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Ash Percentage and Mineral Content of Maize (*Zea mays* L.) Pollen and Style

I. Genotypic Effects[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 and style from three single cross hybrids (*Wf9xH55*, *Ky49xKy27*, *K64xK55*) and two inbred lines (*0h43*, *H55*) were ashed and the content of various mineral elements was determined. The ash percentage of the pollen was 2.93, 2.94, 2.83, 3.70, and 3.77 for *Wf9xH55*, *Ky49xKy27*, *K64xK55*, *0h43*, and *H55* respectively. Significant differences were found between the hybrid and inbred groups but not within groups. The ash percentage of the style was 4.85, 4.60, 4.52, 5.59, and 5.23 for *Wf9xH55*, *Ky49xKy27*, *K64xK55*, *0h43*, and *H55* repectively. Significant differences were present both between and within the hybrid and inbred groups. Averaged over all genotypes the content of *Al*, *Ca*, *Cu*, *Fe*, *K*, *Mg*, *Mn*, *Na*, *P*, and *Zn* was 0.46, 9.20, 0.20, 0.48, 105.09, 11.58, 0.24, 5.93, 69.57, and 1.90 micrograms/gram dry weight of pollen respectively. The content of *Al*, *Ca*, *Fe*, and *K* was altered by genotype. Averaged over all genotypes, the content of *Al*, *Ca*, *Cu*, *Fe*, *K*, *Mg*, *Mn*, *Na*, *P*, and *Zn* was 0.25, 19.33, 0.32, 0.64, 308.80, 23.03, 0.21, 10.78, 52.20, and 1.10 micrograms/gram dry weight of style respectively. The content of *Al*, *Ca*, *Fe*, *K*, *Mg*, and *Mn* was altered by genotype. Based on dry weight, highly significant differences between the pollen and style were obtained for all elements. Also, significant tissue × genotype interactions were found for *Ca*, *Fe*, *K*, *Mg*, *Mn*, *and P*. Averaged over all genotypes, the content of *Al*, *Ca*, *Cu*, *Fe*, *K*, *Mg*, *Mn*, *Na*, *P*, *and Zn* was 13.83, 288.39, 6.43, 15.06, 3287.58, 370.27, 7.48, 185.55, 2176.65, and 58.95 micrograms/gram ash weight of pollen respectively. The content of *Al*, *Ca*, *Fe*, *K*, *Mg*, and *Mn* was altered by genotype. Averaged over all genotypes, the content of *Al*, *Ca*, *Cu*, *Fe*, *K*, *Mg*, *Mn*, *Na*, *P*, and *Zn* was 4.76, 390.28, 6.54, 12.84, 6235.78, 466.59, 4.22, 220.41, 1059.23, and 22.12 micrograms/gram, ash weight of style respectively. The content of *Al*, *Ca*, *Fe*, *Mg*, *Mn*, and *P* was altered by genotype. Based on ash weight, highly significant differences between the pollen and style were found for *Al*, *Ca*, *Fe*, *K*, *Mg*, *Mn*, *P*, and *Zn*. Also, significant tissue × genotype interactions were found for *Ca*, *Fe*, *K*, *Mg*, and *Mn*. The results indicated that the ash percentage and the content of a number of mineral elements in the pollen and style were influenced by the source genotype.

Introduction

Information regarding the ash percentage and mineral content of maize pollen is very limited. The few previous studies were generally confined to analyzing pollen from a single genotype (Anderson and Kulp 1922; Knight *et al.* 1973). However, ash percentage was reported to be influenced by the genotype of the source (Anderson and Kulp 1922). Other characteristics of pollen grains such as fertilization ability (Pfahler 1965, 1967), *in vitro* germination characteristics (Pfahler 1968, 1970, 1971; Pfahler and Linskens 1972) and biochemical composition (Pfahler and Linskens 1970, 1971) were shown to be influenced by the genotype of the pollen or pollen source. Therefore, ash percentage and mineral content of the pollen probably would also be altered by the genotype of the pollen or pollen source.

Very little information is available regarding the ash percentage and mineral content of maize style. A very recent study (Knight *et al.* 1973) reported the *Ca*, *K*, *Mg*, *P*, and *S* content of style collected from one genotype. The effect of genotype on one other characteristic of the style in maize has been found (Pfahler 1967). The fertilization ability of pollen grains was shown to be influenced by the genotype of the style suggesting that the rate of pollen tube growth through the style was altered by the genotype of the style.

Genotypic differences in the ash percentage and mineral content of the pollen and style would contribute to our understanding of the fertilization process. Therefore, this study was undertaken to determine the ash percentage and mineral content of pollen and style from five genotypes.

Materials and Methods

Five genotypes, three single cross hybrids (*Wf9xH55*, *Ky49xKy27*, *K64x55*) and two inbred lines (*0h43*, *H55*) were used. Pollen and style were collected from at least 100 plants of each genotype. Pollen was collected and screened by the method of Pfahler (1965). Styles free of pollen (bagged before emergence) were collected and cut into sections about 1 cm long to promote rapid drying. At the time of collection, the styles had attained maximum length. Immediately after collection, the pollen and style sections were rapidly dried using silica gel as a desiccant and a temperature of 30 °C.

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Approximately 5g of the dried pollen and style from each genotype were accurately weighed in beakers and ashed. The ashing procedure involved heating for 1 h at 200 °C followed by 8 h at 500 °C. The beakers were allowed to cool slowly. After cooling, excess concentrated HCl was added and slowly evaporated. The beaker was then heated for 8 h at 500 °C. A white ash resulted. After careful reweighing, the ash weight was determined and the ash percentage was calculated. The ash was then dissolved in a 0.1 N HCl solution for mineral content determinations. Four replications of the weighing and ashing procedure were made for the pollen and style of each genotype. Therefore, for each tissue in each genotype, four measurements of ash percentage and the content of each mineral element were obtained.

The content of *Al, Ca, Cu, Fe, Mg,* and *Zn* was determined with an atomic absorption spectrophotometer. The content of *K* and *Na* was determined with a flame emission spectrophotometer. The content of *P* was determined colorimetrically using the chlorostannous-reduced molybdophosphoric blue color method in a sulfuric acid system (Jackson 1958).

The content of each element was expressed on the basis of micrograms/gram dry weight of tissue and micrograms/gram ash weight of tissue. Both were necessary because of the large differences found in the ash percentages of the tissues and genotypes.

For each element, a number of analyses of variance was performed. To reduce variance heterogeneity, the data expressed in dry weight of tissue was analyzed separately from that expressed in ash weight of tissue. Initially, an analysis of variance was performed on the data associated with the pollen and style separately to determine genotypic effects wthin the pollen and style. A second analysis of variance was then performed combining the pollen and style data. *F* values for the tissue main effect and the tissue × genotype interaction were obtained from the second analysis.

The minimum differences for significance presented in the tables were obtained by the revised Duncan's ranges using for *p* only the maximum number of means to be compared (Harter 1960).

Results

Ash Percentage

Significant differences were found between the pollen and style with the percentage in the style considerably higher than that in the pollen for all genotypes (Table 1). In the pollen, considerable differences were present between the genotypes with the single cross hybrids consistently lower as a group than the inbred lines. Significant differences were present between groups but not within the hybrid group or the inbred group. A similar situation was found in the style with the single cross hybrids having a lower percentage than the inbred lines. However, significant differences were found not only between groups but within each group. A highly significant tissue × genotype interaction was obtained indicating that the genotypes altered the magnitude of the differences between the pollen and style.

Table 1. *Ash percentage*[a] *of pollen and style from several maize genotypes*

Genotype	Description	Heterozygosity level	Pollen	Style
Wf9xH55	Single cross hybrid	High	2.93	4.85
Ky49xKy27	Single cross hybrid	High	2.94	4.60
K64xK55	Single cross hybrid	High	2.83	4.52
Oh43	Inbred line	Low	3.70	5.59
H55	Inbred line	Low	3.77	5.23

[a] Minimum differences for significance were 0.12 and 0.17 at the 5 and 1% level respectively. Each value represents the mean of 4 measurements.

Element Content

Dry weight of tissue: The content of many elements in the pollen and style were influenced by the genotypes (Table 2). In general, the genotypic influence on each element was present both in the pollen and style or was not present in either the pollen or style as in *Cu, Na, P,* and *Zn*. However, with *Mg* and *Mn* a genotypic influence was found in the style but not in the pollen.

Table 2. *Content (micrograms/gram dry weight) of various mineral elements in the pollen and style. F values associated with the tissue main effect and the tissue × genotype interaction are included*

Element	Pollen	Style	F value: Tissue main effect	F value: Tissue × genotype interaction
Al	0.46[a]	0.25[a]	34.73[c]	1.11
Ca	9.20[a]	19.33[a]	3803.26[c]	73.44[c]
Cu	0.20	0.32	17.15[c]	0.77
Fe	0.48[a]	0.64[a]	94.19[c]	12.38[c]
K	105.09[a]	308.80[a]	3703.06[c]	11.57[c]
Mg	11.58	23.03[a]	365.22[c]	5.69[c]
Mn	0.24	0.21[a]	9.57[c]	4.39[c]
Na	5.93	10.78	34.72[c]	1.62
P	69.57	52.20	40.58[c]	2.84[b]
Zn	1.90	1.10	19.87[c]	1.75

[a] Significant differences (*F* value) at the 1% level between genotype means that were averaged to obtain this value. Each value represents the mean of 20 measurements.
[b,c] *F* value significant at the 5 and 1% level respectively.

As shown by the *F* values associated with the tissue main effect, significant differences between the pollen and style were found for all elements (Table 2). Differences between the pollen and style were not large or consistently in one direction. The element *K* showed the largest percentage difference with the style having 294% more *K* than the pollen.

The tissue × genotype interactions indicated that for *Ca, Fe, K, Mg, Mn,* and *P,* the genotypes altered the magnitude of the differences between the pollen and style (Table 2).

The content of each genotype for those elements which showed either a significant genotype effect or a tissue × genotype interaction is shown in Table 3. Relatively large differences within the pollen and style as a result of the genotypes were found. These

Table 3. *Content (micrograms/gram dry weight) of various mineral elements in pollen and style from each genotype. Only those elements in which a significant genotype effect or a tissue × genotype interaction was indicated in Table 2 are included*

Element	Tissue	Genotype					Minimum differences	
		Wf9xH55	*Ky49xKy27*	*K64xK55*	*Oh43*	*H55*	5%	1%
Al	Pollen	0.35[a]	0.28	0.30	0.70	0.66	0.26	0.36
	Style	0.10	0.15	0.15	0.50	0.33	0.10	0.13
Ca	Pollen	8.17	8.67	9.40	9.68	10.08	0.92	1.29
	Style	15.07	16.50	22.01	23.90	19.19	0.83	1.16
Fe	Pollen	0.41	0.51	0.35	0.60	0.55	0.08	0.12
	Style	0.46	0.54	0.60	0.93	0.68	0.09	0.13
K	Pollen	93.29	117.65	93.80	100.98	119.71	7.18	10.03
	Style	313.06	289.68	278.29	335.43	327.54	24.18	33.77
Mg	Pollen	10.00	15.17	10.60	10.65	11.49		
	Style	23.94	21.13	22.84	24.06	23.19	1.47	2.05
Mn	Pollen	0.21	0.26	0.25	0.26	0.21		
	Style	0.22	0.18	0.21	0.23	0.22	0.01	0.02
P	Pollen	61.94	68.64	62.20	69.60	85.47		
	Style	50.89	49.91	55.20	53.37	51.64		

[a] Each value represents the mean of 4 measurements.

differences were not consistently associated with the heterozygosity level of the genotypes. However, for some elements, the inbred lines had a higher content as a group than the single cross hybrids. The significance of the tissue × genotype interactions indicated that, considering a number of genotypes, the content of the pollen would not reflect the content of the style. The element, *Ca*, showed the largest interaction ($F = 73.44$, Table 2) and will be used as an example. The difference (style content-pollen content) was 6.90 for *Wf9xH55* and 14.22 for *Oh43*.

Ash weight of tissue: The content of many elements in the pollen and style were influenced by the genotypes (Table 4). In general, the genotypic influence was present both in the pollen and style or was not present in either the pollen or style as in *Cu*, *Na*, and *Zn*. However, with *K* and *P*, a genotypic influence was present in either the pollen or style but not both.

Table 4. *Content (micrograms/gram ash weight) of various mineral elements in the pollen and style. F values associated with the tissue main effect and the tissue × genotype interaction are included*

Element	Pollen	Style	*F* value	
			Tissue main effect	Tissue × genotype interaction
Al	13.83[a]	4.76[b]	83.09[c]	1.07
Ca	288.39[b]	390.26[b]	467.78[c]	29.89[c]
Cu	6.43	6.54	0.02	0.68
Fe	15.06[b]	12.84[b]	26.54[c]	8.74[c]
K	3287.58[b]	6235.78	1771.07[c]	6.76[c]
Mg	370.27[b]	466.59[b]	33.78[c]	5.49[c]
Mn	7.48[a]	4.22[b]	152.01[c]	5.54[c]
Na	185.55	220.41	2.56	1.65
P	2176.65	1059.23[b]	182.82[c]	0.75
Zn	58.95	22.12	51.74[c]	1.39

[a,b] Significant differences (*F* value) at the 5 and 1% level respectively between genotype means that were averaged to obtain this value. Each value represents the mean of 20 measurements.

[c] *F* value significant at the 1% level.

As shown by the *F* values associated with the tissue main effect, significant differences between pollen and style were obtained for all elements except *Cu* and *Na* (Table 4). Differences between the pollen and style were not large with *Al* showing the largest difference in terms of percentage. In this case, the pollen had 291% more *Al* than the style.

The tissue × genotype interactions indicated that for *Ca*, *Fe*, *K*, *Mg*, and *Mn*, the genotype altered the magnitude of the differences between the pollen and style (Table 4).

The content of each genotype for those elements which showed either a significant genotype effect or a tissue × genotype interaction is presented in Table 5. Relatively large differences between the pollen and style as a result of the genotypes were found. In general, these differences were not consistently associated with the heterozygosity level of the genotypes. However, some exceptions did occur. The significance of the tissue × genotype interactions indicated that, considering a number of genotypes, the content of the pollen would not reflect the content of the style. The element, *Ca*, showed the largest interaction ($F = 29.89$, Table 4). The difference (style content-pollen content) was 30.77 for *Wf9xH55* and 164.64 for *Oh43*.

Discussion

The results of this study indicated that the genotype influenced the ash percentage of maize pollen. The range in ash percentage reported for maize pollen was from 2.55 (Todd and Bretherick 1942) to 4.90 (Nielsen *et al.* 1955). Only one report indicates genotypic differences. Anderson and Kulp (1922) reported that the ash percentage of yellow dent, white flint

Table 5. *Content (micrograms/grams ash weight) of various mineral elements in pollen and style from each genotype. Only those elements in which a significant genotype effect or a tissue × genotype interaction was indicated in Table 4 are included*

Element	Tissue	Genotype					Minimum difference	
		Wf9xH55	*Ky49xKy27*	*K64xK55*	*Oh43*	*H55*	5%	1%
Al	Pollen	12.04[a]	9.33	10.86	19.15	17.96	7.23	
	Style	2.07	3.27	3.32	9.84	6.22	1.98	2.77
Ca	Pollen	280.22	295.66	333.24	263.19	269.62	30.91	43.16
	Style	310.99	358.57	487.38	427.83	366.64	55.21	77.10
Fe	Pollen	14.08	17.50	12.58	16.56	14.58	22.55	3.56
	Style	9.51	11.77	13.37	16.60	12.96	2.00	2.80
K	Pollen	3193.71	4007.15	3319.17	2739.41	3178.47	224.24	313.14
	Style	6460.73	6296.13	6162.56	6003.26	6256.24		
Mg	Pollen	346.61	517.69	377.66	293.11	316.30	121.48	169.64
	Style	493.98	459.40	505.97	430.59	442.99	28.95	40.43
Mn	Pollen	7.16	8.84	8.67	7.05	5.69	1.99	
	Style	4.48	3.74	4.53	4.16	4.20	0.14	0.20
P	Style	1048.90	1084.87	1221.33	954.65	986.40	109.55	152.98

[a] Each value represents the mean of 4 measurements.

and popcorn was 3.46, 3.83, and 3.13 respectively. Presumably the varieties were open-pollinated so that no differences in heterozygosity levels were present. Therefore, the differences were the result of genetic factors which may or may not be related to the starch endosperm types represented in the study. In the study reported here, the five genotypes were all of the dent type but differed in heterozygosity level. The results indicated that differences in ash percentage were obtained between but not within heterozygosity levels. Apparently, a major factor in the difference was the heterozygosity level rather than genetic factors. The environmental conditions under which the pollen source is grown is another factor which could conceivable alter the ash percentage of pollen. Nielsen *et al.* (1955) collected pollen in each of two successive years and reported an ash percentage of 4.90 for each year. They did not indicate if the same genotype was used in each year. However, if this is assumed, environment in the form of years apparently has little or no influence on the ash percentage of the pollen.

In comparison with pollen, the genotypic influence on the ash percentage of style followed a somewhat different pattern. The results of this study indicated that as in pollen, both inbred lines as a group had a higher ash percentage than the single cross hybrids as a group. However, significant differences were present within the two groups. This suggests that not only heterozygosity level but genetic factors are involved in influencing the ash percentage of the style.

The level of mineral elements in pollen vary widely depending on the species (Todd and Bretherick 1942). In this study, the content of the various elements compare quite closely to those reported for maize pollen by other workers (Anderson and Kulp 1922; Knight *et al.* 1973; Nielsen *et al.* 1955; Todd and Bretherick 1942). No studies are available making direct comparisons among genotypes within a species. The results of this study indicated that for most elements, the genotypic influence is independent of the heterozygosity level of the genotypes. One report suggested that the environmental conditions under which the pollen source is grown may influence the mineral content of the pollen. Nielsen *et al.* (1955) reported that the *P* content was 0.58% ash weight in 1953 and 0.75% ash weight in 1954. No indication was given whether this difference was significant or the same genotype was sampled each year. If both are assumed, then apparently, year differences will alter the content of some elements.

Only one report concerning the mineral content of maize style is available. Knight *et al.* (1973) reported the *Ca*, *K*, *Mg*, *P*, and *S* content of style collected from one genotype and in general, their values correspond closely to those presented in this paper. In the study reported here, no consistent relationship between the heterozygosity level of the genotypes and their mineral content was found indicating that genetic factors were involved.

The role of mineral elements in maize pollen and style is essentially unknown at the present time. *In vitro* germination studies (Pfahler 1968, 1970, 1971) with maize pollen have indicated that the level of *Ca* and *B* required in the artificial medium to produce maximum germination and pollen tube growth varies considerably depending on the genotype of the pollen or pollen source. This suggests that the content of some elements in the pollen may be associated with the level of the same elements in the germination medium required to produce maximum germination and pollen tube growth. The conditions present in the *in vitro* germination medium probably are related to those in the style. Therefore the reported differences in fertilization ability resulting from the pollen

source (Pfahler 1965, 1967) and style (Pfahler 1967) may be related to the mineral content of the pollen and style. The complex relationships between genotype, mineral content, *in vitro* germination characteristics and fertilization ability require further study.

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Professor Dr. P. L. Pfahler
Department of Agronomy
University of Florida
Gainesville, Florida 32611 (USA)

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