Non-random mating in a *Secale cereale* L. (rye) population

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The mating system in an experimental population of rye (*Secale cereale* L.) cv. Merced was studied using eight polymorphic isozyme loci as genetic markers and two progeny samples from a large population: 20 emasculated mother plants (obligately cross-pollinating) and 100 non-emasculated mother plants. Merced rye showed a significant frequency of self-fertilization ($s = 1 - t$) in a previous generation, but in the generation under study the outcrossing frequency was not significantly different from $t = 1$. This fact suggests that environmental factors can affect the rate of outcrossing vs. selfing (self-fertilization). In spite of the outcrossing rate of $t = 1$, the large progenies from emasculated plants (100 seeds per plant) demonstrated that pollination was not completely at random. This represents a violation of the generally accepted mixed-mating model on which most methods used to estimate mating parameters are based. Temporal heterogeneity and the differential ability of gametophytes to fertilize are possible causes of the non-randomness of mating. It is concluded that, although violations of the assumption of the mixed-mating model can bias the estimates, most of the estimated outcrossing values clearly differ from complete outcrossing.

Keywords: isozymes, mating-system, mixed-mating model, rye, *Secale cereale*.

Introduction

Higher plant species can reproduce by one or more reproductive systems: sexual, apomictic or vegetative. Reproductive systems have a decisive effect on the genetic structure of plant populations because they largely determine the amount, distribution and transmission of genetic variation from generation to generation (Allard, 1975). Monoecious sexually-reproducing plants are classified as predominantly selfing or self-fertilizing (with an outcrossing rate lower than 0.10), predominantly outcrossing (self-fertilization rate lower than 0.05), or mixed selfing and outcrossing (Brown, 1989). The mating system is subjected to changes due to environmental, genetic and evolutionary causes (Kahler *et al.*, 1975; Farris & Mitton, 1984; Marshall & Abbott, 1984; Vaquero *et al.*, 1989), thus differences in outcrossing rate between populations or between years in the same population may be observed in plant species. The progeny genotype set is most widely used by plant population geneticists when estimating mating system parameters because such data identify the actual fertilizations which occur in populations. The mixed-mating model, the most widely used method for

estimating mating system parameters from progeny genotype data, assumes that some mating events occur at random between different plants in the population with probability t while other zygotes arise from self-fertilization with probability $s = 1 - t$ (Ritland, 1983; Brown, 1989).

The mixed-mating model assumes that (1) there is no structure in plant genotype distribution in the population, which means that the pollen pool is genetically uniform throughout the population; (2) there is no selection between mating and the time of census; and (3) outcrossing probability is independent of the genotypes of both mother plants and pollen. Violation of any of these premises introduces bias in outcrossing rate estimation. In annual cultivated plants bulk harvesting and random sowing rule out the possibility of population genetic structure. Selection between mating and census is difficult to estimate and cannot usually be ruled out. With regard to the third premise there is experimental evidence that matings are not completely independent of genotypes in maize (Bijlsma *et al.*, 1986). If these assumptions are fulfilled, then it can be accepted that any excess in homogametic fertilizations is due entirely to selfing and estimates of s reflect the true level of self-fertilization in populations. But if they are not fulfilled, estimates of s can be significantly

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different from zero even when there is no selfing at all (Bijlsma *et al.*, 1986).

Cultivated rye (*Secale cereale* L.) is a predominantly outcrossing species with a gametophytic incompatibility system controlled by two multiallelic loci (Trang *et al.*, 1982). It is probable that most of its populations are nearly 100 per cent outcrossed, but self-fertilization rates can range widely (rates as high as 45 per cent have been reported) among 'natural' and synthetic populations, depending upon environmental factors (e.g. plant density), genetic factors such as self-fertility genes, B chromosomes, or temperature-induced pseudocompatibility (Wricke, 1979; Cruz-Pardilla *et al.*, 1989; Vaquero *et al.*, 1989; Gertz & Wricke, 1991).

The main objective of this work was to establish whether there is a differential probability of outcrossing depending upon the genotype of the maternal plant

and/or the pollen in rye, as has been demonstrated in maize. We also examined the effects of seasonal variation on the mating system in rye.

Materials and methods

The Merced cultivar of rye (*Secale cereale* L., $2n = 14$) was used in this work. Merced originated in California and we maintain a population in our experimental fields in León (Spain). A sample of 2790 seeds was randomly extracted from a large seed lot. Seeds were sown 30 cm apart in a plot made up of 62 rows and 45 columns. No replanting was carried out and 1990 plants survived to maturity. Before pollination 25 randomly-chosen plants were emasculated and the remainder allowed to open-pollinate (Fig. 1). The

Fig. 1 Location of the rye plants analysed in this study. Bars indicate the 56 sectors of 32 rows and 24 columns into which the population was subdivided to test genetic heterogeneity in the distribution of allele frequencies.

progeny of each plant was collected individually and mating system analyses were carried out with progeny arrays of 10 seedlings per non-emasculated mother plant and 100 seedlings from emasculated plants. Two samples were assayed: the general population sample formed by the arrays of 100 mother plants taken at random from the population, and the emasculated plant sample formed by 20 out of the 25 emasculated plants. These plants were not included in the general sample.

Seedlings were assayed for eight isozyme loci. The standard starch gel electrophoresis procedures used and the genetic nomenclature of loci and alleles have been described elsewhere (Pérez de la Vega & Allard, 1984; Vaquero *et al.*, 1989, 1990). We have used the nomenclature of the recent review by Melz *et al.* (1992), except for the most generally used synonym of glutamate oxaloacetate transaminase, *Got*, instead of aspartate aminotransferase, *Aat*. According to Melz *et al.* (1992) the nomenclature of the loci is: *Acp2*, *Got3* (syn. *Aat3*), *Amp1*, *Mdh1*, *Mdh2*, *Gpi1*, *Pgm1* and *Pgd3*. According to our previous work the nomenclature is *Acp2*, *Got3*, *Lap*, *Mdh1*, *Mdh2*, *Pgi2*, *Pgm* and *6Pgd2*, respectively. The 6PGD1 zone was monomorphic in this population and has been omitted in the results; 6PGD1 is under the control of two loci (Vaquero *et al.*, 1990) named according to Melz *et al.* (1992) as *Pgd1* and *Pgd2*. Adult allelic and genotypic frequencies, pollen allelic frequencies and single locus outcrossing rates were estimated following the method of Clegg *et al.* (1978). The variances of the single locus outcrossing estimates were calculated by applying the 'jackknife' method of Schoen & Clegg (1986), and the weighted mean of single locus estimates was calculated according to Kahler *et al.* (1984). Multilocus outcrossing rates were determined using the method of moments estimator of Shaw *et al.* (1981).

Results

The Merced seed sample originated from the generation previously studied by Vaquero *et al.* (1989). Except for the *Acp2* locus, no significant allelic frequency differences between the two seedling generations were observed (*Amp1* was not included in the previous study). However, when genotypic frequencies were compared, significant differences were observed between the two generations at the seedling stage. There were no significant differences between the two generations of adult plants and between the seedlings analysed by Vaquero *et al.* (1989) and the adult plants analysed here (the same generation). As will be further discussed, the differences in genotypic frequencies may be due to differential self-fertilization rates in the two

generations. For these comparisons only the 1000 seedling progeny (progeny 1 in Table 1) for the 100 non-emasculated plants were considered. The progeny of the 20 emasculated plants (progeny 2) were not considered since the small sample of only 20 maternal genotypes introduces a bias in the genetic structure of the progeny.

The study of 100 seedling progeny from each of 20 emasculated Merced plants allowed us to deduce unambiguously the genotypes of these 20 mother plants which are shown in Table 2. Genotypes were first directly deduced and no disagreements were found when they were subsequently deduced by the maximum likelihood method (Clegg *et al.*, 1978). The expected progeny genotype distribution at each locus in each plant was estimated from the deduced maternal genotype and the allelic frequencies in the general population; when these distributions were compared with the observed genotypes by means of a χ^2 test, several significant deviations were observed (Table 2).

The single locus estimates of the outcrossing rates (t) are also shown in Table 1. The weighted means were 0.980 (SD 0.015) and 1.015 (SD 0.137) for progenies 1 and 2, respectively. The multilocus estimates (Shaw *et al.*, 1981) were, respectively, 0.989 (SD 0.024) for progeny 1 and 0.915 (SD 0.018) for the progeny from emasculated plants. Neither the weighted mean of the single locus estimates nor the multilocus estimates differed significantly from an outcrossing frequency of $t = 1$; although there was some heterogeneity among single locus estimates. This 100 per cent outcrossing contrasts with the outcrossing rate obtained in the previous generation (Vaquero *et al.*, 1989) in which a significant rate of self-fertilization (the lowest estimate was 9 per cent) was observed.

Allelic frequencies were estimated from the 100 adult mother plant genotypes (general population) and compared with the allelic frequencies of the pollen fertilizing emasculated homozygous plants (Table 3). Noticeable differences of approximately 0.05 were observed for the *Amp1*, *Pgd3* and *Pgm1* loci. However, when comparing the observed and expected genotypic frequencies of the progenies from emasculated plants, highly significant differences between the observed and expected frequencies were obtained for all loci except *Acp2* and *Mdh2* (Table 4). Expected frequencies in Table 4 were deduced from allelic frequencies in the general population of surviving adult plants. Differences were also highly significant for *Got3* and *Pgd3* when the frequencies of fertilizing pollen deduced from progenies of homozygous emasculated plants were used (data not shown).

Data in Table 4 also indicate that there were some correlations between fertilizing events depending upon

Table 1 Deduced* maternal and observed progeny genotypic frequencies and single locus outcrossing estimates (*t*)

Locus		Maternal	Progeny 1	Progeny 2
<i>Acp2</i>	11	0.81	0.821	0.825
	12	0.19	0.164	0.170
	22		0.015	0.005
	<i>t</i>		0.934	1.019
SD			0.007	0.031
<i>Amp1</i>	11	0.63	0.670	0.681
	12	0.34	0.282	0.282
	22	0.03	0.048	0.036
	<i>t</i>		0.983	0.966
SD			0.004	0.082
<i>Got3</i>	11	0.73	0.684	0.700
	12	0.24	0.289	0.262
	22	0.03	0.027	0.038
	<i>t</i>		1.040	0.831
SD			0.004	0.061
<i>Gpi1</i>	11	0.90	0.874	0.832
	13	0.10	0.121	0.163
	14		0.001	
	33		0.004	0.005
<i>t</i>			1.001	1.026
	SD		0.009	0.012
<i>Mdh1</i> †	11	1.00	0.997	1.000
	12		0.002	
	22		0.001	
<i>Mdh2</i>	11	0.83	0.802	0.764
	12	0.14	0.188	0.224
	22	0.03	0.010	0.012
<i>t</i>			0.964	1.028
	SD		0.006	0.024
<i>Pgd3</i>	11	0.62	0.596	0.677
	12	0.33	0.345	0.296
	22	0.05	0.059	0.027
<i>t</i>			0.961	0.999
	SD		0.006	0.027
<i>Pgm1</i>	11	0.58	0.590	0.666
	12	0.28	0.278	0.269
	13	0.03	0.037	0.011
	14		0.002	0.002
	15	0.05	0.045	0.010
	22	0.03	0.038	0.039
	23	0.02	0.006	0.002
	25	0.01	0.001	0.001
	33		0.001	
	35		0.001	
	55		0.001	
<i>t</i>			0.993	0.918
	SD		0.003	0.076
<i>N</i>		100	1000	2000

the enzymatic locus. For example, *Got3* pollen carrying the allele 2 fertilized at high frequencies, as can be deduced from the high frequency of the 12 and 22 progeny from mothers 11 and 12, respectively, and this occurs irrespective of homogametic or heterogametic matings since the χ^2 for homogametic (i.e. 1-1 or 2-2) vs. heterogametic matings (i.e. 1-2 or 2-1) is non-significant. A similar result is observed for allele 3 of *Gpi1* among the progeny of 11 maternal plants. Differences were not significant for 13 and 33 maternal plants, but heterogametic matings were significantly more frequent than expected ($\chi^2_1 = 8.79$, $P < 0.01$). By contrast, the pollen carrying allele 1 fertilized at high frequencies in loci *Amp1*, *Pgm1* and *Pgd3*, but while for the *Pgd3* locus there is no significant difference between homogametic and heterogametic matings there is a significant excess of homogametic matings for *Amp1* and *Pgm1* ($\chi^2_1 = 12.92$, $P < 0.001$ and $\chi^2_1 = 13.74$, $P < 0.001$ respectively). *Pgm1* infrequent alleles (3, 4, 5) are generally under-represented in pollen fertilizing emasculated plants (data not shown). Neither the differences between observed and expected progeny (Table 4) nor the differences between homogametic vs. heterogametic matings are significant for *Acp2* and *Mdh2*.

Discussion

The rye population tested here fitted the hypothesis of complete outcrossing, as is expected in a species with an incompatibility system. However, it has been demonstrated that outcrossing rates in plant species, including rye, change depending upon both genetic and environmental factors (Bijlsma *et al.*, 1986; Cruz-Pardilla *et al.*, 1989; Vaquero *et al.*, 1989). In rye it has been demonstrated that compatibility genes and pseudocompatibility due to environmental circumstances, in particular high temperatures at the time of flowering, can facilitate self-pollination (Gertz & Wricke, 1991; Wricke, 1979). Thus, differences in outcrossing rates between cultivars and between years in the same cultivar are expected. Merced, which in this generation has an outcrossing rate of 100 per cent, showed an averaged single-locus self-fertilization rate close to 9 per cent, and a multilocus estimate even higher (≈ 20 per cent), in the previous generation. The difference in self-fertilization rates between Merced

*Maternal frequencies and outcrossing rates were deduced from the observed progeny frequencies by Clegg *et al.*'s (1978) method.

†*Mdh1* single locus outcrossing frequency was not estimated. SD = standard deviation, *N* = number of individuals.

Table 2 Deduced genotypes of the 20 emasculated rye plants

Plant	<i>Acp2</i>	<i>Amp1</i>	<i>Got3</i>	<i>Gpi1</i>	<i>Mdh1</i>	<i>Mdh2</i>	<i>Pgd3</i>	<i>Pgm1</i>
1	11	12	11	11	11	11	11	11
2	11*	11*	12	11	11	11	12	12
3	12*	11	11*	11	11	11	11	11
4	11	11*	12	13	11	11	11	12*
5	11	12	11	11	11	11	11	12
6	11	11*	11*	11*	11	11	11	12
7	11*	12	12	11*	11	11	11	11
8	11	11*	11	11*	11	11	12	11
10	11	11*	11	11	11	11	11*	11
11	11*	12*	12	11*	11	11	11	11
12	11*	11*	12*	11	11	11	11	11
13	11	11*	11	33	11	11	22*	12
15	11	11	12	11	11	11*	11	11
17	11	11	11	11*	11	12	11	12
18	12*	12*	11	11*	11	12	11	11
19	11	12*	11	11	11	12	11*	12
20	11	11	11	11	11	12	11	11
22	11	12	11	13*	11	11*	12*	11
23	11	11*	11	11	11	22	12*	11*
25	12*	12	11*	11	11	11	11*	11*

*Significant χ^2 deviation between the observed and the expected progeny genotypes according to the pollen frequencies in the general population.

Table 3 Estimates of adult allelic frequencies and observed allelic frequencies in functional pollen* of Merced rye

Locus	Allele	Adult	Pollen
<i>Acp2</i>	1	0.905	0.904
	2	0.095	0.096
<i>Amp1</i>	1	0.800	0.847
	2	0.200	0.153
<i>Got3</i>	1	0.850	0.836
	2	0.150	0.164
<i>Gpi1</i>	1	0.950	0.925
	3	0.050	0.075
<i>Mdh2</i>	1	0.900	0.901
	2	0.100	0.099
<i>Pgd3</i>	1	0.785	0.823
	2	0.215	0.177
<i>Pgm1</i>	1	0.760	0.810
	2	0.185	0.177
	3+4+5	0.055	0.013

*Adult frequencies were estimated from the genotypes of the 100 non-emasculated mother plants; pollen frequencies were deduced from the progeny genotypes of emasculated homozygous plants.

generations could be due to differences in the climatic conditions of the two years. The previous generation was grown in a dry year (the rainfall during the 30-day period in which flowering occurred was 10.3 mm, and the annual rainfall was 256.1 mm) with relatively high temperatures during the time of flowering (mean of 17.1°C) while the present generation was grown under normal rainfall for León (25.9 mm during flowering time) and cooler temperatures (mean of 16.6°C).

The genotypic differences observed between the two generations of Merced rye seedlings agree with the different estimates of self-fertilization rates. In the previous generation of Merced seedlings there was a significant deficit of heterozygotes at several loci, which fits the estimated rate of self-fertilization (Vaquero *et al.*, 1989), while in this generation no significant differences exist, the outcrossing rate being the estimated value of $t \approx 1$. In general, heterozygote deficits disappeared from the seedling to the adult plant stage in Merced and other rye varieties analysed by Vaquero *et al.* (1989). This also occurs in other species (Farris & Mitton, 1984; Morgante *et al.*, 1991). Thus, genotype frequencies between generations of adult plants can be more similar than between adult plants and their seedling progenies depending upon the genetically and/or environmentally variable outcrossing rate.

Table 4 Observed and expected† progenies in emasculated plants

Progeny	Maternal genotype‡					
	Observed			Expected		
	11	12	22	11	12	22
<i>Acp2 11</i>	1536	115		1538.50	135.75	
<i>Acp2 12</i>	164	175		161.50	150.00	
<i>Acp2 22</i>		10			14.25	
Total	1700	300			$\chi^2_4 = 8.65$	
<i>Got3 11</i>	1170	230		1190.00	255.00	
<i>Got3 12</i>	230	294		210.00	300.00	
<i>Got3 22</i>		76			45.00	
Total	1400	600			$\chi^2_4 = 26.17^{***}$	
<i>Amp1 11</i>	1016	347		960.00	320.00	
<i>Amp1 12</i>	184	381		240.00	400.00	
<i>Amp1 22</i>		72			80.00	
Total	1200	800			$\chi^2_4 = 20.31^{***}$	
<i>Mdh2 11</i>	1348	179		1350.00	180.00	
<i>Mdh2 12</i>	152	203	93	150.00	200.00	90.00
<i>Mdh2 22</i>		18	7		20.00	10.00
Total	1500	400	100		$\chi^2_6 = 1.28$	
<i>Gpi1 11</i>	1571	93		1615.00	95.00	
<i>Gpi1 13</i>	129	103	94	85.00	100.00	95.00
<i>Gpi1 33</i>		4	6		5.00	5.00
Total	1700	200	100		$\chi^2_6 = 25.52^{***}$	
<i>Pgm1 11</i>	1053	280		988.00	266.00	
<i>Pgm1 12</i>	247	337		312.00	350.00	
<i>Pgm1 22</i>		83			84.00	
Total	1300	700			$\chi^2_4 = 19.05^{***}$	
<i>Pgd3 11</i>	1231	124		1177.50	157.00	
<i>Pgd3 12</i>	269	237	86	322.50	200.00	78.50
<i>Pgd3 22</i>		39	14		43.00	21.50
Total	1500	400	100		$\chi^2_6 = 28.79^{***}$	

†According to the maternal genotype and the general frequencies of pollen in the population.

‡11, 13 and 33 for *Gpi1*. *Pgm1* has been reduced to a two-allele locus; *Mdh1* has been omitted from the table due to its uniformity. Subscripts indicate the degrees of freedom.

** $P \leq 0.01$; *** $P \leq 0.001$.

If the mixed-mating model accurately estimated the real outcrossing rate of a population, the estimates of outcrossing rates made from the same set of progeny arrays would be the same for all loci, within sampling error, because all loci are transmitted together in each gamete. In practice, however, mixed-mating model estimates, especially in predominantly outcrossing species (Bijlsma *et al.*, 1986; Vaquero *et al.*, 1989), are often significantly different for different marker loci, which indicates that one or more of the assumptions

made in formulating the estimator of outcrossing rate is not valid in the population under study. Violations of the assumptions of the mixed-mating model can produce outcrossing estimates either higher or lower than the real outcrossing rate in a population.

The results of this generation of Merced indicate that, although outcrossing estimates in normal and emasculated plants do not differ significantly from 1, there is some heterogeneity in pollen sampling in the population (Table 3), and data from emasculated plants indicate that homogametic or heterogametic fertilizations are favoured depending upon the locus (Table 4). Similar results of differential pollen sampling have been reported in detasseled maize plants (Bijlsma *et al.*, 1986). There are several other causes, in addition to self-fertilization, that can result in non-random sampling of pollen: (1) temporal and/or spatial heterogeneity; (2) gametophytic selection; and (3) postmating selection.

Temporal heterogeneity can affect pollen sampling if specific alleles of the marker loci themselves influence the time of flowering, or if alleles of the marker loci are associated with alleles of loci which affect it. Although differences among plants in the time of flowering in Merced were not obvious, this hypothesis cannot be completely rejected since no experiment has been designed to test for it in our material. It is unlikely that several genetically unlinked (Vaquero *et al.*, 1990) enzymatic loci have a direct influence on, or are closely linked to, loci influencing the time of flowering. Spatial heterogeneity is also unlikely since all Merced seeds from the previous generation were mixed and a random bulk sample was sown. Hence, there was little, if any, opportunity for non-random localized neighbourhood structure. This hypothesis was further reinforced since a test for genetic heterogeneity yielded non-significant results. The test was carried out by estimating the F_{st} statistic of Nei (1977) among 56 subsamples of all analysed plants included in sectors of 32 rows and 24 columns (Fig. 1). The significance of allelic differences for each locus was calculated using the χ^2 test of Workman & Niswander (1970), and no χ^2 value was significant.

Differential survival of zygotes (postmating selection) with different genotypes from fertilization to the time of the seedling assay (15 days after germination under controlled conditions) is another unlikely cause of nonrandomness. Since germination rates were higher than 98 per cent it is unlikely that selection operating in controlled temperature chambers during early seedling stages had much effect on altering seedling genotypic frequencies. Similar conclusions have been obtained in other plant species (Kahler *et al.*, 1975; Clegg *et al.*, 1978; Bijlsma *et al.*, 1986) as well as in rye (Pérez de la Vega & Allard, 1984).

The last cause is gametophytic selection, that is to say the differential ability of gametophytes to fertilize depending upon their own genotype, the genotype of maternal plants or interactions between both. The influence of a particular allele of a marker locus (or other closely linked gene) on the gametophyte's ability to fertilize could be the cause of allele 2 of *Got3* fertilizing more than expected in both homozygous and heterozygous mother plants; of allele 3 of *Gpi1* being more frequent than expected among fertilizing pollen in homozygous plants; and of homogametic matings being significantly more frequent than expected in *Pgm1* and *Ampl*. Differential ability to fertilize could be influenced by genetic linkage with rye self-incompatibility genes; loci *Pgd3* and *Mdh1* are located in the same chromosome arm (1RL) as the incompatibility locus *S1*, while the *Gpi1* locus is on arm 1RS. However, it is unlikely that, in such an open-pollinating and relatively large population as Merced, genetic recombination will allow for a high disequilibrium between particular alleles in isozymatic loci and the numerous alleles in rye incompatibility loci (a second locus, *S2*, is located on chromosome 2R). Furthermore, loci located in chromosomes other than 1R and 2R (see Melz *et al.*, 1992, for rye linkage maps) show similar deviations (Table 4).

Our results agree with previous reports that estimates of the mating system in predominantly outcrossing species are frequently heterogeneous throughout marker loci (Pérez de la Vega & Allard, 1984; Bijlsma *et al.*, 1986; Vaquero *et al.*, 1989), which indicates that violations to the assumptions of the mixed-mating model are occurring. In our rye material the most likely violation is gametophytic selection correlated with marker-locus genotype, although temporal variability cannot be completely ruled out. Similar results have been claimed as the cause of non-random mating in some populations of maize (Bijlsma *et al.*, 1986). Positively correlated matings are expected to bias estimates of t , causing values of $t < 1$ or $t > 1$ depending upon whether homoallelic or heteroallelic crosses are favoured, respectively. This fact must not be interpreted in the sense that true selfing does not exist. True self-fertilization can exist in open-pollinating species without a self-incompatibility system, and even in those species with one, although the estimates of $s = 1 - t$ may not be completely due to true self-pollination but biased by other causes. However, it is likely that average single-locus and multilocus self-fertilization estimates of 20, 30 or even 40 per cent are at least partially due to true selfing, especially if these cultivars are able to produce a high level of seed-set under forced selfing (Cruz-Pardilla *et al.*, 1989; Vaquero *et al.*, 1989). Taking into account that self-fertilization rates in rye, and in other species, are

influenced by genetic and environmental factors, it is also expected that estimates may vary from year to year and between cultivars. As Bijlsma *et al.* (1986) stated, the complexity of interactions determining sexual reproduction in plants requires more complex factorial experiments to identify and measure all the factors involved in mating at the population level.

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