets.

Structure of Niger sample

The morphological distribution according to the first principal component is composed of a majority of monodii-like plants followed by a long tail of hybrid-like plants (Table 3). A minimum appears in class 5. Joint discriminant analysis of morphological and enzyme

Table 1. Senegalese sample: principal component analysis on morphological characters
First component frequency distribution. Controls as additional elements.

Class	1		2		3		4		5		6		Total
Limb pubescence	V	G	V	G	V	G	V	G	V	G	V	G	
Senegalese sample	23	0	14	0	1	4	0	9	0	3	1	0	55
Monodii controls		4		3		0		0		0		0	7
Hybrid controls		0		0		0		3		3		0	6

Limb hairy: V, Glabrous: G.

Table 2. Means for heading dates and morphological characters of groups identified in the wild samples from Senegal and Niger.

Character	Controls		Senegal		Niger	
	Monodii	Hybrid	SS	HH	SS	HH
CY	49	70	112	82	90	83[a]
LT	104	163	130	161	132	186
NT	46	33	35	24	31	18
VG	1	0.78	1	0.06	0.59	0.58[a]
LF	37	44	38	50	39	47
lf	15	26	13	25	19	27
LC	12	27	10	26	15	29
DR	10	26	9	25	15	31
Ipi	0.4	5.1	1	4.3	1	3.5
LS	104	68	69	56	72	83
AR	19	7	28	9	16	23[a]
GL	57	47	51	44	45	47[a]
NE	1	1.6	1	2	1.1	1.8
LG	26	29	23	26	24	30
DG	13	18	12	16	12	17
VI	3.3	2	3.4	2.5	2.7	1.5
OS	3	1.4	1.8	1.5	1.8	1.4

[a]Difference not significant at 5% level between SS and HH.

Table 3. Wild Niger sample: principal component analysis on morphological characters. First component frequency distribution. Controls as additional elements.

Class n°	1	2	3	4	5	6	7	8	9	10	11	12	13
Wild Niger	10	15	11	11	3	5	4	1	1	0	1	0	1
Monodii controls	0	3	2	2	0	0	0	0	0	0	0	0	0
Hybrid controls	0	0	0	0	0	1	1	3	1	0	0	0	0

characters improves differentiation of the two groups (Table 4). The first SS group (50 plants) includes the previous morphological classes (1 to 5). The second HH group (12 plants) includes the previous morphological classes (6 to 13). The cut-off point seen in class 8 of Table 4 agrees well with the location of controls.

Heading date and limb pubescence play no part in discrimination of the two groups (Table 2).

Inside the HH group, fixation indexes are generally negative but non-significant (tests are not powerful due to small sample size) (Table 5). The multilocus F of the HH group is significantly lower than that of the SS group. This agrees well with the fact that in a hybrid group like-HH allelic frequencies differ between male and female gametes. In contrast, fixation indexes in the SS group show a tendency to be positive, although tests

Table 4. Wild Niger sample: Joint discriminant analysis on morphological and enzyme characters. Frequency distribution along the discriminant axis. Controls as additional elements.

Class n°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Wild Niger	6	8	9	11	4	7	6	0	1	3	3	1	1	1	0	1	0	0	0	1	62
Monodii controls	0	2	1	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Hybrid controls	0	0	0	0	0	0	0	1	0	1	0	3	1	0	0	0	0	0	0	0	6

Table 5. Niger sample: fixation index for each locus and multilocus of the SS and HH groups.

Group	Est A7	Adh A4	Pgm A2	Got A1	Pgd A1	Cat A1	F multilocus	Heterogeneity test probability
SS	0.03	−0.2	0.28[a]	0.30[a]	−0.01	0.11	0.073(0.066)	0.24
HH	−0.27	−0.17	−0.2	−0.14	−0.14	0.17	−0.108(0.029)	0.93

[a]Significant at 5% level-standard error in parentheses.

Table 6. Niger sample: correlations between heading date (CY) and enzyme markers in the two groups: SS (below the diagonal) and HH (above the diagonal).

HH / SS	CY	Est A7	Adh A4	Pgm A2	Got A1	Pgd A1	Cat A1
CY	—	−0.051	0.384	0.056	−0.098	0.313	−0.154
Est A7	−0.139	—	−0.151	−0.666[a]	−0.25	0.25	−0.431
Adh A4	0.002	0.514[a]	—	−0.201	0.255	0.25	−0.19
Pgm A2	0.094	−0.014	0.086	—	0	−0.408	0.522
Got A1	−0.007	0.002	−0.215	−0.046	—	−0.556	−0.104
Pgd A1	−0.13	0.125	0.028	0.379[a]	−0.133	—	−0.217
Cat A1	−0.273	0.081	0.097	0.099	0.107	−0.022	—

[a]Significant at the 5% level.

Table 7. Niger: enzyme allele frequencies of SS and HH groups compared to that of wild and cultivated millets from Niger

Group	Total	Est A7	Adh A4	Pgm A2	Got A1	Pgd A1	Cat A1
Pastoral monodii	9	24.2	64.4	100	39.3	76.2	55
Agricultural monodii	8	23.3	61.9	95.4	25.6	69.5	64.4
SS	50	50.4	36.7	87.3	22	68	77.7
HH	12	19.6	15.4	83.3	12.5	87.5	72.1
Cultivated	18	26.5	36.8	51.3	0	86.7	69.6

Table 8. Multilocus measure of gametic disequilibrium.

Group	S^2k/Ho	Observed s^2k	L 5%
SS	1.35	1.14	1.84
HH	1.15	2.75[a]	2.06

[a]Above L 5%, significant at 5% level.

Table 9. Backcross V × (T × V): principal component analysis on morphological characters. First component frequency distribution. Controls as additional elements.

Class n°	1	2	3	4	5	6	7	8	9	10	11
Backcross	2	5	11	3	12	9	3	2	1	1	1
Control V.	2	6	0	0	0	0	0	0	0	0	0
Control F_1 V × T	0	0	0	0	0	0	0	1	1	6	2

V: Senegalese monodii inbred line-T cultivated inbred line from Tiotande.

are only significant for Got and Pgm. The test of Fi heterogeneity is not significant. This trend may express a vicinism effect: in the SS group, crosses have occurred mainly between related neighbouring plants. Heading date does not explain this apparent inbreeding. No correlation is significant between heading date and enzyme genotype (Table 6).

Correlations between allele frequencies show no evidence of any clear gametic

Curiously, no trace of recombinant pollens appears, as if the many hybrids produced each year did not contribute to the active pollen population. This fact cannot be explained by the hypothesis that the whole domestication syndrome is controlled by a unique gene. Previous observations have been made upon a cross between a wild Senegalese line (called Violaceum) and a cultivated line (Tiotande) (Marchais and Tostain, 1985). The morphological segregation then observed in the backcross monodii × (cultivated × monodii) shows a continuum with a mode of recombinant phenotypes surrounded by a few parental phenotypes at each extremity (Table 9).

This absence of recombination between wild and cultivated gametes is currently unexplained: why do Senegalese monodii plants not possess the glabrous limb gene? Why in Niger is the Got A1 allele not eliminated from monodii plants?

Conclusion

The current results show that Brunken's typology is accurate under natural conditions although it has not been observed in controlled crosses studied to date. This fact expresses the isolation of monodii plants from cultivated germplasm, as observed by Tostain (1991) in enzyme electrophoresis of seeds.

The underlying mechanism remains to be discovered. Genetic barriers are not strong enough to prevent annual production of monodii × cultivated hybrids in wild populations. Other genetic and ecological factors must intervene to eliminate hybrids, for instance, pollen competition to the advantage of wild pollen (Marchais and Tostain, 1985), flowering asynchronism, predation of hybrid plant seeds (bigger and less enclosed in floral bracts than monodii seeds) by birds, rats, ants etc.

The search for more precise information cannot be conducted within the framework of traditional germplasm collecting missions as described in Tostain *et al.* (1986). Specific missions must be organized during successive years at a few selected sites where wild and cultivated millets grow sympatrically. These missions will allow analysis of adult plants *in situ* and their offspring in station for morphological and enzyme characters, using methods described by Allard, Clegg, Brown, Weir, Ritland, etc. (Brown *et al.*, 1990). However, many practical and technical difficulties are likely to be encountered during *in situ* analyses at remote sites (hundreds of kilometers from laboratories).

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