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4. Intervarietal differentiation of maize pollen.

Considerable differences in values for quantitative characters of maize pollen and pollen of maize-related taxa have already been demonstrated (Tsukada and Rowley, 1964, Banerjee and Barghoorn, 1970). Attempts to discriminate between the pollen of maize and that of Euchlaena mexicana (teosinte) and Tripsacum spp. are complicated by the wide range of values for any given character. Pollen diameter, pore-axis ratio, spinule density and spinule distribution have proven to be useful characters when taken together. Little work has been done, however, on variation at the varietal level.

Rumbaugh and Whalen (1972) reported that significant size differences exist among pollen grains from some maize genotypes, particularly in the case of tetraploid varieties.

This report outlines some preliminary aspects of a study being carried out to determine the feasibility of characterizing the pollen on the basis of multivariate analysis of a number of characters.

Pollen samples were collected from plants of 11 stocks (see Table 1) grown in the field or in the greenhouse. Samples were immediately transferred to a deep freeze where they were stored at -10°C.

Micrographs of pores and of areas of the spore wall were taken at a magnification of 5000, 10,000 and 20,000 diameters in a Cambridge Mark 2a scanning electron microscope. For this purpose, samples were fixed to glass and coated with a gold-palladium alloy.

Spinule density was calculated for 5 to 8 pollen grains per stock by counting the number of spinules in a 176 sq. cm. area from 20,000X micrographs. This corresponded to an actual area on the pollen of $44u^2$. The mean basal diameter of spinules was calculated from 20,000X micrographs and based on 40 spinules per grain. Data for 5 to 8 pollen grains were recorded for each stock.

Table 1

Sample number	Stock	Growing conditions
1	'Seneca' 60 su_1/su_1	field August 1972
2	'Seneca' 60 su_1/su_1	field August 1972
3	'Seneca' 60 su_1/su_1	field August 1972
4	'Seneca' 60 su_1/su_1	greenhouse October 1972
5	'Seneca' 60 su_1/su_1	greenhouse October 1972
6	Seneca Chief su_1/su_1	field August 1972
7	Seneca Chief su_1/su_1	field August 1972
8	Seneca Chief su_1/su_1	field August 1972
9	9-tester yg c sh bz wx	field August 1972
10	W23 4N	field August 1972
11	W23 4N	field August 1972
12	9 tester yg C sh wx	field August 1972
13	9 tester yg C sh wx	field August 1972
14	ABPHYL	field August 1972
15	CO106	field August 1972
16	8174-6 st/st	field August 1972
17	8200-1 am/+	field August 1972
18	O_2	greenhouse October 1972
19	v_1	greenhouse October 1972

Pore diameter was defined as the maximum distance separating the spinules on either side of the pore. Measurements were made for 8 to 10 grains per stock at 10,000 or 20,000X.

Pollen samples were also examined with a Phillips 75 transmission electron microscope. For this purpose, they were stained with $KMnO_4$ and embedded in hard Spur plastic prior to thin sectioning.

Measurements of pollen size were carried out for several stocks. The maximum diameter in the plane of the aperture and perpendicular to it was recorded for pollen which had been allowed to imbibe in 0.5M sucrose for 1 hour.

To determine differences in properties of imbibition and strength of intine at the pore, data were collected on a number of pollen samples which showed extent of intine rupture after 1 hour in 4, 3.5, 3, 2.5, 2, 1.5 and 1M sucrose.

The values representing spinule density listed in Table 2 show considerable discrimination between stocks. Within a variety, density values did not show significant differences between plants either under the same or differing culture conditions (see samples 1-4); 63% of the sample pairs which might have been expected to differ were discriminated at $p \leq 0.05$.

Measurements of spinule diameter resulted in 26% of intervarietal sample pairs being discriminated at $p \leq 0.05$ (see Table 3). No significant differences were demonstrated between plants of the same variety resulting from the same or different growing conditions. There was, however, a difference between measurements taken from different electron microscope preparations of the same sample (see Table 3, sample pair 1a - 1b).

The character pore diameter (see Table 4) proved capable of discriminating at $p = 0.05\%$ or better, 43% of those sample pairs representing varietal differences. Sample pairs 1-2, 1-4, 2-3, 2-4, 3-4, 6-7, 6-8, 7-8, representing samples of a single variety from different plants grown under the same or differing environmental conditions, did not differ significantly at $p \leq 0.05$.

Of the characters analyzed in detail, spinule density and pore diameter proved useful in discrimination of pollen of the varieties tested. In only one instance did intra-varietal samples differ significantly. For the varieties studied, it appears that values for these characters are relatively consistent within a given variety and differ between varieties, to an extent which allows for discrimination among a number of varieties with a probability of 0.999.

While it was shown that the values for spinule diameter differed considerably between varieties, differences were significant primarily at the $p = 0.05$ and $p = 0.02$ levels. This might suggest that the varietal differences in spinule diameter values are insufficient to allow for confident discrimination between varieties. However, the level of resolution obtainable with this material on the scanning electron microscope was such that considerable error variance was accumulated in measuring this character.

Table 2

Spinule density on surface of pollen grain: expressed as number of spinules per 44 sq.u.

Sample number	$\bar{X}$	S^2	Sample number										
			1a°	1b°	4	5	6	9	10	12	14	15	16
1a	208.00	23.56											
1b	190.60	13.90	-										
4	220.60	34.39	-	-									
5	189.00	38.11	-	-	-								
6	130.60	20.71	.001	.001	.002	.01							
9	105.80	12.47	.001	.001	.001	.002	.1						
10	151.40	19.34	.01	.01	.01	.1	-	.01					
12	119.20	17.46	.001	.001	.001	.01	-	-	.05				
14	167.60	39.17	.1	-	.1	-	.1	.002	-	.05			
15	141.60	19.16	.002	.002	.01	.05	-	.01	-	.1	-		
16	114.40	16.99	.001	.001	.001	.01	-	-	.02	-	.05	.05	
17	147.80	12.19	.001	.001	.01	.05	-	.001	-	.02	-	-	.05

°Separate scanning electron microscope preparation of sample 1.

Table 3

Spinule diameter: expressed in microns x 10^{-1}

Sample number	$\bar{X}$	S^2	Sample number 1a°	1b°	4	5	6	9	10	12	14	15	16	17
1a	3.26	0.24												
1b	3.54	0.15	.05											
4	3.48	0.18	-	-										
5	3.64	0.53	.1	-	-									
6	3.29	0.14	-	.02	.1	.05								
9	3.53	0.58	-	-	-	-	-							
10	3.81	0.42	.05	-	.1	-	.05	-						
12	3.81	0.29	.02	.1	.05	-	.01	-	-					
14	3.26	0.44	-	-	-	-	-	-	.1	.1				
15	3.69	0.26	.05	-	-	-	.05	-	-	-	-			
16	3.65	0.18	.05	-	-	-	.02	-	-	-	-	-		
17	3.43	0.27	-	-	-	-	-	-	-	-	-	-	-	
19	2.97	0.01	-	.02	.01	.02	.01	-	.05	.01	-	.05	.01	.1

°Separate scanning electron microscope preparations of sample 1.

Table 4

Pore diameter expressed in microns: measured as the maximum distance separating spinules on either side of the pore

Sample number	$\bar{X}$	s^2	Sample number 1	2	3	4	6	7	8	9	11	13	14	15	16	17
1	8.18	0.58														
2	8.26	0.96	-													
3	8.97	0.90	.05	.1												
4	8.50	0.82	-	-	-											
6	8.76	0.95	-	-	-	-										
7	8.76	0.72	-	-	-	-	-									
8	9.30	0.76	.01	.05	-	.1	-	-								
9	8.15	1.18	-	-	-	-	-	-	.05							
11	10.29	1.81	.01	.02	.1	.05	.05	.05	-	.01						
13	7.64	0.82	-	-	.002	.1	.02	.01	.001	-	.001					
14	6.52	0.62	.001	.002	.001	.001	.001	.001	.001	.01	.001	.01				
15	7.93	0.86	-	-	.1	-	.1	.1	.01	-	.01	-	.01			
16	8.73	1.67	-	-	-	-	-	-	-	-	.1	.1	.01	-		
17	7.67	0.88	-	-	.01	.1	.05	.02	.02	-	.002	.002	.02	-	-	
18	7.09	0.98	.05	.1	.002	.05	.01	.01	.001	.1	.01	-	-	-	.1	-

It is felt that the character spinule diameter will prove to be useful, if an improved measuring technique can be found. Preliminary investigations of ultrathin sections of pollen viewed through a transmission electron microscope suggest that this may represent an improved technique.

Early investigations of pollen size and tendency towards intine rupture lead us to believe that these characters will also contribute to intervarietal discrimination.

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1. The taxonomy of Zea mays (Gramineae).

The origin of maize has long been disputed. Of the various theories, the oldest postulates its direct origin by ancient human selection from a wild grass of the genus Euchlaena, the "Teosinte" of Mexico and Guatemala; i.e., maize is simply regarded as a highly domesticated and variable cultivar of Euchlaena. The morphological steps, first clearly outlined by G. N. Collins (J. Agr. Res. 17: 127-135, 1919), were discussed but not accepted by P. C. Mangelsdorf (Bot. Mus. Leafl. Harvard Univ. 12: 33-75, 1945) and amplified by W. C. Galinat (An. Rev. Gen. 5: 447-478, 1971) and myself (H. H. Iltis, The Maize Mystique, 5 pp. mimeo. MS. 1970; cf.