Theoretical and Applied Genetics 42, 136–140 (1972)

In Vitro Germination and Pollen Tube Growth of Maize (*Zea mays* L.) Pollen

VI. Combined Effects of Storage and the Alleles at the Waxy (*wx*), Sugary (su_1) and Shrunken (sh_2) Loci[1]

P. L. PFAHLER and H. F. LINSKENS

Department of Agronomy, University of Florida, Gainesville, Florida (USA), and Department of Botany, University of Nijmegen, Nijmegen (The Netherlands)

Summary. Pollen grains containing either the *Wx*, *wx*, Su_1, su_1, Sh_2 or sh_2 alleles were stored for 0, 1, 2, 3, 4 or 5 days at 2 °C. After each storage period, a portion of each genotype was cultured on a 15% sucrose, 0.6% bacto-agar, 0.03% calcium nitrate and 0.01% boric acid medium, while another portion was placed on receptive silks, the number of kernels produced being a measure of fertilization ability. Regardless of the allele present in the pollen grain, 1 day of storage greatly increased the germination percentage and significantly increased pollen tube length. After 4 days of storage, there was no *in vitro* germination but some fertilization ability was found. The experiment was designed so that comparisons free from genetic background effects could be made between alleles at each locus. Significant differences at each storage period and a differential response to storage were obtained at some loci for germination percentage, ruptured percentage, pollen tube length and fertilization ability. A relationship between dominance of the allele and response to storage was detected only for fertilization ability. Since alleles at these loci affect the biochemical composition of pollen grains containing them, the results suggest that differences in *in vitro* germination characteristics and fertilization ability may be associated with biochemical composition.

Introduction

Previous studies with alleles at various loci in maize have indicated that the genotype of the pollen grain influences its *in vitro* germination characteristics (Pfahler 1971), its fertilization ability (Brink and MacGillivray 1924; Jones 1924) and its biochemical composition (Pfahler and Linskens 1970; Pfahler and Linskens 1971). However, no information is available on the relationship between pollen genotype, *in vitro* germination characteristics and fertilization ability. The use of the alleles at the waxy, sugary and shrunken loci would appear ideal for a study of this relationship since significant differences in the water-soluble polysaccharide content (Pfahler and Linskens 1971) and the amino acid content and distribution (Pfahler and Linskens 1970) in pollen grains containing them have been reported.

Maize pollen remains viable for only a short period even under optimum conditions (Johri and Vasil 1961; Jones and Newell 1948; Walden 1967). This suggests that the pollen grains are in a highly active metabolic state and, as a result, biochemical changes probably occur over time. Therefore, it would be desirable to add storage as a variable in a study of the relationship between pollen genotype, *in vitro* germination characteristics and fertilization ability, to more fully examine the effect of these alleles.

[1] Journal Series Paper No. 3950, Florida Agricultural Experiment Station.

The purpose of this study was to compare the *in vitro* germination characteristics and fertilization ability of pollen grains containing the normal and mutant alleles at the waxy, sugary and shrunken loci, stored for various periods.

Material and Methods

Six homozygous genotypes, *WxWx*, *wxwx*, Su_1Su_1, su_1su_1, Sh_2Sh_2 and sh_2sh_2 were used as pollen sources in this study. The homozygous dominant (*WxWx*, Su_1Su_1 and Sh_2Sh_2) and homozygous recessive (*wxwx*, su_1su_1 and sh_2sh_2) genotypes at each of the three loci were obtained as follows, using the waxy locus as an example. A *wxwx* source was crossed to a single cross Wf9 × H55 (*WxWx*) to produce *Wxwx*. This heterozygote was crossed to Wf9 × H55 to produce progeny in a .5 *WxWx*: .5 *Wxwx*. At least 40 plants in this progeny were selfed. Selfed ears having at least 200 kernels were examined. If no segregation for the recessive allele was observed on an ear, all kernels were considered to be *WxWx* and were planted to produce plants for use as the *WxWx* pollen source. If segregation on an ear approached a .75 *Wx*– : .25 *wxwx* ratio, the *wxwx* kernels were selected and planted to produce plants for the *wxwx* pollen source.

From the above crossing scheme, it is apparent that the homozygous dominant and the homozygous recessive genotypes at the same locus have the same genetic background except for the linkage block surrounding the locus involved. Therefore, comparisons within the same locus should be relatively free of genetic background effects. All six genotypes are related to the extent that Wf9 × H55 was used as the homozygous dominant parent in all crosses with each locus. The relationship between the original homozygous recessive sources used as parents

to produce the initial heterozygotes cannot be determined.

Pollen grains from at least 40 plants of *WxWx*, *wxwx*, Su_1Su_1, su_1su_1, Sh_2Sh_2 and sh_2sh_2 were collected (Pfahler 1965) on each of three dates, May 27, June 8 and June 17, 1970. Immediately after collection, a portion from each genotype was removed for *in vitro* germination and fertilization ability tests. The remainder was placed in an open container at about 2 °C with the relative humidity above the surface approaching 100%. The *in vitro* germination medium contained 15% sucrose, 0.6% bacto-agar, 0.3% calcium nitrate, $Ca(NO_3)_2 \cdot 4\,H_2O$ and 0.01% boric acid, H_3BO_3. Six plates were inoculated and all activity was stopped on two plates at 1, 2 and 3 hours after inoculation. Fertilization ability of pollen grains from each genotype was estimated by placing enough pollen grains to produce maximum numbers of kernels on receptive silks of 7 ears. At 1, 2, 3, 4 and 5 days of storage, a portion of the pollen grains from each genotype was removed and identical *in vitro* germination and fertilization tests were conducted.

For the *in vitro* germination portion of this study, methods of medium preparation, inoculation procedures, killing and preservation, data collection and statistical analyses have been presented in previous papers (Pfahler 1967b, 1968, 1970).

Fertilization ability was measured by counting the number of kernels produced on each pollinated ear. Appropriate statistical analysis was then applied.

Results

Significant differences in germination percentage were found among pollen grains containing the various alleles at different storage periods (Table 1). For all alleles, an increase in storage from 0 to 1 day greatly increased the germination percentage. Further increases in storage resulted in a decrease, the magnitude of the decrease being associated with the allele and locus involved. The effect of storage was most pronounced between alleles at the waxy locus. At 0 day, *Wx* was significantly higher than *wx*, while at 1 day, no significant difference was present. Increasing the storage time to 2 days decreased the germination to percentage to be consistent of *Wx* sharply while that of *wx* decreased only slightly. At 3 days, no germination was obtained with *Wx* but a substantial percentage was found for *wx*. Significant differences between the alleles at the sugary and shrunken loci as a result of storage were present but were less pronounced. The response patterns resulting from storage were not associated with genetic dominance relationships between the alleles at any locus.

A distinctly different pattern was observed for ruptured percentage compared with germination percentage (Table 1). At 0, 1 and 2 days, the ruptured percentage for all alleles remained low and relatively constant, even though the germination percentage fluctuated considerably. After 2 days, the ruptured percentage increased, the amount depending on the allele involved. Rupturing was apparently associated with loss of germination. In general, for all alleles the first substantial increase in ruptured percentage was noted in the storage period in which greatly reduced or no germination was observed. With further increases in storage beyond this period, further increases in the ruptured percentage were obtained. As with germination percentage, genetic dominance relationships did not alter the response to storage of the alleles at any locus.

Pollen tube length was altered by storage in much the same manner as germination percentage (Table 1). Storage for 1 day significantly increased length regardless of the allele or locus involved but further increases in storage reduced the length. Differences between alleles at some loci were obtained. At the waxy locus, the dominant allele *Wx* was significantly longer at 0 and 1 day than the recessive allele *wx*, but at 2 days, no difference was found. At 3 days, no germination of *Wx* was observed but the germi-

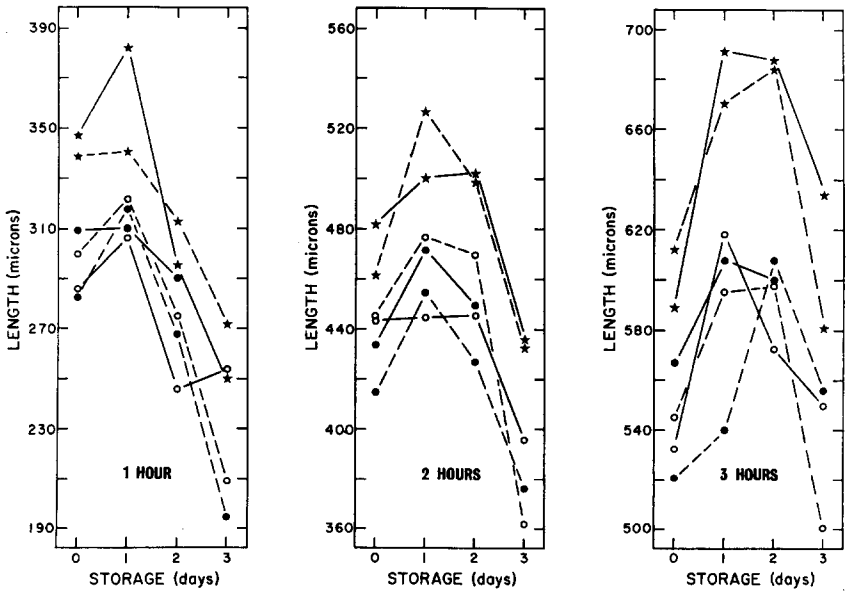

Fig. 1. The effect of storage on the pollen tube length of pollen grains containing various alleles at 1, 2 and 3 hours after inoculation. Closed circles, waxy locus; open circles, sugary locus; and stars, shrunken locus. Solid line, dominant allele (*Wx*, Su_1 or Sh_2); broken line, recessive allele (*wx*, su_1 or sh_2). Each point represents the mean of 120 measurements. Minimum differences for significance (Harter 1960) 5% = 23 and 1% = 30

Table 1. *Storage effects on the in vitro germination characteristics and fertilization ability of pollen grains containing various alleles at three loci*

Category	Locus	Allele	Storage (days at 2 °C)					
			0	1	2	3	4	5
Germinated (%)*	waxy	*Wx*	44.5 (41.8)	69.3 (56.5)	41.1 (39.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
		wx	35.1 (36.3)	70.2 (57.0)	65.8 (54.2)	30.8 (33.5)	0.0 (0.0)	0.0 (0.0)
	sugary	Su_1	30.3 (33.3)	57.1 (49.1)	36.0 (35.8)	22.9 (21.3)	0.0 (0.0)	0.0 (0.0)
		su_1	34.1 (35.6)	60.1 (50.9)	57.2 (49.2)	34.1 (35.2)	0.0 (0.0)	0.0 (0.0)
	shrunken	Sh_2	35.4 (36.2)	63.9 (53.3)	63.7 (53.1)	40.1 (39.1)	0.0 (0.0)	0.0 (0.0)
		sh_2	46.2 (42.8)	69.7 (56.7)	63.2 (52.8)	36.3 (36.7)	0.0 (0.0)	0.0 (0.0)
Ruptured (%)*	waxy	*Wx*	14.0 (21.6)	11.0 (18.6)	13.5 (20.6)	26.0 (30.6)	82.8 (65.7)	84.3 (66.8)
		wx	8.1 (16.1)	9.8 (17.7)	12.8 (20.6)	16.1 (22.9)	23.7 (28.9)	60.7 (51.3)
	sugary	Su_1	11.6 (19.6)	15.8 (22.9)	13.0 (20.3)	39.3 (37.1)	50.7 (45.5)	57.1 (49.1)
		su_1	11.3 (19.2)	14.3 (21.6)	13.4 (21.1)	18.2 (24.7)	26.8 (29.8)	49.5 (44.7)
	shrunken	Sh_2	8.8 (16.7)	11.5 (18.8)	11.6 (19.6)	11.2 (18.7)	17.4 (24.3)	52.6 (46.5)
		sh_2	8.9 (17.0)	9.2 (17.0)	11.5 (19.4)	12.9 (20.3)	19.3 (25.6)	42.8 (40.5)
Pollen tube length (μ)**	waxy	*Wx*	437	463	447			
		wx	406	437	435	376		
	sugary	Su_1	420	453	422	400		
		su_1	429	464	450	357		
	shrunken	Sh_2	472	524	495	439		
		sh_2	472	512	500	429		
Fertilization ability+	waxy	*Wx*	323	371	361	125	0	0
		wx	381	416	363	61	43	0
	sugary	Su_1	287	354	282	127	12	0
		su_1	342	377	443	245	80	30
	shrunken	Sh_2	392	425	413	208	62	0
		sh_2	491	392	498	316	155	66

* Each value represents the mean of 36 measurements. Minimum differences for significance (Harter 1960) among means in parentheses (arcsin transformation) at the 5 and 1% level respectively: germinated = 2.3 and 3.0; and ruptured = 2.2 and 2.9.

** Each value represents the mean of 360 measurements and includes all hours after inoculation. Minimum differences for significance (Harter 1960) were 13 and 17 at the 5 and 1% level respectively.

+ Number of kernels per ear with each value representing the mean of 21 ears. Minimum difference for significance (Harter 1960) were 75 and 98 at the 5 and 1% level respectively.

nation of *wx* was relatively high and substantial tube length was found. With the sugary locus, no significant differences were found at 0 and 1 day, but at longer storage periods, significant differences were obtained. At this locus at 2 days, su_1 was significantly longer than Su_1, but at 3 days Su_1 was significantly longer than su_1. No significant differences were obtained between the lengths of Sh_2 and sh_2 at any storage period. As was found with germination and ruptured percentages, genetic dominance relationships were not associated with the response to storage of the alleles at any locus.

Differences between the alleles at the various loci were found in relation to length at 1, 2 and 3 h after inoculation (Fig. 1). In general, at all hours after inoculation, the lengths after 1 day of storage were increased regardless of the allele or locus involved. Increasing storage beyond 1 day resulted in reduced lengths, the size of the decrease being associated with the allele and locus involved. Genetic dominance relationships between alleles at the same locus were not a factor in their response to storage.

Differences between alleles in fertilization ability were obtained (Table 1). The results indicated that fertilization ability was not completely related to ability to germinate on the artificial medium used, since relatively large numbers of kernels were obtained from pollination with grains that displayed no

in vitro germination capacity. For fertilization ability, genetic dominance relationships affected the response of the alleles to storage. At longer storage periods, kernels were produced by pollen grains containing the recessive allele at all loci, whereas no kernels were produced by grains containing the dominant allele.

Discussion

The results of this study indicate that pollen genotype, represented by different alleles at certain recognized loci, can greatly influence *in vitro* germination characteristics and fertilization ability. The waxy, sugary and shrunken loci chosen for this study are some of a large number of loci that alter the appearance and biochemical composition of the endosperm. Most studies (Andrew et al. 1944; Cameron and Cole 1959; Creech 1965; Kramer et al. 1958; Laughnan 1953; Pfahler et al. 1957) of these endosperm mutants have emphasized their effect on the appearance and/or the carbohydrate characteristics of the endosperm. Recently, the opaque-2 (Mertz et al. 1964) and floury-2 (Nelson et al. 1965) loci were shown to influence the content and distribution in the endosperm of amino acids, especially lysine and tryptophan. The only reported effect of the alleles at the waxy locus is the alteration of the amylose content of starch, with the recessive allele, *wx*, inhibiting the production of amylose in both endosperm and pollen starch (Creech 1965; Demerec 1924; Kramer et al. 1958). Alleles at the sugary and shrunken loci not only drastically change the physical appearance of the mature endosperm but also influence the carbohydrate content and distribution in the endosperm (Andrew et al. 1944; Cameron and Cole 1959; Creech 1965; Kramer et al. 1958; Laughnan 1953). No information is available on the effect of alleles at the sugary and shrunken loci on the amino acid content and distribution in the endosperm. Recent studies with pollen grains containing the alleles at the waxy, sugary and shrunken loci have indicated that these alleles also influence the carbohydrate content (Pfahler and Linskens 1971) and amino acid content and distribution (Pfahler and Linskens 1970). Therefore, these alleles are known to alter the biochemical composition of pollen grains.

Biochemical and phenotypic studies with alleles at these loci indicate that dominance is complete (Andrew et al. 1944; Cameron and Cole 1959; Dunn et al. 1953; Kramer et al. 1958; Neuffer et al. 1968). Therefore pollen grains containing the dominant alleles, *Wx*, Su_1 and Sh_2, should have the same biochemical pathways intact. In this study, the *in vitro* germination characteristics and fertilization ability of *Wx*, Su_1 and Sh_2 differed greatly. As indicated earlier, Wf9 × H55 was the source of all these dominant alleles. As a result of the crossing and selection procedures the *Wx*, Su_1 and Sh_2 pollen sources should contain, on average, about 75% Wf9 × H55 genetic background. Apparently, differences in the genetic background among the original sources of the recessive alleles were a major factor in altering *in vitro* germination characteristics and fertilization ability. Differences in the amino acid content and distribution in pollen grains because of genetic background were reported for the sugary locus (Pfahler and Linskens 1970).

Differences between alleles at each locus were the result of two factors: the allele at the locus under study and the linkage block surrounding the locus. As indicated earlier, the alleles at all three loci alter the carbohydrate content (Pfahler and Linskens 1971) and amino acid content and distribution (Pfahler and Linskens 1970) in the pollen grains, and these biochemical factors or their interaction may be responsible for the differences. However, other factors such as enzymes which could be directly or indirectly associated with these biochemical differences may also have an influence. The effect of the linkage block surrounding each locus cannot be accurately determined since its size or contribution is not known for each locus. The waxy and sugary loci are adjacent to the centromere on chromosomes 9 and 4 respectively (Neuffer et al. 1968). The shrunken locus is distal to the centromere on chromosome 3 (Neuffer et al. 1968).

The factors involved in the response to storage are completely unknown. An unexpected finding in this study was the large increase in germination percentage and pollen tube length after 1 day of storage. The results also suggest that fertilization ability was increased after 1 day of storage although the method used to measure fertilization ability was too imprecise to definitely establish differences. In a previous study (Pfahler 1967a), storage was found to alter the fertilization ability of pollen grains from a number of hybrids. The method of pollen collection used in this study (Pfahler 1965) ensures that mature pollen grains are obtained such as would be released normally. No reports are available on changes in the biochemical composition of pollen grains as a result of storage. However, the suspected increase in fertilization ability as a result of storage may have evolutionary significance since it would enhance or at least allow gene transfer through pollen over distance and time.

Undoubtedly fertilization is a very complex process involving many interactions. Not only the biochemical composition of the pollen grains is involved but also the conditions that promote and maintain pollen tube growth. These results indicate that *in vitro* germination capacity and fertilization ability were not completely related, especially as the storage period was extended. The measurement of fertilization ability is, at best, very difficult and the methods used are therefore imprecise. However, some fertilization was obtained using pollen grains

that showed no germination on the medium used in this study.

Acknowledgements

Sincere appreciation is expressed to H. S. Anspach for technical assistance.

Literature

1. Andrew, R. H., Brink, R. A., Neal, N. P.: Some effects of the waxy and sugary genes on endosperm in maize. J. Agr. Res. **69**, 355–371 (1944). — 2. Brink, R. A., Mac Gillivray, J. H.: Segregation for the waxy locus in maize pollen and differential development of the male gametophyte. Amer. J. Bot. **11**, 465–469 (1924). — 3. Cameron, J. W., Cole, D. A. Jr.: Effects of the genes su_1, su_2 and *du* on carbohydrates in developing maize kernels. Agron J. **51**, 424–427 (1959). — 4. Creech, R. G.: Genetic control of carbohydrate synthesis in maize endosperm. Genetics **52**, 1175–1186 (1965). — 5. Demerec, M.: A case of pollen dimorphism in maize. Amer. J. Bot. **11**, 461–464 (1924). — 6. Dunn, G. M., Kramer, H. H., Whistler, R. L.: Gene dosage effects on corn endosperm carbohydrate. Agron. J. **45**, 101–104 (1953). — 7. Harter, H. L.: Critical values for Duncan's multiple range test. Biometrics **16**, 671–685 (1960). — 8. Johri, B. M., Vasil, I. K.: Physiology of pollen. Bot. Rev. **27**, 325–381 (1961). — 9. Jones, D. F.: Selective fertilization among gametes from the same individuals. Proc. Nat. Acad. Sci. U.S.A. **10**, 218–221 (1924). — 10. Jones, M. D., Newell, L. C.: Longevity of pollen and stigmas of grasses: Buffalograss, *Bachloe dactyloides* (nutt.) Engelm. and corn, *Zea mays* L. J. Amer. Soc. Agron. **40**, 195–204 (1948). — 11. Kramer, H. H., Pfahler, P. L., Whistler, R. L.: Gene interactions in maize affecting endosperm properties. Agron. J. **50**, 207–210 (1958). — 12. Laughnan, J. R.: The effect of the sh_2 factor on carbohydrate reserves in the mature endosperm of maize. Genetics **38**, 485–499 (1953). — 13. Mertz, E. T., Bates, L. S., Nelson, O. E.: Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science **145**, 279–280 (1964). — 14. Nelson, O. E., Mertz, E. T., Bates, L. S.: Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science **150**, 1469–1470 (1965). — 15. Neuffer, M. G., Jones, L., Zuber, M. S.: The mutants of maize. Crop Sci. Soc. Amer. Publ., Madison, Wisconsin (1968). — 16. Pfahler, P. L.: Fertilization ability of maize pollen grains. 1. Pollen sources. Genetics **52**, 513–520 (1965). — 17. Pfahler, P. L.: Fertilization ability of maize pollen grains. II. Pollen genotype, female sporophyte and pollen storage interactions. Genetics **57**, 513–521 (1967a). — 18. Pfahler, P. L.: *In vitro* germination and pollen tube growth of maize (*Zea mays* L.) pollen. I. Calcium and boron effects. Can. J. Bot. **45**, 839–845 (1967b). — 19. Pfahler, P. L.: *In vitro* germination and pollen tube growth of maize (*Zea mays*) pollen. II. Pollen source, calcium and boron interactions. Can. J. Bot. **46**, 235–240 (1968). — 20. Pfahler, P. L.: *In vitro* germination and pollen tube growth of maize (*Zea mays*) pollen. III. The effect of pollen genotype and pollen source vigor. Can. J. Bot. **48**, 111–115 (1970). — 21. Pfahler, P. L.: *In vitro* germination and pollen tube growth of maize (*Zea mays*) pollen. IV. Effects of the fertility restoring Rf_1 locus. Can. J. Bot. **49**, 55–57 (1971). — 22. Pfahler, P. L., Linskens, H. F.: Biochemical composition of maize (*Zea mays* L.) pollen. I. Effects of the endosperm mutants, waxy (*wx*), shrunken (sh_2) and sugary (su_1) on the amino acid content and distribution. Theor. Appl. Genet. **40**, 6–10 (1970). — 23. Pfahler, P. L., Linskens, H. F.: Biochemical composition of maize (*Zea mays* L.) pollen. II. Effects of the endosperm mutants, waxy (*wx*), shrunken (sh_2) and sugary (su_1) on the carbohydrate and lipid percentage. Theor. Appl. Genet. **41**, 2–4 (1971). — 24. Pfahler, P. L., Kramer, H. H., Whistler, R. L.: Effect of genes on birefringence endpoint temperature of starch grains in maize. Science **125**, 441–442 (1957). — 25. Walden, D. B.: Male gametophyte of *Zea mays* L. I. Some factors influencing fertilization. Crop Sci. **7**, 441–444 (1967).

Received October 5, 1971

Communicated by H. F. Linskens

Professor Dr. P. L. Pfahler
Department of Agronomy
University of Florida
Gainesville, Florida 32601 (USA)

Professor Dr. H. F. Linskens
Department of Botany
University of Nijmegen
Toernooiveld
Nijmegen (The Netherlands)