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Evidence of a partial reproductive barrier between wild and cultivated pearl millets (*Pennisetum glaucum*)

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Summary

The occurrence of seed malformation in association with reduced thousand grain weight and germination ability has been observed in crosses between cultivated female plants and wild male plants. A survey of 16 cultivated accessions (*P. glaucum* subsp. *glaucum*) and 11 wild accessions (*P. glaucum* subsp. *monodii*) ranging over the whole species diversity showed this postzygotic incompatibility was general, but its intensity varied greatly with the cultivated female accession used and very little with the wild male parent origin. About 15% of the 123 cultivated × wild crosses observed gave normal seeds. Seed malformation has never been observed in crosses between cultivated accessions and appeared independent of genetic distances between the parents. The reciprocal crosses between wild female plants and cultivated male plants gave normal-looking seeds with good germination but consistently reduced thousand grain weight. Both seed malformation and seed small size are an expression of a genetic imbalance. These slight reproductive barriers seem to have been built during the domestication process.

Abbreviation: ICRISAT – International Crop Research Institute for the Semi-Arid Tropics

Introduction

As a general rule, wild and cultivated pearl millets can be crossed easily with a high seed set (Bilquez & Lecomte, 1969; Brunken, 1977; Pernès et al., 1980; Mohindra & Minocha, 1991). F_1 hybrids are vigorous and fertile with normal meiosis. No barrier has been till now observed to gene flow between both forms. But in the course of 1988, at Niamey (Niger), during unrelated experiments on crosses between cultivated plants of pearl millets used as female and wild plants used as male, many cases of abnormal seeds were observed too frequently to be attributed to environmental causes. The abnormal seeds were small, crumpled and germinated badly. A decisive observation was made on a heterogeneous seed bulk harvested on a cultivated spike pollinated by a wild plant. This seed bulk could be easily divided into two parts: one with crumpled seeds and one with normal plump seeds. Enzymatic analysis of esterases showed that all the plump seeds were selfed and all the crumpled seeds were hybrids indicating that seed malformation could express some post zygotic incompatibility between cultivated and wild subspecies of pearl millet.

A systematic study was then started in order to

estimate the frequency and intensity of this phenomenon in cultivated × wild crosses, but also in cultivated × cultivated, wild × wild and wild × cultivated crosses.

Materials and methods

A set of 16 cultivated and 11 wild accessions (Table 1) was chosen in order to include the main geographical and enzymic groups of millets as determined by Tostain (Tostain & Marchais, 1989; Tostain, 1992). Tostain's data (allelic frequencies at 12 loci coding for 8 enzyme systems) were used to compute Nei's distance between each pair of accessions. Wild accessions (W2, W6, W7, W10 and W11) were collected in pastoral areas devoid of cultivated millets, and including exclusively wild plants (i.e. spontaneous and no trace of domesticated characters) true to *P. glaucum* subsp. *monodii* (Brunken, 1977). The other wild accessions were collected on monodii plants in spontaneous populations growing be-

Table 1. Characteristics of the accessions used in crosses and the type of crosses in which they are involved

Botanical type	Enzymatic group	Code	Origin	Degree of inbreeding	Type of crosses
Pennisetum glaucum subsp. *glaucum* (cultivated)	West Africa early maturing	C1	Senegal	So	a b c d
		C2	Mali	inbred	a b d
		C3	Mali	So	a b d
		C4	Niger	inbred	a b d
		C5	Niger	So	a b c d
		C6	Togo	inbred	a b d
	West Africa late maturing	C7	Mali	So	a d
		C8	Mali	inbred	a d
		C9	Niger	So	a b c d
		C10	Togo	inbred	a b d
	East Africa early maturing	C11	Sudan	So	a b d
		C12	Chad	So	a b d
	Southern Africa early maturing	C13	Zambia	So	a b c d
		C14	Botswana	So	a
	Southern Africa late maturing	C15	Tanzania	inbred	a b d
	India early maturing	C16	Gujerat	So	a b d
Pennisetum glaucum subsp. *monodii* (wild)	Western	W1	Senegal	So	a b c e
		W2	Mauritania	So	a b c e
		W3	Mali	So	a c e
	Centre	W4	Niger	So	a b c e
		W5	Niger	So	a b e
		W6	Mali	So	a b c e
		W7	Mali	So	a c e
		W8	Burkina Faso	So	c e
	Western Chad	W9	Chad	So	a b c e
	Darfour	W10	Chad	So	a b e
		W11	Sudan	So	a b e

So: original sample, a = C × W, b = Two-way ANOVA, c = W × C, d = C × C, e = W × W.

side cultivated fields. Their offspring segregate (Marchais & Tostain, 1992) into a majority wild (monodii) group and a minority group comprising botanical types exhibiting domesticated characters at a level intermediate between wild and cultivated types, classified by Brunken (1977) as *P. glaucum* subsp. *stenostachyum*. In the current study, only wild offspring were used.

A first experiment using the local cultivar C5 as female and the pastoral wild sample W6 as male compared a set of C5 × W6 crosses to a set of intracultivar C5 × C5 (C×) crosses and to a set of selfed seeds (Cs). The harvested seeds of each crossing were assessed for morphological appearance (PHENO = 1: normal, 2: defective, 3: very defective), the 1000 grain weight in grams (P1000), and germination rate measured in Petri dishes on 150 seeds (GERM).

A second experiment was designed in order to make the maximum number of crossings without repetition between all the cultivated (C) and wild (W) accessions of Table 1. The plants were grown at the ICRISAT station of Niamey from September to December 1989. The crosses obtained were: 123 C × W, 13 C × C, 13 W × C and 27 W × W. In addition, each cultivated accession was observed at least on a selfed spike (Cs) and sometimes on intracultivar crosses (C×). This set of crosses includes a subset of 12 cultivated accessions each pollinated by the same 8 wild accessions allowing a two-way ANOVA (analysis of variance) with one repetition. The variables recorded were the same as in the first experiment.

The materials harvested on cultivated plants and those harvested on wild plants were analyzed separately. A synthetic variable expressing the degree of seed malformation was sought by principal component analysis of the materials described by the 3 variables PHENO, P1000 and GERM. In practice, the first principal component (CP1) was found to be adequate. Each cross was represented by the difference from its 'normal' mother value (CP1-T) as follows: a reference value T was computed for each mother as the mean value of its selfed samples (Cs). Statistical treatments were applied to these adjusted values (CP1-T). In this way, the effects of different males on different females, distinct in terms of the original variables, could be compared. The statistical analyses were performed using STAT-ITCF software (Institut Technique des Céréales et des Fourrages, 8 avenue du Président Wilson, 75116 Paris).

Results

Crosses C × W and C × C

The principal component analysis dealing with all the C × W, C × C, C× and Cs families gave a CP1 opposing the C × W crosses to other families (Tables 1 and 2), whereas the second component displayed an intra-family variation (not shown). The degree of seed malformation PHENO was correlated moderately (– 0.58) with P1000 and highly with GERM (– 0.82). CP1 was highly correlated with PHENO (– 0.91), P1000 (0.82) and GERM (0.93). CP1 expressed 79% of the total variance.

C5 × W6 cross

The families C5 × C5 and C5s displayed similar distributions (means and standard errors) for PHE-

Table 2. Frequency distribution of the family C5s, C5×, and C5 × W6 in the classes cut along the variable CP1-T. Means with standard errors in parentheses of the 3 original variables and of CP1-T. N = number of crosses

Family	Classes for CP1-T																N	Pheno	P1000	Germ	CP1-T
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21					
C5s													1	1	3		5	1	6.36 (1.1)	0.93 (0.04)	0 (0.26)
C5×											1	1	0	1	1	1	5	1	7.66 (1.3)	0.85 (0.19)	– 0.22 (0.48)
C5 × W6	4	3	7	4	1	1											20	3	4.06 (0.7)	0.36 (0.10)	– 2.84 (0.33)

Table 3. Frequency distribution of the families Cs, C×, C × C, and C × W in the classes cut along the variable CP1-T. Means with standard errors in parentheses of the 3 original variables, of CP1-T and of D, the NEI's distance between parents of a cross. N = number of crosses

Family	Classes for CP1-T																							N	Pheno	P1000	Germ	CP1-T	D
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23						
Cs																3	1	3	1	3	5	2	1	19	1	7.6 (2.6)	0.89 (0.08)	0 (0.3)	
C×																1	1	2	1	1	1			7	1	8.02 (1.2)	0.89 (0.17)	−0.24 (0.4)	
C × C															2	0	0	0	3	4	3	1		13	1	8.59 (2.4)	0.87 (0.12)	0.047 (0.5)	0.07 (0.04)
C × W	4	8	9	5	11	10	4	12	8	7	6	7	5	4	4	7	1	2	1	1	1	1		123	2.46 (0.7)	5.35 (2.29)	0.36 (0.31)	−2.55 (1.3)	0.09 (0.05)

NO, P1000, GERM and CP1-T, which characterize the normal seeds and which differed strikingly from the C5 × W6 distributions (Table 2). The 20 C5 × W6 repetitions gave very crumpled seeds with low germination rates and 1000 grain weight. Qualitatively, the same level of seed malformation was observed whichever individual plants were used in both accessions. Clearly, seed malformation had a genetic origin. This first experiment corroborated the existence in a particular cross of post zygotic incompatibility between cultivated and wild plants.

Global analysis of the C × W and C × C crosses

The hybrid constitution of all the C × W crosses was checked by observing in the field about 10 plants per cross for botanical characters: all the plants observed were true hybrids. Hence, every normal seed observed in a C × W cross may not be attributed to wrong crossing.

The families Cs, C× and C × C gave normal seeds with similar (CP1-T) distributions (Table 3). Consequently, a common error variance was estimated for these 3 families: $s^2 = 0.21$ with 23 df. This variance led to an estimate of 2.5% for the probability of a C × C cross being distributed in a class with a number less than 15. All the C × W crosses distributed in the classes numbered from 1 to 14 can therefore be considered abnormal. Hence, only 18 C × W crosses out of 123 (about 15%) may be said to be normal i.e. similar to C × C crosses.

Table 4. Characteristics of the C × W crosses not different from C × C crosses. The limit at 5% level of significance the C × C crosses is CP1-T = − 0.97

Crosses	Pheno	P1000	Germ	CP1-T
C1 × W1	1	8.60	0.70	− 0.52
C1 × W5	1	5.30	0.96	− 0.72
C2 × W5	1	9.36	0.62	− 0.11
C2 × W11	2	7.78	0.73	− 0.91
C3 × W1	1	9	0.82	− 0.48
C3 × W2	1	10	0.67	− 0.55
C3 × W3	1	8	0.84	− 0.31
C6 × W1	2	9.5	0.82	− 0.59
C6 × W4	2	9.5	0.76	− 0.69
C6 × W6	2	10	1.00	1.06
C7 × W3	1	5.36	0.61	− 0.31
C13 × W4	1	6.64	0.87	0.75
C13 × W11	1	5.31	0.88	0.50
C14 × W3	1	4.8	0.96	0.11
C14 × W6	1	4.60	0.90	− 0.04
C14 × W11	1	4.20	0.95	− 0.04
C15 × W1	2	4.35	0.80	− 0.68
C15 × W10	2	6.60	0.61	− 0.91

The cultivated mothers of normal C × W crosses originate either from West Africa (C1, C2, C3, C6, C7) where they grow sympatrically with wild millets or from southern Africa (C13, C14, C15) where wild millet is absent (Table 4). The wild male parents of these same crosses originate from the pastoral area (W2, W6, W10) or from the agricultural area (W1, W3, W4, W5, W11).

The other 105 C × W crosses show a variable degree of seed malformation. There is no difference between wild millet from the agricultural area and those from the pastoral area in the ability to induce seed malformation. All the cultivated accessions showed this incompatibility with a wild male parent except the cultivar C14 from Botswana which was pollinated by W3, W6 and W11. In conclusion, the occurrence of seed malformation, at present, follows no evident geographical rule.

Enzymic distances between pairs of parents were not significantly correlated with seed malformation (correlation = 0.168). Furthermore, seed malformation was not expressed in any C × C cross although the distances between cultivated accessions displayed the same range of variation as the cultivated-wild pairs distances (Table 3). For instance, marked seed malformation (CP1-T = − 4.2) appeared in C1 × W6 (D = 0.041) but not in C4 × C15 (D = 0.093). Genetic divergence does not, therefore, explain seed malformation.

Two-way ANOVA 12C × 8W

The accessions used in ANOVA are given in Table

Table 5. Two-way ANOVA 12 C × 8 W on the variable CP1-T

Source of variation	SS	df	MS	F	Probability
Total	179.89	93	1.93		
Cultivated female	101.92	11	9.27	44.11	0.00
Wild male	7.71	7	1.07	5.09	0.01
Interaction	70.51	75	0.94	4.28	0.01
Controls variance	4.82	23	0.21		

1. In spite of the absence of repetitions, the 3 mean squares for the female, male and interaction effects have been tested by the error mean square computed for the Cs, C× and C × C families (Table 5). The 3 effects were significant but female effects were much greater than male effects. The cultivated millets roughly fell into two groups of incompatibility to wild millet (Table 6). The very incompatible millets were the late maturing cultivars from Togo (C10), Niger (C9), and the early maturing cultivars from Niger (C4), Sudan (C11) and Chad (C12). Their C × W seeds exhibited a very low mean germination rate (from 1% to 25%) and a strong decrease of quality measured by CP1-T/T (between 73 and 92%). The moderately incompatible group (the remainder) had a mean germination rate of C × W seeds ranging from 35 to 67%, which is satisfactory for the usual laboratory studies. Nevertheless, even the less incompatible millet C6 displayed a mean CP1-T value significantly below zero. In this ANOVA then, no cultivated accession appeared totally compatible to wild millet. The male effects were very slight. Only the W11 accession from Sudan induced a lower seed malformation than the other wild accessions (Table 7).

The presence of interactions is not confirmed by Tukey's test of additivity (Dagniélie, 1975, p. 171). This discrepancy with the test against the error mean square computed for Cs, C×, and C × C families can be explained if the normal C × W crosses observed with nevertheless incompatible cultivated parents are caused by intra-cultivar variability in incompatibility to wild millet. This issue will be solved by crosses with repetitions in order to discriminate between the effects of intra-cultivar variability and the existence of interaction between specific cultivated-wild couples of accessions.

Table 6. Two-way ANOVA 12 C × 8 W: Mean values of the C × W crosses made with the same parent C, for the 3 original variables and CP1-T. Relative decrease of grain quality (CP1-T/T)

Code	Pheno	P1000	Germ	CP1-T/T	CP1-T*
C6	2	8.95	0.62	19.35	−0.90 a
C15	2	4.89	0.55	29.82	−1.36 a b
C13	2.38	4.35	0.35	34.65	−1.58 a b
C2	2.25	7.19	0.45	37.30	−1.70 a b
C16	2.75	8.16	0.67	44.10	−2.01 a b
C3	2	6.9	0.53	49.56	−2.26 b c
C1	2.25	5.41	0.51	51.53	−2.35 b c d
C12	3	3.89	0.11	73.46	−3.35 c d e
C9	3	5.71	0.25	75.58	−3.46 c d e
C11	2.88	3.15	0.05	77.41	−3.53 d e
C4	2.75	3.89	0.13	81.14	−3.70 d e
C10	3	5.65	0.01	92.32	−4.21 e

* The means for CP1-T marked by the same alphabetical letter are not statistically different at the 5% level of significance.

Crosses W × C and W × W

The accessions used in these crosses are given in Table 1. All crosses produced normal-looking seeds (PHENO = 1) with good germination ability. But the W × C crosses consistently produced smaller seeds than their respective controls, the W × W crosses using the same W female accession (Table 8). In Table 8, the values P1000 and GERM of each cross W × C or W × W are adjusted to their control values by the ratio P1000/T and GERM/T where T is for each female accession the mean value of the W × W crosses made with that female accession. As an average, W × C seed weight makes 67% of the W × W seed weight.

Discussion

When the 3 variables PHENO, P1000, and GERM

Table 7. Two-way ANOVA 12 C × 8 W: Mean values of the C × W crosses made with the same male parent W, for the 3 original variables and for CP1-T

Code	Pheno	P1000	Germ	CP1-T*
W11	2.16	6.06	0.53	−2.01 a
W1	2.33	6.02	0.40	−2.34 a b
W5	2.36	5.54	0.38	−2.41 a b
W4	2.42	5.88	0.38	−0.47 a b
W2	2.58	5.88	0.33	−2.58 a b
W6	2.58	5.76	0.35	−2.67 a b
W9	2.58	5.36	0.25	−2.84 b
W10	2.75	4.96	0.29	−2.96 b

* The means of CP1-T marked by a same alphabetical letter are not statistically different at the 5% level of significance.

were used simultaneously, seed malformation was detected in 85% of 123 crosses between the subspecies glaucum and monodii, independently of the geographic and genetic origins of the parents. It has never been observed in intra-subspecific crosses.

The question arises as to why this frequent phenomenon has not been detected up to now in the many studies of crosses between wild and cultivated millets. One answer lies in the use of W × C crosses (wild female parent) in which seed malformation is very slight and without evident disadvantages (Bilquez & Lecomte, 1969; Belliard et al., 1980; Pernès et al., 1980). Another answer lies in the use of exceptional cultivars compatible to wild pollen: Brunken (1977) used a cultivar very close to our Botswana accession C14 and Marchais & Tostain (1985) used the cultivar Tiotandé, grown in Senegal during the cool season with irrigation in the absence of wild plants, which gave plump seeds with wild pollens (no P1000 and GERM data were collected). In the other studies (Niangado, 1981; Rey Herme, 1982; Joly Ichenhauser, 1984; Dujardin & Hanna, 1989; Robert et al., 1991; Mohindra & Minocha, 1991) failure to detect this phenomenon was perhaps due to the fact that seed malformation was not sufficiently harmful to be noticed in their experimental conditions. It cannot be excluded that the hard natural Niger environment increases the expression of seed malformation comparatively to the mild conditions of the previous studies. Thörn (1992) has shown the influence of environment on the normal development of embryos in the *Hordeum vulgare* × *Hordeum bulbosum* interspecific crosses.

According to the present results, intra-cultivar variability in incompatibility to wild millet is suspected in some cases which need further research.

The detrimental effect of subspecific glaucum × monodii crosses was clearly more marked when the female parent was the cultivated type. This reciprocal effect may be due either to cytoplasmic effects or to different endosperm genotypes as in crosses between *Oryza longistaminata* and *Oryza sativa* (Chu & Oka, 1970).

The small size of W × C seeds is also an expression of some genetic imbalance. Its detrimental effect under natural conditions is perhaps more serious than it seems in the laboratory if seed size influences seed establishment vigour (Zhang & Maun, 1990) and survival ability in hard environments (Krannitz et al., 1991).

Preliminary histological observations of crumpled C × W seeds have shown on the same spike some heterogeneity and different types of anomalies: delayed seed growth, embryo and (or) endosperm degenerescence beginning 10 days after fertilization, reduced starch accumulation. This syndrome also observed on defective kernel mutants of maize (Knowles et al., 1992; Neuffer & Sheridan, 1980; Sheridan & Neuffer, 1980) and on Triticale seeds (Varghese & Lelley, 1983) is considered as the expression of imbalanced genotypes. Finally, it must be noticed that the few C × W seeds succeeding to establish give vigorous and fertile adult plants.

Conclusions

Seed malformation observed in C × W crosses, and small seed size observed in W × C crosses, probably contributes to maintenance of the genetic integrity of the two glaucum and monodii subspecies as shown by Tostain (1992) and Marchais & Tostain (1992) even in areas where both subspecies grow together.

This seed malformation is a slight reproductive barrier of great evolutionary significance: it gives strength to the taxonomy of Brunken (1977) and af-

Table 8. Comparison of W × W and W × C crosses. N = number of crosses. Standard errors in parentheses

Crosses	N	Pheno	P1000	P1000/T	Germ	Germ/T
W × W	27	1	2.17 (0.67)	0.99 (0.19)	0.53 (0.21)	0.99 (0.35)
W × C	13	1	1.44 (0.49)	0.66 (0.14)	0.62 (0.30)	1.07 (0.48)
Probability of no difference				0.00		0.30

fords the evidence that the domestication process can also lead to speciation. Cultivated millet is on the way becoming a species distinct from its wild progenitor. This result is quite new by comparison with the survey made in 1973 by Harlan et al. (1973) on the comparative evolution of cereals. At that time, it was concluded that the domestication process showed no trace of speciation.

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