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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 Fatty Acid Distribution[1]

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Summary. Proline was the most abundant amino acid with a mean value of 186.28 μ moles/mg dry pollen. The other amino acids tested were below 33 μ moles/mg dry pollen. The mutant *wx* significantly increased aspartic acid, valine, histidine and an unknown but significantly decreased α aminobutyric acid. The mutant sh_2 significantly increased glutamic acid, proline, lysine, histidine and an unknown but significantly decreased aspartic acid and α aminobutyric acid. The effect of su_1 was altered by the genetic background involved. In one genetic background, su_1 did not significantly increase any amino acid but significantly decreased alanine and α aminobutyric acid. However, in a distinctly different background, su_1 significantly increased α aminobutyric acid but significantly decreased aspartic acid and glutamic acid. Apparently the genetic background is capable of producing major shifts in the amino acid pattern in addition to the action of these mutants.

The fatty acids, palmitic and linolenic were the most common with percentages of 54.1 and 34.4 respectively. The mutants tested did not affect the fatty acid distribution.

Introduction

The use of pollen to study the relationship between gene action and biochemistry offers unique advantages. Firstly, pollen grains are independent, highly reduced single cells that are very active metabolically and are capable of growth and cell wall synthesis. Secondly, their haploid nature makes possible a more critical isolation and examination of the action of specific alleles without the confounding effects of dominance.

In maize, various mutants which alter the physical appearance of the endosperm have been identified and described (Neuffer et al. 1968). Most of these endosperm mutants have been shown to alter carbohydrate synthesis in the endosperm (Creech 1965). A few have drastically shifted the amino acid pattern in the endosperm (Mertz et al. 1964; Nelson et al. 1965). One mutant, waxy, affects the starch composition of the endosperm and pollen grains in the same manner (Demerec 1924). Also, the action of the waxy allele on pollen starch is independent of the plant on which the pollen grain is produced. Therefore, the biochemistry of the pollen grain may be directly influenced by the alleles it contains and as a result, differences in biochemical composition could be associated with the actions of specific alleles.

The purpose of this study was to investigate the effect of three endosperm mutants on the amino acid content and fatty acid distribution of pollen grains.

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Materials and Methods

Plant Material Produced at: Department of Agronomy, University of Florida, Gainesville, Florida.

Biochemical Analyses Performed at: Department of Botany, University, Nijmegen, the Netherlands.

Description and derivation of pollen sources: Large quantities of pollen grains from at least 15 plants within each of nine pollen sources (Table 1) were collected by the method of Pfahler (1965). Immediately after collection, the pollen grains were rapidly dried using silica-gel as a desiccant and a temperature of 30 °C.

A description of the pollen sources is presented in Table 1. With the exception of pollen source 9 (group E) which was a normal dent single cross hybrid (Wf9 × H55), the two lines within each group were obtained by backcrossing. Using *wx* as an example (pollen sources 1 and 2, group A), a homozygous recessive source (*wxwx*) was crossed with a homozygous dominant (*WxWx*) single cross hybrid producing a heterozygote (*Wxwx*). This heterozygote (*Wxwx*) was backcrossed to the original *wxwx* source. The resulting ear contained both *wxwx* and *Wxwx* kernels. The *wxwx* (pollen source 1, group A) and *Wxwx* (pollen source 2, group A) kernels were separated and the plants each produced were used as pollen sources. Theoretically, the genetic background of the homozygotes and heterozygotes within the same group should be identical except for the linkage block containing the mutant involved. Therefore, comparisons within groups should be unaffected by genetic background differences.

To obtain an estimate of genetic background effects, comparisons of the effects of su_1 in two distinctly different genetic backgrounds were included. As indicated in Table 1, the two backgrounds were designated group C (pollen sources 5 and 6) and group D (pollen sources 7 and 8).

Amino acid determination: The amino acids were extracted from intact pollen grains using the medium (30 ml double distilled water: 1 ml thiodiglycol: 70 mg citric acid p.a.: 70 ml 96% ethanol) and procedure developed by Linskens and Tupý (1966). Norleucine was added

initially in the extraction procedure as an internal standard. Amino acid estimations were made on an automatic amino acid analyzer. Extractions and estimates from each pollen source were made in duplicate.

Fatty acid analysis: Pollen grains were ground with quartz sand in a 2 parts chloroform: 1 part methanol extraction medium (Folch et al. 1957). The chloroform phase obtained after the addition of water to the supernatant was dried and methylated using methanolic potassium hydroxide, boron fluoride and hexane. The hexane phase obtained after the addition of a saturated sodium chloride solution was evaporated to dryness. This sample was redissolved in hexane and spotted on thin layer chromatography plates (20 cm × 20 cm) coated with silica-gel G (Merck 7731). The solvent system used was petroleum ether-diethyl ether-acetic acid (45:5:0.5, v/v/v). The plates were exposed to iodine vapors to indicate the bands. The band containing the ester forms of the fatty acids (located using suitable standards) was eluted from the silica-gel with ether and dried. This residue was redissolved in ether and introduced into a gas chromatograph (flame ionization detector in a Becker type 1452/SH, column dimensions 0.80 mm × 3 mm filled with 12% HI-EFF-2 AP in Chromosorb W (AW) 100 to 120 mesh, column temperature 175 °C, N_2 flow at end of column 42 ml/min). The fatty acid peaks were identified using appropriate standards and the area beneath the peaks were measured by planimetry and the percentages calculated. Extractions and estimates from each pollen source were made in duplicate.

Statistical analysis: An analysis of variance of each amino acid and fatty acid was performed including all nine pollen sources. The minimum differences for significance presented in Table 2 were obtained by means of the revised Duncan's ranges using for p only the maximum number of means to be compared (Harter 1960).

Conversions: Since the pollen from the heterozygous pollen sources contained approximately equal number of normal and mutant grains, direct comparisons between the homozygous and heterozygous sources within each group (Table 2) would not indicate the full extent of the difference between the two alleles at each locus. Therefore, to present this difference more clearly and simply, the results were in some cases, converted into percentages using the following formula with *wx* as an example:

$$\frac{wx}{Wxwx - (wx - Wxwx)} \times 100 .$$

This conversion was made for each amino acid within every group (Table 3). A value of 100 indicates that the mutant had no effect on the particular amino acid in comparison to normal. A value above 100 indicates an increase and below 100, a decrease because of the mutant. The significance levels in Table 3 were obtained from the appropriate comparisons between the homozygous and heterozygous pollen sources in Table 2.

Table 1. *Description and designation of the pollen sources*

Pollen source	Group	Genotype*		Designation
		Pollen source	Pollen	
1	A	$wxwxSh_2Sh_2Su_1Su_1$	all $wxSh_2Su_1$	*wx*
2	A	$WxwxSh_2Sh_2Su_1Su_1$	$.5WxSh_2Su_1$:.$5wxSh_2Su_1$	*Wxwx*
3	B	$WxWxsh_2sh_2Su_1Su_1$	all $Wxsh_2Su_1$	sh_2
4	B	$WxWxSh_2sh_2Su_1Su_1$	$.5WxSh_2Su_1$:.$5Wxsh_2Su_1$	Sh_2sh_2
5	C	$WxWxSh_2Sh_2su_1su_1$	all $WxSh_2su_1$	su_1(C)
6	C	$WxWxSh_2Sh_2Su_1su_1$	$.5WxSh_2Su_1$:.$5WxSh_2su_1$	Su_1su_1(C)
7	D	$WxWxSh_2Sh_2su_1su_1$	all $WxSh_2su_1$	su_1(D)
8	D	$WxWxSh_2Sh_2Su_1su_1$	$.5WxSh_2Su_1$:.$5WxSh_2su_1$	Su_1su_1(D)
9	E	$WxWxSh_2Sh_2Su_1Su_1$	all $WxSh_2Su_1$	N

* For the 3 endosperm mutants studied.

Table 2. *Amino acid content (μ moles/mg dry pollen) of the pollen grains obtained from the various pollen sources*

Amino acid	Pollen source									Mean	F value and significance level	Minimum differences	
	wx	*Wxwx*	sh_2	Sh_2sh_2	su_1(C)	Su_1su_1 (C)	su_1(D)	Su_1su_1 (D)	N			.05	.01
Aspartic acid	5.39	4.70	4.68	5.02	3.21	2.98	4.34	5.61	4.61	4.50	91.15**	0.33	0.48
Threonine-serine	32.55	29.20	38.17	35.18	28.25	25.16	36.43	38.36	32.35	32.85	9.56**	5.29	7.65
Glutamic acid	9.92	9.04	11.09	9.43	7.16	6.55	9.71	11.30	7.55	9.08	14.71**	1.55	2.25
Proline	176.88	167.19	217.39	161.80	194.47	183.51	193.82	211.70	169.76	186.28	4.66*	32.10	—
Glycine	1.37	1.10	0.98	1.00	1.10	0.88	1.65	2.06	0.95	1.23	3.29*	0.76	—
Alanine	11.52	12.40	14.46	15.44	12.18	14.10	18.76	19.21	18.82	15.21	39.62**	1.71	2.48
Valine	1.26	1.10	1.20	1.11	1.23	1.15	1.60	1.59	1.37	1.29	19.76**	0.15	0.22
Isoleucine	0.22	0.17	0.21	0.20	0.15	0.17	0.26	0.23	0.13	0.19	2.36	—	—
Leucine	0.57	0.45	0.53	0.53	0.39	0.44	0.56	0.58	0.42	0.50	3.85*	0.13	—
Tyrosine	0.24	0.18	0.28	0.20	0.14	0.13	0.20	0.23	0.20	0.20	3.83*	0.09	—
Phenylalanine	0.54	0.43	0.53	0.52	0.42	0.45	0.51	0.46	0.53	0.49	0.34	—	—
Ethanolanine	5.80	5.62	6.69	6.50	6.88	7.89	7.46	7.64	5.00	6.61	3.06	—	—
α Amino-butyric acid	1.87	2.50	2.93	4.60	1.72	3.35	4.05	3.52	3.97	3.17	59.29**	0.44	0.66
NH_3	7.52	8.23	12.43	12.62	8.43	9.97	10.24	15.33	6.62	10.15	0.75	—	—
Lysine	0.80	0.73	1.38	0.79	0.46	0.32	0.96	0.89	0.66	0.89	76.25**	0.24	0.35
Histidine	1.74	1.01	1.50	0.97	0.71	0.46	0.88	1.02	0.87	1.02	25.47**	0.27	0.39
Arginine	0.39	0.28	0.46	0.33	0.20	0.26	0.43	0.43	0.15	0.33	2.87	—	—
Unknown+	10.53	7.19	8.79	5.92	3.04	1.87	3.52	3.49	8.33	5.85	21.30**	2.32	3.36

* $.01 < P < .05$. — ** $P < .01$. — + Glycine amide, β amino-n-butyric acid, α aminocapyryrllic acid, OH lysine, methyl histidine, anserine, 5-hydroxytrytophan, kyneurenine, 6-hydroxytrytophan or homocarnosine.

Table 3. *Effect (expressed as a percentage) of the endosperm mutants on the amino acid content of pollen grains. A value of 100 indicates no effect, above 100 an increase and below 100 a decrease because of the mutant*

Amino acid	Endosperm mutant			
	wx	sh_2	su_1(C)	su_1(D)
Aspartic acid	134**	87*	117	63**
Threonine-serine	126	119	128	90
Glutamic acid	122	143*	121	75*
Proline	112	205*	113	84
Glycine	165	96	167	67
Alanine	87	88	76*	95
Valine	134*	118	115	101
Isoleucine	183	111	79	130
Leucine	173	100	80	93
Tyrosine	200	233	117	77
Phenylalanine	169	104	88	124
Ethanolanine	107	106	77	95
α Aminobutyric acid	60*	47**	35**	135*
NH_3	84	97	73	50
Lysine	121	234**	256	117
Histidine	621**	341**	338	76
Arginine	229	230	63	100
Unknown+	274*	288*	434	102

* $.01 < P < .05$. — ** $P < .01$. — + Glycine amide, β amino-n-butyric acid, α aminocapyryrllic acid, OH lysine, methyl histidine, anserine, 5-hydroxytrytophan, kyneurenine, 6-hydroxytrytophan or homocarnosine.

Results

Amino acid content: Wide variation was found among the various amino acids tested (Table 2). Proline was most abundant with a mean of 186.28 μ moles/mg dry pollen, and isoleucine was the least abundant with a mean of 0.19. Most of the other amino acids ranged between 0.19 and about 33 μ moles/mg dry pollen.

The various pollen sources tested altered the amino acid content of the pollen (Table 2). The effect of the mutants was not consistent with one or even a group of amino acids. For example, *wx* significantly increased aspartic acid, valine, histidine and the unknown and significantly decreased α aminobutyric acid. On the other hand, sh_2 significantly increased glutamic acid, proline, lysine, histidine and the unknown and significantly decreased aspartic acid and α aminobutyric acid. In some cases, the alterations produced by these mutants were relatively large. In the case of histidine, the difference between *wx* and *Wxwx* was 0.73 μ moles/mg dry pollen (Table 2) or the presence of the *wx* allele resulted in over a 6-fold (621 — Table 3) increase in this amino acid. The presence of sh_2 doubled the content of proline (Table 3).

The effects of su_1 were greatly modified by the group or genetic background involved. In group C, the only effect of su_1 was to significantly decrease alanine and α aminobutyric acid (Tables 2 and 3). However, in group D, su_1 significantly decreased aspartic acid and glutamic acid but produced a significant increase in α aminobutyric acid. Apparently the genetic background was responsible for these differences in the effects of su_1 and is an important factor to be considered.

Fatty acid distribution: As indicated in Table 4, unsaturated palmitic and saturated linolenic are the most common acids with percentages of 54.1 and 34.4 respectively. The remaining acids were present only in relatively small percentages. For all of the acids measured, no significant differences were found resulting from pollen sources. Thus, for the pollen sources and mutants tested, pollen genotype was not a factor in fatty acid distribution.

Discussion

The results reported here indicate that substantial differences in the biochemical nature of pollen grains are present and can be influenced by pollen genotype. Possibly these biochemical differences associated with pollen genotype are at least partially responsible for the reported differences between pollen sources in fertilization ability and *in vitro* germination characteristics. *In vivo* studies (Jones 1922, 1924; Sprague 1933) have indicated that aberrant ratios obtained at the *wx* and su_1 locus in maize were produced by differences between pollen transmission of the two

Table 4. *Distribution (% of total extracted) of fatty acids from pollen grains obtained from the various pollen sources*

Fatty acid	Carbon number	Pollen source									
		wx	*Wxwx*	sh_2	Sh_2sh_2	su_1(C)	Su_1su_1(C)	su_1(D)	Su_1su_1(D)	N	Mean*
Lauric	12	1.8	1.7	2.4	1.7	1.7	1.8	2.8	2.2	1.7	2.0
Myristic	14	0.4	0.3	0.2	0.1	0.1	0.2	0.3	0.2	0.1	0.2
Pentadecanoic	15	1.0	0.8	0.3	0.2	0.3	0.4	0.7	0.3	0.3	0.5
Palmitic	16	56.2	44.9	55.5	50.6	49.1	50.7	56.8	58.1	64.6	54.1
Heptadecanoic	17	1.0	0.2	0.3	0.3	0.1	0.4	0.5	0.2	0.2	0.4
Stearic	18	1.3	0.9	1.6	1.5	1.4	1.4	1.8	1.5	1.6	1.5
Oleic	18:1	1.6	1.8	2.1	2.1	1.9	1.8	1.9	2.0	2.1	1.9
Linoleic	18:2	3.8	5.0	5.2	6.1	5.1	4.5	3.9	3.8	3.8	4.6
Linolenic	18:3	32.9	44.1	31.6	36.7	39.8	38.3	30.4	31.0	24.7	34.4
Eicosanoic	20	0.8	0.6	1.2	1.0	0.7	0.9	1.0	0.9	0.9	0.9

* Differences among pollen sources were not significant at the .05 level for any fatty acid.

alleles. In their studies, the ability of the pollen grain to germinate on or establish a pollen tube in the style was influenced by its genotype. Other *in vivo* studies (Jones 1920; Pfahler 1965, 1967) dealing with pollen mixtures of various maize genotypes have shown that fertilization ability is related to pollen genotype but no specific mechanism or factor was established. *In vitro* studies (Pfahler 1968) have indicated that various maize pollen sources require different concentrations of calcium nitrate and boric acid for optimum germination. It is apparent from these studies that pollen genotype influences both fertilization ability and *in vitro* germination characteristics. However, the biochemical differences found in the study reported here cannot be directly correlated because of the complexity of the fertilization process.

At the present time, knowledge regarding the number or complexity of biochemical pathways influenced by the mutants used in this study is very limited. However, two other endosperm mutants, opaque-2 (O_2) and floury-2 (fl_2) have been shown to alter the amino acid pattern in the endosperm. The mutant O_2 was associated with a 69% increase in the lysine content of the endosperm (Mertz et al. 1964) while fl_2 increased lysine to the O_2 level but also substantially increased methionine (Nelson et al. 1965). No information regarding the biochemical pathways altered by these mutants or the effect of this enhanced amino acid content on subsequent protein synthesis was derived from these studies with endosperm. However, it appears that some mutants are capable of altering the amino acid pattern in the endosperm.

Some information regarding the amino acid content of pollen grains is available but no association with gene action was attempted. A high content of free proline in pollen grains from a number of species was not found to be related to any particular pollination system or group and thus was assumed to be involved in more fundamental reactions of the sexual process (Britikov et al. 1964). In the results reported here, proline was by far the most common amino acid in maize pollen and it was doubled in the presence of sh_2. Other amino acids which were altered by the mutants were aspartic acid, glutamic acid, proline, alanine, valine, α aminobutyric acid, lysine, histidine and the unknown. However, the mechanisms or pathways altered by these mutants are not known.

Knowledge regarding the fatty acid distribution in pollen grains is quite limited. Fatty acid methylesters in maize pollen were considered to function as growth substances (Fathipour et al. 1967; Fukui et al. 1958). Hoeberichts and Linskens (1968) reported that in Petunia pollen, the main free fatty acid was palmitic being 42% of the total. In the results reported here, palmitic was the major fatty acid, but linolenic was present in considerable quantities. In this study, the mutants did not affect the fatty acid distribution, indicating that either the mutants tested were not involved in these biochemical pathways or this distribution was essential for the pollen grains to function in the fertilization process.

The results reported here indicate that the biochemical nature of pollen grains is altered by their genotype. Since very little is known about the biochemical pathways or enzyme systems involved in amino acid content or fatty acid distribution, no definite relationship between gene action and biochemistry can be established at this time. However, as more information becomes available, the specific function of these alleles should become more apparent.

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Zusammenfassung

Prolin war die am reichlichsten vorkommende Aminosäure mit einem mittleren Gehalt von 186,28 Mikromol per mg trockenen Pollen. Die anderen Aminosäuren erreichten weniger als 33 Mikrogramm per mg trockenen Pollen.

Die Mutante *wx* zeigte einen signifikant erhöhten Gehalt an Asparaginsäure, Valin, Histidin, sowie einer nicht identifizierten Komponente, während der Gehalt an α-Aminobuttersäure signifikant erniedrigt war. Die Mutante sh_2 ist gekennzeichnet durch einen signifikant erhöhten Gehalt an Glutaminsäure, Prolin, Lysin, Histidin, sowie einer unbekannten Fraktion; der Gehalt an Asparaginsäure und α-Aminobuttersäure war dagegen signifikant erniedrigt. Die Wirkung des mutierten Gens su_1 wurde durch das übrige Genom, in dem es sich befand, geändert. In dem einen genetischen Milieu verursachte su_1 keine signifikante Erhöhung des Gehaltes irgend einer Aminosäure, während der Gehalt an Alanin und α-Aminobuttersäure signifikant erniedrigt war. In einem anderen genetischen Milieu jedoch zeigte su_1 eine signifikante Erhöhung der α-Aminobuttersäure; Asparaginsäure und Glutaminsäure waren signifikant erniedrigt.

Offensichtlich ist das übrige Genom zusätzlich zu der Wirkung der genannten Mutanten in der Lage, wesentliche Verschiebungen im Verteilungsmuster der Aminosäuren zu verursachen.

Von den Fettsäuren wurden am häufigsten Palmitin- und Linolen-Säure mit einem Gehalt von 54,1 bzw. 34,4% gefunden. Die untersuchten Endosperm-Mutanten zeigten keinen Einfluß auf die Fettsäureverteilung im Pollen.

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