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Selfing rates of pearl millet (*Pennisetum typhoides* Stapf and Hubb.) under natural conditions

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Abstract. The selfing rate of pearl millet (*Pennisetum typhoides* Stapf and Hubb.) has been determined under natural conditions. This species is said to be allogamous. Nine test plants homozygous for a particular allele on the alcohol dehydrogenase: ADH A locus (A_1A_1) were sown 2.5 m one from each other interspersed among 300 plants homozygous for the same locus (A_2A_2); these nine plants served as indicators of selfing. In the 20 spikes produced by these a test plants, the selfing rates varied between 2.2% and 21.7%. Selfing rates were not significantly different within spikes of the same plant, except for one individual. There was no significant correlation between the rate of selfing and the density of the pollen shadow (estimated from the number of spikes producing pollen during the female phase of test plants) or variation in protogyny.

Key words: Pearl millet – Selfing – Protogyny – Isozymes – Natural conditions

Introduction

Pearl millet is a cereal originating from Sahelian Africa. It is usually considered to be allogamous because of its protogynous flowering (Rao 1949; Burton 1974), but the assumption that it is strictly allogamous seems doubtful: Gupta and Dhinan (1977) on cultivars from India and Sarr (1987) on African cultivars have shown that variations in a protogyny index has a genetic basis and that it can also be modified by environmental parameters. Depending on environmental conditions and/or the degree of protogyny, the female and male phase can overlap and lead to some selfing. Moreover, Sarr (1987) and Robert (1989) have shown that, under controlled hand-pollinations, competition occurs between pollen grains from different genetic sources, with the result that self-pollen usually fertilizes most of the ovules.

Under natural conditions, the different morphs of pearl millet (wild plants, precocious or late cultivars, and hybrids between wild and cultivated plants) evolve in sympatry, especially in Western Africa (Clement 1985), and gene flows can occur between the different compartments. These flows can be modified whenever some genotypes present high selfing rates.

Consequently, it is very likely that pearl millet is not strictly allogamous. Our aim in the investigation reported here was to determine the selfing rate of this species when grown under natural conditions. High selfing rates may have important consequences on the evolution of pearl millet populations.

Material and methods

Plants studied

Nine plants homozygous for a particular allele on the ADH A locus (A_1A_1) were used as indicators of selfing. These test plants were generated from a cross between two lineages, 'Ligui' and 'Massue', which was then selfed for two generations. 'Ligui' (a cultivar from Tchad) is homozygous for a different allele of ADH A (A_2A_2), and its indifference to photoperiod enables it to bloom under a long-day regimen. Furthermore, Sarr (1987) has shown that this lineage has a tendency to self. 'Massue' originates from a Mauritania oasis and carries the A_1A_1 allele of the ADH A locus. Despite two successive selfings of this hybrid, test plants were not entirely homozygous.

In the experimental plot, these test plants were surrounded by plants originating from Northern Africa that had undergo three cycles of free crosses in Nièvre, France, in order to get adapted to temperate climatic conditions. They were homozygous for the locus ADH A (A_2A_2).

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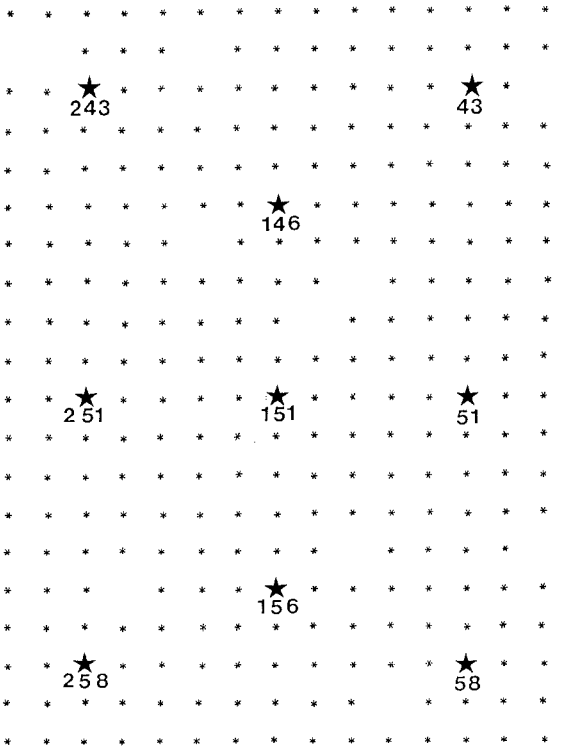

Fig. 1. Map of the experimental plot. The *black asterisks* (*) represent ADH A_2A_2 plants that produce 100% ADH A_2 pollen grains; the black stars (★) represent the test plants that produced ADH A_1 pollen grains; a *blank* indicates that a plant died or did not flower

The experimental plot

Figure 1 shows the position of the 9 test plants within the 300 plants sown in the experimental plot located at Paris l'Hôpital (Saône et Loire, France). Seeds were sown in a greenhouse in Orsay, France, on May 15, 1989; seedlings were planted on June 5, 0.5 meters apart. They were neither watered nor treated against pathogens. The plants were harvested twice, at the beginning and at the end of September.

Estimation of selfing rates

Seeds of the 9 test plants were scored for ADH by electrophoresis (as described in Trigui et al. 1985). ADH from seedlings was extracted in a sodium ascorbate buffer (pH 7.4) supplemented with 20% sucrose. The electrophoresis was run on starch gels with a lithium-borate buffer (pH 7.2). Analysis of the electrophoresis data was based on the hypothesis that heterozygous seeds issued from outcrosses, whereas homozygote seeds came from selfing. A total of 2,071 seeds sampled from 20 spikes were analysed; this represented about 100 seeds per spike.

In order to rule out the possibility that some homozygote seeds came from outcrosses with other test plants, we measured the pollen dispersal distance of the test plants. Seeds of A_2A_2 plants which surrounded the test plants and could outcross with the latter (i.e. were in the female phase when the test plants were in the male phase) were scored for ADH, and the proportions of heterozygous (A_1A_2) seedlings were recorded. The results show that A_2A_2 plants which were 0.5 m from the test plants produced on average 9.8% heterozygous seeds (8 spikes analysed; 6.7–13.2% of the heterozygous seeds); plants that were at a 1-m distance produced on average 3.0% heterozygous seeds (7 spikes analysed; 0–4.3% of the heterozygous seeds), and the plants that were at a 1.5-m distance had on average only 0.2% heterozygous seeds (9 spikes analysed; 0–2.1% of the heterozygous seeds). Consequently, since 2 test plants were separated by at least 2.5 m, no outcrosses between the different test plants could occur.

Measurements of reproductive biology parameters

The influorescence of pearl millet consists of groups of spikelets gathered by involucres. A spikelet consists of one pedicellate hermaphrodite flower and one male flower. The hermaphrodite flower has three stamens, an unilocular ovary with one style, and a bifidous stigma (Ferraris 1973; Sarr 1987).

The beginning of the female phase (first receptive stigmas) and male phase (first mature anthers) was recorded for all of the spikes. The delay between the male and female phases, measured in days, is defined as the protogyny index.

Total stem height, the weight, length, and width of the spikes, total seed weight within each spike, and the weight of 1,000 seeds were also recorded for each plant. The global proportion of viable seeds was about 95% and did not differ significantly between spikes.

Results

Under the prevailing temperate conditions, seed set was satisfactory. Flowering occurred between July 12 and the beginning of September, and 968 spikes were harvested in the experimental plot.

Parameters quantifying male and female reproductive efforts

The flowering characteristics of the test spikes are given in Table 1. Each test plant produced 1–4 spikes, with female flowering occurring between days 79 and 102 and male flowering occurring between days 81 and 105. The seed-set rate of the spikes (defined as the ratio of spike weight to weight of the seeds) varied between 0.53 and 0.92, with a high value indicating good seed set because most of the female flowers were fertilized. Seed-set rate was not correlated to the density of the pollen cloud ($r=0.170$; ns) or to the date of female flowering of the test plants ($r=-0.124$; ns). Thus, seed set may not be limited by pollen availability.

The number of spikes that were likely to give pollen to the test plants varied greatly. For example, since female flowering lasted for approximately 5 days in all of the spikes examined, spike $(156)_2$ could receive pollen from 378 spikes, whereas spike $(58)_4$ could receive pollen from only 38 spikes (Table 1). These are the most extreme values recorded during the experiment.

Seed weight produced by the test plants varied between 2 g and 21.5 g and the weight of 1,000 seeds varied between 1.8 g and 7.5 g. Values taken by the protogyny variation varied between 2 days and 6 days, they were not correlated to any of the parameters examined (Table 1).

Table 1. Floral biology parameters of the spikes examined

Plant[a]	Flowering[b]		S.S.R.[c]	Pollen pool[d]	Weight (g)		P.V.[e]
	(days)				Seed weight for each spike	Weight of 1,000 seeds	
$(43)_1$	79	84	0.678	322	9.7	5.1	5
$(51)_1$	79	85	0.588	322	2.0	7.2	6
$(51)_2$	82	88	0.778	351	9.2	4.6	6
$(51)_3$	83	88	0.742	368	13.5	5.1	5
$(58)_1$	85	90	0.865	367	19.8	5.8	5
$(58)_2$	94	98	0.726	170	9.0	4.8	4
$(58)_3$	99	105	0.531	84	5.2	3.1	6
$(58)_4$	102	?	0.769	38	3.0	1.8	?
$(146)_1$	80	84	0.741	350	21.5	7.4	4
$(146)_3$	91	97	0.726	249	5.3	2.9	6
$(151)_1$	87	91	0.538	340	4.9	6.1	4
$(151)_2$	97	101	0.681	115	4.9	3.8	4
$(156)_1$	82	86	0.777	351	17.4	6.4	4
$(156)_2$	84	89	0.780	378	16.3	7.2	5
$(156)_3$	86	91	0.757	347	14.3	6.6	5
$(243)_1$	79	81	0.824	322	10.3	6.5	2
$(251)_1$	89	93	0.794	288	16.2	7.5	4
$(251)_2$	96	99	0.810	125	8.1	5.3	3
$(251)_3$	97	100	0.920	115	9.2	4.3	3
$(258)_1$	82	88	0.530	351	4.4	5.3	6

[a] The number identifying the test plant is enclosed in parentheses; the subscript indicates the spike number with spike 1 being the earliest to flower, spike 2, the second one to flower, etc....
[b] The values in the first column are the number of days it took for female flowering to begin and those in the second are the number of days for male flowering to start in each spike.
[c] Seed-set rate, defined as the ratio between spike weight and weight of seeds.
[d] Number of spikes contributing to the pollen pool during the female flowering of the test plants
[e] Protogyny variation (days)

Selfing rates

Table 2 gives the selfing rates of the 20 spikes analysed. Spike $(251)_2$ had the lowest value (2.1%); spike $(43)_1$, the largest one (21.7%). A homogeneity χ^2 test carried out on all results showed that the selfing rates differed significantly between individuals ($\chi^2 = 82.2$; $df = 19$; $P < 0.001$).

Six plants bore several spikes, which led us to examine the homogeneity of selfing rates between spikes of the same plant. These selfing rates did not differ significantly from 1 spike to another in plants 51, 58, 151, 156, and 251 (Table 2). In only 1 plant (146) were the selfing rates significantly different between the 2 spikes, with the most precocious spike having the highest selfing rate. The low selfing-rate value in the second spike occurred when the density of the allopollen cloud was the lowest; if the density of the allopollen cloud had been correlated to the selfing-rate value, we would have expected higher or at least equal values of selfing rates between these two spikes. Such was not the case, which led us to find other explanations for this phenomenon. Mean temperatures were lower at the end of August than during the first part of the experiment. This cooling might have affected the viability of the pollen grains. Self-pollen would be the most sensitive, whereas outcross pollen, which had undergone three cycles of adaptation to temperate climates, would be more resistant to low temperatures. Robert et al. (1989) have shown that some pollen grains are less competitive than others under cool temperatures. In our case, self-pollen might be very sensitive to low temperatures.

Most of the variability in selfing rates was due to variations observed between the 9 test plants, rather than to within-plant variation, as shown by the results of a one-way ANOVA on selfing rates:

mean square value between plants: 87.3; $F = 7.69$; $df = 8$; $P = 0.0046$;
mean square value within plants: 29.2; $F = 2.57$; $df = 3$; $P = 0.1273$;
mean square value of the residual: 11.3; $df = 8$.

Influence of floral biology parameters on selfing rate in pearl millet

We looked for correlations between parameters other than genetic ones and the selfing-rate values.

Table 2. Selfing rates in the spikes of the test plants

Plant[a]	Number of seeds issued from		Selfing rate (%)	χ^2[b]
	Selfing	Outcross		
$(43)_1$	28	101	21.7	–
$(51)_1$	6	90	6.3	
$(51)_2$	3	93	3.1	1.56 ns
$(51)_3$	3	92	3.2	(2 *df*)
$(58)_1$	17	89	17.9	
$(58)_2$	23	96	19.3	
$(58)_3$	26	102	20.3	3.51 ns
$(58)_4$	18	126	12.5	(3 *df*)
$(146)_1$	15	128	10.5	8.26**
$(146)_3$	3	136	2.2	(1 *df*)
$(151)_1$	7	41	14.6	0.30 ns
$(151)_2$	9	39	18.8	(1 *df*)
$(156)_1$	5	89	5.3	
$(156)_2$	12	83	12.6	3.10 ns
$(156)_3$	15	128	10.5	(2 *df*)
$(243)_1$	5	86	5.5	–
$(251)_1$	7	89	7.3	
$(251)_2$	2	94	2.1	3.48 ns
$(251)_3$	3	89	3.3	(2 *df*)
$(258)_1$	12	78	13.3	–

[a] As defined in Table 1

[b] Level of significance of the chi-squire test: ** $P<0.01$; ns, non-significant

Female flowering data

Late flowering spikes might have had higher selfing rates, since the density of the pollen shadow was low at this time and few ADH A_1A_1 spikes were able to give pollen. There were no correlations between the flowering date and the selfing rate ($r=-0.097$; ns). The same was true for the density of the pollen shadow ($r=-0.091$; ns), and also for male flowering date of the test plants ($r=0.124$; ns).

Protogyny values

These varied between 2 and 6 days. A high selfing rate was expected in spikes that had low values of protogyny variation, but such was not the case ($r=-0.212$; ns). There was a significant correlation between seed-set rate and selfing rate ($r=-0.443$; $P=0.049$).

Seed weight of each spike ($r=-0.032$; ns), spike weight ($r=-0.052$; ns), and the weight of 1,000 seeds ($r=-0.143$; ns) were not correlated to selfing rate.

Therefore, parameters of floral biology or plant environment do not modify the outcrossing rates of pearl millet under our experimental conditions.

Conclusion

The results obtained from our investigation show that, under natural conditions, selfing rates can be high in pearl millet. We found that these rates could vary between 2.1% and 21.7% depending on the plant examined. Of course, these results cannot be extrapolated to pearl millet in general since we have been working on only a few genotypes. Another interesting result is that selfing rates are very different from one plant to another, but not within 1 individual plant (except for 1 plant in our experiment).

Kahler et al. (1984) estimated outcrossing rates (t) in maize from the allelic frequencies of eight isozymes. Values taken by t varied between 0.82 and 0.92; outcrossing rates differed when different isozymes were used, possibly due to homogamy phenomena. In pearl millet, the outcrossing rates observed during our experiment are comparable to those obtained by these authors. The high proportion of homozygous offspring could not be due to outcrosses between the different test plants because pollen from test plants could not fertilize spikes that were farther than 1 meter away, and 2 test plants were separated from each other by at least 2.5 m.

Factors involved in establishing these high selfing rates can be placed into three categories: (1) environmental (2) plant morphology, and (3) genetic.

Environmental factors

Although some spikes borne on different test plants were in bloom the same day, i.e. under the same environmental conditions, their selfing rate was different, which suggests that environmental factors play a negligible role on this phenomenon. This is in agreement with the lack of a significant correlation between selfing rate and female flowering date ($r=-0.097$; ns). According to Brown et al. (1990), selfing rates in anemophilous outcrossing species (such as pearl millet) are influenced only to a minor extent by environmental fluctuations. Instead, selfing rates are mainly controlled by the structure of the reproductive organs. Our results corroborate this hypothesis.

Parameters of plant morphology

In other outcrossed species, selfing rates vary in conjunction with several factors. In corn, Pollak et al. (1984) found that outcrossing rates are different and vary according to the rank of the spike (primary or secondary spike) or to the genotype of the plant examined. Although it seemed likely that protogyny variation and pollen-cloud density would act upon selfing rates, such was not the case in our experiment. The amount of pollen produced in the experimental plot was certainly enor-

mous, so it is very likely that pollen was not a limiting factor.

Several characteristics of the test plants and spikes (female-flowering date, male-flowering date, protogyny, seed weight of each spike, spike weight, and weight of 1,000 seeds) are not correlated to selfing rates; seed-set rate is only weakly negatively correlated to this trait ($r = -0.443$, $P = 0.049$). This does not enable us to draw valid hypotheses on the influence of plant morphology on selfing rates.

Genetic factors

The results obtained by Sarr (1987) showed that when the 'Ligui' lineage is crossed with another lineage, distortions of segregation occur which favour the 'Ligui' lineage. This lineage also has a tendency to self, whereas this is not the case for the 'Massue' lineage. Our results show that selfing rates depend on the plant examined; thus on its genotype: an ANOVA carried out on selfing rates showed that variation between plants was highly significant ($P = 0.0046$), whereas within-plant variation (i.e. between spikes) was not. Furthermore, the selfing rates between spikes of the same plant did not differ significantly (except for 1 plant). These results are in agreement with the explanation that selfing rates have a genetic basis. An analysis of the selfed progeny of the test plants will allow us to test this hypothesis.

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