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THE EFFECT OF FORCED SHEDDING ON POLLEN TRAITS, SEEDSETTING, AND TRANSMISSION AT VARIOUS MAIZE (ZEA MAYS L.) ENDOSPERM MUTANT LOCI

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SUMMARY

Under field conditions, attached maize tassels shed pollen daily between 800–1200 h with all or most of the pollen grains in the tassel released in 7–10 days. Detached tassels with their cut ends submerged in water and exposed to low light intensity, high humidity, and 25–30 °C, shed pollen continuously with all or most of the pollen grains in the tassel released in three days. After cutting at 1600 h on day 0, pollen grains from 15 detached tassels from three heterozygous (*Wx wx, Su su, Sh_2 sh_2*) populations at the waxy, sugary-1, and shrunken-2 loci, were collected at the end of eight collection periods (A = 1600 h, day 0–1000 h, day 1; B = 1000 h, day 1–1600 h, day 1; C = 1600 h, day 1–2200 h, day 1; D = 2200 h, day 1–1000 h, day 2; E = 1000 h, day 2–1600 h, day 2; F = 1600 h, day 2–2200 h, day 2; G = 2200 h, day 2–1000 h, day 3; H = 1000 h, day 3–1600 h, day 3). The volume of pollen released and pollen grain diameter was measured at each collection period. On the resulting selfed ears, seed number ear^{-1} and deviations from the genetic ratio at collection period A at each locus were used as an index of seedset and pollen transmission, respectively. The volume of pollen grains released, pollen grain diameter, relative starch content of the pollen grains, and seedset decreased at the later collection periods, but the amount and the collection period where the decrease occurred depended on the locus. Significant increases in the transmission frequency of the recessive allele were found in collection periods B, C, D, E, and F at the waxy locus and collection period B at the shrunken locus. Apparently, less mature pollen grains are released at the later collection periods and this immaturity alters seedset and pollen transmission. This forced shedding procedure may be useful in amplifying pollen transmission differences and, in so doing, improve the selectivity and efficiency of pollen genotype selection programs.

1. INTRODUCTION

Artificial manipulation of the normal pollen transmission process may be useful in amplifying pollen transmission differences among pollen grains containing different alleles. In maize, extended pre-pollination pollen storage at 2 °C and pre-pollination stylar treatments increased pollen transmission differences between alleles at various endosperm mutant loci (PFAHLER 1974, PFAHLER et al. 1986). Under field conditions, attached maize tassels usually shed pollen daily between 800–1200 h, with all or most of the pollen grains in the tassel released in 7–10 days (KIESSELBACH 1949). Detached tassels with their cut ends submerged in water and exposed to low light intensity, high humidity, and 25–30 °C, were observed to shed pollen continuously, with all or most of the pollen grains in

the tassel released in three days. The effect of this forced shedding procedure on pollen traits and transmission has not been examined.

The purpose of this study was to determine the effect of forced shedding on various pollen traits, seedsetting ability, and the pollen transmission of alleles at the waxy, sugary, and shrunken loci in maize.

2. MATERIALS AND METHODS

On each of two dates, 15 tassels from each of three heterozygous (*Wx wx, Su su, Sh_2 sh_2*) populations at the waxy (*wx*), sugary-1 (su_1), and shrunken-2 (sh_2) loci were cut (about 35 cm below the lowest branch) from field-grown plants at 1600 h (day 0). Each tassel selected was just beginning to exsert anthers, and any leaves were removed. The cut ends of the tassels were then submerged in water. The three groups of tassels were placed on separate sheets of paper and exposed to low light intensity, high humidity, and 25–30 °C conditions. At the end of eight collection periods (A = 1600 h, day 0–1000 h, day 1; B = 1000 h, day 1–1600 h, day 1; C = 1600 h, day 1–2200 h, day 1; D = 2200 h, day 1–1000 h, day 2; E = 1000 h, day 2–1600 h, day 2; F = 1600 h, day 2–2200 h, day 2; G = 2200 h, day 2–1000 h, day 3; H = 1000 h, day 3–1600 h, day 3), the papers were replaced. The pollen sample obtained during the collection period was screened to remove the anthers. The total volume of pollen grains shed during the collection period was then determined, using a graduated cylinder. A small portion of each sample was placed in a killing and fixing solution (PFAHLER 1967) for diameter measurements. Within 30 min after collection, the remaining pollen grains were used to pollinate (with an excess amount of pollen grains) eight ears of the same population from which the pollen grains were collected. Normal, mutant, and total seed number were obtained on each ear.

Fifty pollen grains from each collection period and locus were measured, using a microprojector at 400x.

An analysis of variance was performed on each locus for pollen diameter and seedset. Total seed number ear^{-1} was used as an index of seedset. Significant differences from collecting period A at each locus were obtained, by means of the revised Duncan's ranges, using for P only the maximum number of means to be compared (HARTER 1960).

Chi-square tests on the normal:mutant seed number were performed within each locus and collection period. The expected numbers were derived, using the ratio obtained in collection period A at each locus. To more accurately identify deviations in pollen transmission, the recessive transmission frequency (RTF) was determined from the formula, recessive frequency/0.5.

3. RESULTS

The total volume of pollen grains shed at each collection period was strongly influenced by the locus (*table 1*). At A. the volume at the waxy and shrunken

Table 1. The effect of pollen collection period on the volume and diameter of pollen grains produced at the waxy, sugary, and shrunken loci.

Locus	Collection period[1]	Volume (ml)	Diameter (μm)[2]
Waxy	A	13	88.5
	B	12	89.0
	C	15	89.2
	D	19	86.3*
	E	10	84.1**
	F	5	85.5**
	G	3	83.0**
	H	2	81.9**
Sugary	A	21	86.7
	B	12	89.7**
	C	22	87.2
	D	22	86.5
	E	10	86.9
	F	7	85.4
	G	6	86.3
	H	3	85.9
Shrunken	A	10	87.2
	B	9	88.7
	C	8	88.1
	D	16	86.7
	E	10	86.5
	F	8	85.2*
	G	8	84.6**
	H	5	84.9*

[1] A = 1600 h, day 0–1000 h, day 1; B = 1000 h, day 1–1600 h, day 1; C = 1600 h, day 1–2200 h, day 1; D = 2200 h, day 1-1000 h, day 2; E = 1000 h, day 2–1600 h, day 2; F = 1600 h, day 2–2200 h, day 2; G = 2200 h, day 2–1000 h, day 3; and H = 1000 h, day 3–1600 h, day 3.
[2] *,** Significant from collection period A of the locus at the 0.05 and 0.01 probability levels, respectively.

loci was about 50% of that at sugary locus. At the waxy locus, an increase was found at C and D with an extremely sharp decrease at G and H. At the sugary and shrunken loci, the volume at the early periods remained relatively constant, but a sharp decrease was observed at the later collection periods.

The effect of collection period on pollen diameter was greatly affected by the locus (*table 1*). At the waxy and, to a lesser extent, shrunken loci, a progressive decrease in diameter was found at successive collection periods. At the sugary locus, a different pattern emerged, with a highly significant increase obtained only at B.

The starch content of the pollen grains after staining, with a KI-I_2 solution, was observed to be considerably less at the later collection periods at all loci.

Collection period influenced seedset at the later collection periods (*table 2*). At all loci, no significant differences were present at A, B, C, and D. Thereafter, a rapid decrease occurred with the rate dependent on the locus. At the waxy

Table 2. The effect of pollen collection period on seedset and pollen transmission at the waxy, sugary, and shrunken loci. MSN (NE) = mean seed number ear^{-1} (number of ears); TCS (H) = total chi-square value (heterogeneity chi-square value); and RTF = recessive transmission frequency.

Locus	Collection period[1]	MSN (NE)[2]	TCS (H)[3]	RTF[4]
Waxy	A	317 (16)	0.00 (43.44**)	0.410
	B	477 (16)	32.06**(39.28**)	0.462**
	C	511 (16)	11.91**(52.21**)	0.440**
	D	520 (16)	27.20**(36.02**)	0.456**
	E	464 (16)	27.42**(24.14)	0.459**
	F	388**(16)	5.39* (33.15**)	0.386*
	G	176**(15)	0.45 (8.38)	0.399
	H	18** (9)	3.44 (3.71)	0.293
Sugary	A	587 (16)	0.00 (20.26)	0.508
	B	627 (16)	0.19 (16.94)	0.512
	C	538 (16)	0.56 (15.61)	0.515
	D	569 (16)	0.00 (16.26)	0.509
	E	482* (15)	1.07 (16.09)	0.519
	F	477* (15)	2.75 (11.83)	0.525
	G	289**(16)	1.45 (18.71)	0.524
	H	129**(15)	0.88 (14.82)	0.490
Shrunken	A	533 (16)	0.00 (18.51)	0.434
	B	551 (13)	12.09**(17.87)	0.467**
	C	537 (13)	1.66 (12.27)	0.446
	D	531 (15)	0.37 (18.25)	0.428
	E	551 (14)	2.89 (9.31)	0.450
	F	382**(16)	0.23 (20.17)	0.429
	G	382**(15)	0.04 (19.66)	0.436
	H	185**(13)	2.24 (27.81**)	0.459

[1] A = 1600 h, day 0–1000 h, day 1; B = 1000 h, day 1–1600 h, day 1; C = 1600 h, day 1–2200 h, day 1; D = 2200 h, day 1–1000 h, day 2; E = 1000 h, day 2–1600 h, day 2; F = 1600 h, day 2–2200 h, day 2, G = 2200 h, day 2–1000 h, day 3; and H = 1000 h, day 3–1600 h, day 3.

[2] *,** Significant from collection period A of the locus at the 0.05 and 0.01 probability levels, respectively.

[3] *,** Chi-square values significant at the 0.05 and 0.01 probability levels, respectively. The total chi-square values for each collection period were derived using the ratio present at collection period A and had one degree of freedom. The heterogeneity chi-square values involve only the ears (samples) within each collection period and locus, and had (number of ears-1) degrees of freedom.

[4] *,** Significant from collection period A of the locus at the 0.05 and 0.01 probability levels, respectively.

locus, the seedset at H was extremely low relative to the other loci.

Pollen transmission was greatly influenced by collection period, but the effect was very locus specific (*table 2*). At the waxy locus, highly significant increases in the recessive transmission frequency were found at B, C, D, and E, but a highly significant decrease was obtained at F. No changes in the recessive transmission frequency were present at the sugary locus as a result of collection date. At the shrunken locus, a highly significant increase in the recessive transmission frequency was found at B.

4. DISCUSSION

The results of this study indicated that forced shedding in maize altered to total volume of pollen released, the diameter and relative starch content of the pollen grains, the seedsetting ability of the pollen grains, and the recessive transmission frequency. No information is available as to what processes are disrupted as a result of this procedure. Obviously, the removal of the tassel from the plant eliminates the transfer of various substances (carbohydrates, nitrogenous compounds, minerals, growth hormones, etc.) which would occur during normal pollen maturation. The absence of the transfer was probably expressed at the later collection periods in the lower starch content and reduced seedsetting ability of the pollen grains. However, most of the pollen grains in the tassel were released before seedsetting ability was affected. In the forced shedding procedure used in this study, no obvious disruption in the normal pollen release sequence was observed, but the cyclical nature of this process was disturbed so that an almost continuous release of the pollen grains resulted. The pollen release sequence (lodicule expansion, lemma and palea movement, anther exsertion, filament elongation, anther pore opening followed by pollen grain release) has been described (KIESSELBACH 1949), but nothing is known about the physiological mechanisms involved or their interactions with the environment. The environment (low light intensity, high humidity, high temperature) to which the detached tassels were exposed probably contributed to the general response. Another major variable was the genetic factors or loci involved. All three heterozygous populations have a common parent (*Wf9xH55*) which served as a source of the dominant allele at each locus. As a result, the populations were phenotypically similar in most respects. Biochemical differences among mature pollen grains containing the dominant and recessive alleles at these three loci, have been reported (LINSKENS & PFAHLER 1973; PFAHLER & LINSKENS 1970, 1971). Differences in the starch type and germination rate of pollen grains containing alleles at the waxy locus have been reviewed for certain species (ERIKSSON 1969). In the study reported here, pollen diameter and the recessive transmission frequency at the waxy locus was particularly sensitive to collection period during the forced shedding procedure. Apparently, this procedure can influence the recessive transmission frequency at specific loci. However, since no information is available about pollen development, the pollen release sequence, and the potential genetic interactions, the influence of forced shedding on pollen transmission cannot be accurately determined. Possibly, the addition of various compounds to the medium in which the cut ends of the tassels are placed may amplify differences in the transmission rates of alleles at various loci. However, additional research in this complex area is essential.

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