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THE EFFECT OF PRE-POLLINATION STYLAR TREATMENTS ON SEEDSET AND POLLEN TRANSMISSION AT VARIOUS MAIZE (*ZEA MAYS* L.) ENDOSPERM MUTANT LOCI

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SUMMARY

Aqueous solutions containing various organic compounds (phytic acid; lipase; thiourea; cellulase; α-amylase; quinaldic acid; gibberellic acid; D-methionine; L-methionine; maleic hydrazide; papain) and different solute-osmotic concentration combinations (sucrose at 5, 10, 15, 20, 25 atm; mannitol at 5, 10, 15, 20, 25 atm; NaCl at 10 atm.) were sprayed on the styles (silks) of three heterozygous (*Wx wx; Su su; Sh_2 sh_2*) populations at the waxy, sugary-1, and shrunken-2 loci about 2 h before selfing. Seed number ear^{-1} and deviations from the genetic ratio of the control at each locus were used as an index of seedset and pollen transmission, respectively. Seedset was not affected by any organic compound at any locus. Increasing osmotic concentrations generally decreased seedset, but the magnitude of the reduction depended on the solute and locus. The transmission of the recessive allele was significantly altered by some organic compounds, but the direction and magnitude depended on the compound and the locus. In general, the transmission of the recessive allele was increased. The effect of the solute-osmotic concentration solutions on pollen transmission was quite inconsistent with the direction and magnitude of any changes depending on the locus, solute, and concentration. The results indicated that pre-pollination stylar application of water does not alter seedset, and the addition of various agents in the water may amplify pollen transmission differences among alleles at specific loci. Therefore, this procedure may be useful in directing and improving the efficiency of pollen genotype selection programs.

1. INTRODUCTION

Most aspects of the pollen transmission process from microporogenesis to zygote formation are not clearly understood. As a result, little information is available to indicate the effect of the artificial manipulation of this process on the differential transmission of genetic elements. Extended mature pollen storage at 2 °C prior to pollination enhanced pollen transmission differences between alleles at various endosperm mutant loci in maize (PFAHLER 1974a). Apparently, artificial manipulation of the complex process may amplify pollen transmission differences and improve the direction and effectiveness of pollen genotype selection.

This study was conducted to determine the effect of aqueous solutions containing various organic compounds and solute-osmotic concentration combinations applied to the styles (silks) prior to pollination with fresh pollen on seedset

and pollen transmission of the alleles at the waxy, sugary, and shrunken loci in maize.

2. MATERIALS AND METHODS

Twelve aqueous solutions, all containing 500 mg l^{-1} of Tween 80 as a surfactant, were used (*table 1*). The concentration of each organic compound in each solution was 300 mg l^{-1}. Between 800–1000 h on each of two days, each of these 12 solutions was sprayed (to runoff with an aerosol sprayer) on five styles (silks) from 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. Immediately after spraying, a rubber band was placed around each earshoot bag and ear below the sprayed silk to reduce evaporation. Between 1000–1200 h, each sprayed silk was pollinated with excess quantities of fresh pollen from the same heterozygous population as the silk. Normal, mutant, and total seed number was obtained on each ear.

In another experiment, 12 aqueous solutions containing various solute-osmotic concentration combinations with 500 mg l^{-1} of Tween 80 added as a surfactant in all solutions, were used (*table 2*). The procedure (spraying, populations, pollinations, number of ears, classification) was identical to that described in the last paragraph, except that two different dates were involved.

Total seed number ear^{-1} was used as an index of seedset. An analysis of variance was performed on each locus and experiment. Significant differences from the control at each locus and experiment 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 experiment. The expected numbers were derived, using the ratio obtained in the control of each locus and experiment. To more accurately identify deviations in pollen transmission, the recessive transmission frequency (RTF) was determined from the formula, recessive frequency/0.5.

3. RESULTS

Stylar treatments with various organic compounds did not affect seedset, but did alter pollen transmission (*table 1*). Their effects on pollen transmission depended on the locus. At the waxy locus, lipase and D-methionine significantly increased the recessive transmission frequency. At the sugary locus, phytic acid and lipase significantly increased the recessive transmission frequency, but D-methionine produced a significant decrease. At the shrunken locus, 8 out of the 11 compounds significantly increased the recessive transmission frequency. Among all compounds, only lipase and D-methionine produced significant changes in the recessive transmission frequency at all loci. Both compounds significantly increased the recessive transmission frequency, except for D-methionine at the sugary locus.

Table 1. The effect of pre-pollination stylar applications of aqueous solutions containing various organic compounds 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	Solution	MSN(NE)[1]	TCS(H)[2]	RTF[3]
Waxy	Control	451 (10)	0.00 (14.73)	0.435
	Phytic acid	340 (10)	0.25 (6.57)	0.442
	Lipase	418 (9)	9.26**(14.16)	0.476**
	Thiourea	475 (9)	0.05 (19.53*)	0.432
	Cellulase	424 (10)	2.96 (19.59*)	0.457
	α-Amylase	386 (10)	0.05 (6.71)	0.432
	Quinaldic acid	520 (8)	0.81 (11.04)	0.447
	Gibberellic acid	422 (10)	1.47 (8.24)	0.420
	D-Methionine	454 (9)	4.14* (7.26)	0.461*
	L-Methionine	442 (10)	0.18 (6.30)	0.430
	Maleic hydrazide	390 (10)	2.07 (8.47)	0.454
	Papain	416 (10)	1.18 (11.88)	0.449
Sugary	Control	482 (10)	0.00 (12.92)	0.485
	Phytic acid	451 (10)	7.42**(10.81)	0.520**
	Lipase	496 (10)	8.49** (8.70)	0.521**
	Thiourea	388 (10)	0.45 (17.04)	0.476
	Cellulase	417 (10)	1.17 (9.92)	0.500
	α-Amylase	457 (10)	0.53 (13.12)	0.495
	Quinaldic acid	463 (10)	0.02 (9.62)	0.484
	Gibberellic acid	444 (10)	0.52 (14.69)	0.495
	D-Methionine	499 (10)	4.91* (13.02)	0.458*
	L-Methionine	447 (10)	0.43 (6.67)	0.477
	Maleic hydrazide	397 (10)	0.19 (8.05)	0.491
	Papain	399 (10)	0.06 (5.03)	0.489
Shrunken	Control	302 (10)	0.00 (7.70)	0.430
	Phytic acid	314 (10)	0.29 (12.86)	0.422
	Lipase	375 (10)	11.70** (8.05)	0.476**
	Thiourea	322 (9)	4.37* (15.28)	0.462*
	Cellulase	237 (10)	7.33** (9.82)	0.476**
	α-Amylase	334 (10)	2.46 (8.93)	0.452
	Quinaldic acid	264 (10)	3.41 (16.60)	0.459
	Gibberellic acid	348 (10)	4.71* (14.93)	0.460*
	D-Methionine	358 (9)	6.17* (10.97)	0.466*
	L-Methionine	337 (9)	11.65** (2.66)	0.481**
	Maleic hydrazide	336 (10)	4.58* (10.18)	0.460*
	Papain	431 (8)	15.77**(13.05)	0.485**

[1] The F values among the seed number ear^{-1} means within each locus were not significant.
[2] *,** Chi-square values significant at the 0.05 and 0.01 probability levels, respectively. The total chi-square values for each solution were derived using the ratio present in the control at that locus and had one degree of freedom. The heterogeneity chi-square values involve only the ears (samples) within each solution and locus and had (number of ears-1) degrees of freedom.
[3] *,** Significant from the control of the locus at the 0.05 and 0.01 probability levels, respectively.

Stylar treatment with various solute-osmotic concentration solutions altered both seedset and pollen transmission (*table 2*). At the waxy and shrunken loci, seedset was significantly decreased with increasing concentrations of sucrose and mannitol. However, at equal concentrations, the reduction was more pronounced with mannitol than with sucrose. The highly significant reduction in seedset with NaCl-10 was about equal at both loci. Although no significant differences among any of the solutions were found at the sugary locus, a similar tendency was apparent. The effect of the solutions on pollen transmission depended on the locus. At the waxy locus, mannitol at 10 and 20 atm significantly reduced the recessive transmission frequency. At the sugary locus, sucrose at 10 and 15 atm and mannitol at 25 atm significantly increased the recessive transmission frequency. At the shrunken locus, a significant decrease in recessive transmission frequency was found with sucrose at 25 atm and mannitol at 5 and 15 atm. No change in recessive transmission frequency was found at any locus with NaCl at 10 atm.

4. DISCUSSION

The results of this study indicated that pre-pollination stylar treatment with water and Tween 80 had little or no effect on seedset. Although no unsprayed treatment was included, the seedset of the control (sprayed with water and Tween 80) was about equal to the seedset obtained in these populations when pollinated with no pre-pollination stylar treatment. In maize, the presence of external water during pollen germination was assumed to be detrimental, since almost 100% of the pollen grains ruptured when submerged in a liquid *in vitro* germination medium (BAIR & LOOMIS 1941, COOK & WALDEN 1965, PFAHLER 1967). To avoid this problem, the addition of agar was essential to partially solidify the medium and, in so doing, control the degree of pollen grain submersion in the medium. Apparently, treatment with water and Tween 80 did not affect seedset, because either this type of treatment had no effect on pollen germination and silk penetration by the pollen tube, or the excess quantities of pollen applied to the silk overcame any disruption resulting from this treatment.

The results of this study also showed that various agents in the aqueous solutions would alter seedset and pollen transmission. Little information is available as to the morphological, biochemical, and germination differences present among pollen grains solely as the result of their individual genotypes. Various biochemical and germination differences among pollen grains containing the alleles at certain endosperm mutant loci in maize have been linked to pollen genotype *per se* (PFAHLER 1974b, 1975, 1978). However, the relationship between these differences and *in vivo* germination characteristics is yet to be established. Artificial manipulation of this process by extended pre-pollination pollen storage at 2°C was found to alter the recessive transmission frequency (PFAHLER 1974a). With this lack of basic information, selection of an agent to include in the aqueous solution for the specific purpose of altering the transmission frequency of a certain allele at a particular locus is impossible. In the study reported

Table 2. The effect of pre-pollination stylar applications of aqueous solutions containing various solutes and osmotic concentrations 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	Solution[1]	MSN(NE)[2]	TCS(H)[3]	RTF[4]
Waxy	Control	386 (10)	0.00 (11.23)	0.469
	S-5	366 (10)	0.01 (15.84)	0.470
	S-10	315 (9)	2.66 (10.19)	0.443
	S-15	238**(10)	0.27 (14.33)	0.460
	S-20	243* (10)	0.06 (12.59)	0.464
	S-25	192**(10)	0.92 (5.21)	0.450
	M-5	297 (10)	3.25 (9.22)	0.441
	M-10	176**(10)	4.29* (9.99)	0.427*
	M-15	239** (8)	0.17 (9.76)	0.461
	M-20	190**(10)	4.63* (12.03)	0.427*
	M-25	147**(10)	2.69 (2.33)	0.432
	NaCl-10	202**(10)	0.20 (3.58)	0.460
Sugary	Control	342 (10)	0.00 (10.35)	0.436
	S-5	250 (10)	0.27 (9.39)	0.445
	S-10	303 (10)	15.84** (6.01)	0.496**
	S-15	362 (10)	15.88** (5.60)	0.491
	S-20	281 (10)	0.89 (20.63*)	0.422
	S-25	240 (10)	0.51 (7.87)	0.447
	M-5	379 (9)	0.72 (8.50)	0.448
	M-10	320 (7)	1.57 (10.86)	0.458
	M-15	238 (9)	1.20 (7.90)	0.417
	M-20	258 (7)	0.90 (16.84**)	0.455
	M-25	190 (10)	17.40**(12.03)	0.515**
	NaCl-10	232 (9)	1.51 (7.32)	0.458
Shrunken	Control	414 (8)	0.00 (10.61)	0.455
	S-5	332 (9)	0.31 (6.57)	0.446
	S-10	360 (8)	2.08 (2.48)	0.432
	S-15	236* (10)	0.25 (14.27)	0.464
	S-20	246* (10)	1.75 (8.93)	0.438
	S-25	236* (8)	5.28* (9.55)	0.411*
	M-5	327 (10)	4.96* (8.15)	0.422*
	M-10	202** (9)	0.58 (15.41)	0.440
	M-15	153** (9)	9.17** (4.05)	0.387**
	M-20	162**(10)	1.73 (6.89)	0.428
	M-25	90** (9)	0.04 (3.60)	0.449
	NaCl-10	194**(10)	1.01 (29.92**)	0.436

[1] Solution-osmotic concentration: S-5 = sucrose – 5 atm; S-10 = sucrose – 10 atm; S-15 = sucrose – 15 atm; S-20 = sucrose – 20 atm; S-25 = sucrose – 25 atm; M-5 = mannitol – 5 atm; M-10 = mannitol – 10 atm; M-15 = mannitol – 15 atm; M-20 = mannitol – 20 atm; M-25 = mannitol – 25 atm; NaCl-10 = NaCl (sodium chloride) – 10 atm.

[2] *,** Significant from the control 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 solution were derived using the ratio present in the control at that locus and had one degree of freedom. The heterogeneity chi-square values involve only the ears (samples) within each solution and locus and had (number of ears-1) degrees of freedom.

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

here, only lipase and D-methionine, among all compounds tested, produced significant changes in the recessive transmission frequencies at all loci. The effect of these compounds on *in vivo* germination processes is unknown. To further complicate the problem of agent selection, the results reported here also suggested that certain compounds were effective only at specific loci. Our lack of knowledge about the mechanisms operating in this area complicates any interpretation of the various solute-osmotic concentration solutions tested. In the study reported here, increasing osmotic concentrations generally decreased both seedset and, in some combinations, altered recessive transmission frequency. However, the effects were solute and locus specific. Apparently, the alleles at some loci influenced the response of the pollen grains to certain solutes at different concentrations, but the mechanism(s) are unknown at this time.

If pollen genotype selection is to be used as a supplement to sporophytic selection in the improvement of crop species, a relationship between pollen genotype, pollen competitive ability, and sporophytic traits must be present. Recent reports have indicated that this relationship exists in certain species (MULCAHY 1975, OTTAVIANO et al. 1980, SACHER et al. 1983, SEARCY & MULCAHY 1985, TANKSLEY et al. 1981, ZAMIR 1983, ZAMIR et al. 1981, 1982). Apparently, pollen genotype selection within genetically heterogenous pollen populations based on the comparative competitive ability of the pollen grains would be effective in altering the resulting sporophytic population. The study reported here indicated that any differences in pollen competitive ability among pollen grains containing alleles at various qualitative loci, could be amplified by pre-pollination stylar treatments. Thus, artificial manipulation of the fertilization process would improve the effectiveness of pollen genotype selection. Other areas, from microsporogenesis to zygote formation, should be explored.

REFERENCES

BAIR, R. S. & W. E. LOOMIS (1941): The germination of maize pollen. *Science* **94**: 168–169.

COOK, F. S. & D. B. WALDEN (1965): The male gametophyte of Zea mays L. *Can. J. Bot.* **43**: 779–786.

HARTER, H. L. (1960): Critical values for Duncan's new multiple range test. *Biometrics* **16**: 671–685.

MULCAHY, D. L. (1975): The biological significance of gamete competition. In: D. L. MULCAHY (ed.): *Gamete competition in plants and animals*, pp. 1–4. North Holland, Amsterdam.

OTTAVIANO, E., M. SARI-GORLA & D. L. MULCAHY (1980): Pollen tube growth rates in Zea mays: implications for genetic improvement in crops. *Science* **210**: 437–438.

PFAHLER, P. L. (1967): In vitro germination and pollen tube growth of maize (Zea mays) L.) pollen. I. Calcium and boron effects. *Can. J. Bot.* **45**: 839–845.

— (1974a): Fertilization ability of maize (Zea mays L.) pollen grains. IV. Influence of storage and alleles at the shrunken, sugary and waxy loci. In: H. F. LINSKENS (ed.): *Fertilization in higher plants*, pp. 15–25. North Holland, Amsterdam.

— (1974b): Pollen genotype studies in maize (Zea mays L.). In: H. F. LINSKENS (ed.): *Fertilization in higher plants*, pp. 3–14. North Holland, Amsterdam.

— (1975): Factors affecting male transmission in maize (Zea mays L.). In: D. L. MULCAHY (ed.): *Gamete competition in plants and animals*, pp. 115–124. North Holland, Amsterdam.

— (1978): Biology of the maize male gametophyte. In D. B. WALDEN (ed.): *Maize breeding and genetics*, pp. 517–530. Wiley, New York.

SACHER, R. F., D. L. MULCAHY & R. C. STAPLES (1983): Developmental selection during self pollination of Lycopersicum × Solanum F_1 for salt tolerance of F_2. In: D. L. MULCAHY & E. OTTAVIANO (eds.): *Pollen: biology and implications for plant breeding*, pp. 329–334. Elsevier, New York.

SEARCY, K. B. & D. L. MULCAHY (1985): The parallel expression of metal tolerance in pollen and sporophytes of Silene dioca (L.) Clairv., S. alba (Mill.) Krause and Mimulus guttatus DC. *Theor. Appl. Genet.* **69**: 597–602.

TANKSLEY, S. D., D. ZAMIR & C. M. RICK (1981): Evidence for extensive overlap of sporophytic and gametophytic gene expression in Lycopersicum esculentum. *Science* **213**: 453–455.

ZAMIR, D. (1983): Pollen gene expression and selection: applications in plant breeding. In: S. D. TANKSLEY & T. J. ORTON (eds.) *Isozymes in plant genetics and breeding, part A*, pp. 313–330. Elsevier, Amsterdam.

—, S. D. TANKSLEY & R. A. JONES (1981): Low temperature effect on selective fertilization by pollen mixtures of wild and cultivated tomato species. *Theor. Appl. Genet.* **59**: 235–238.

—, — & — (1982): Haploid selection for low temperature tolerance of tomato pollen. *Genetics* **101**: 129–137.