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Genotypic Effects on the Amino Acid Relationships in Maize (*Zea mays* L.) Pollen and Style

H.F. Linskens and P.L. Pfahler
University of Nijmegen, Nijmegen (the Netherlands) and University of Florida, Gainesville, Florida (USA)

Summary. The free amino acid content of the pollen grains and the style from three single cross hybrids (Wf9 × H55, Ky49 × Ky27, K64 × K55) and two inbred lines (Oh43, H55) was determined. No tyrosine was detected in the pollen grains of any genotype. Significant differences between pollen genotypes were found for aspartic acid, threonine, serine, lysine and histidine with no effect resulting from the vigor of the pollen source. No proline was found in the style of any genotype. Significant differences between style genotypes were obtained for threonine, serine, glutamic acid, leucine, tyrosine, ethanolanine, α aminobutyric acid and histidine with a relationship between vigor of the style genotype present for tyrosine, ethanolanine and α aminobutyric acid. The relationship between the pollen and style level for each amino acid as influenced by genotype was analyzed. A significant negative correlation was found only for threonine. Apparently, a complementary relationship between the pollen and style exists for some amino acids. Proline and tyrosine are available only from the pollen and style respectively. Threonine levels are balanced with varying contributions from both pollen and style depending on the genotype.

Key words: Amino Acids - Maize - *Zea mays* - Pollen - Style - Genotypes - Male Transmisssion

Introduction

Knowledge regarding the pollen-style interaction and its relationship to differential male transmission of genetic information is extremely limited. Fertilization ability or in vivo studies in maize have shown that male transmission rates are influenced both by the pollen and style genotype (Pfahler 1965, 1967, 1974).

At the present time the specific processes in the pollen-style interaction which could possibly influence male transmission rates are unknown. However, during the initial phases of germination and tube growth, an exchange of numerous biologically-active substances including free amino acids occurs between the pollen grain and style (Linskens 1967; Linskens and Schrauwen 1969; Stanley and Linskens 1965). Free amino acids represent a valuable reserve material which can be utilized for direct incorporation into essential proteins, as a source of energy and nitrogen and as a mediator in many important physiological reactions. The specific functions of free amino acids in the pollen-style interactions are unknown. However, the large amounts of certain free amino acids in the pollen grains of most species suggest that their presence and activities are of major importance (Britikov 1975; Britikov et al. 1964, 1970; Tupý 1964). Recent studies (Linskens and Pfahler 1973; Pfahler and Linskens 1970) have indicated that pollen genotype influences the content of certain free amino acids in ungerminated maize pollen grains. Therefore, the amount and balance of free amino acids in the pollen and style may be a factor in differential male transmission.

This study reports the free amino acid content of the ungerminated pollen grains and unpollinated style from three hybrids and two inbreds of maize. The influence of genotype and vigor on each amino acid was determined in both the pollen and style. The relationship between the pollen and style for those free amino acids found to be under genotypic control was analyzed in an effort to elucidate pollen-style interactions which could contribute to differential male transmission.

Material and Methods

Pollen grains and styles were collected from at least 25 plants in each of three single cross hybrids, Wf9 × H55(W), Ky49 × Ky27(Ky), K64 × K55(K), and two inbreds, Oh43(O), H55(H). The hybrid group was more vigorous than the inbred group. Pollen was collected and screened by the method of Pfahler (1965).

Unpollinated styles (bagged before emergence) were collected and cut into 1 cm sections to hasten

Table 1. Amino acid content (μ moles/mg dry weight) of pollen grains and styles from each genotype

Amino acid	Tissue	Genotype					F value	Minimum differences	
		W	Ky	K	O	H		5%	1%
Aspartic acid	Pollen	6.9	9.3	5.7	4.2	9.5	20.84^{++}	1.9	3.1
	Style	7.5	7.3	7.1	5.0	5.2	4.59		
Threonine	Pollen	12.2	25.1	21.2	11.0	19.0	14.36^{++}	6.0	9.5
	Style	12.4	6.4	6.5	15.0	11.1	85.91^{++}	1.5	2.5
Serine	Pollen	43.7	32.4	37.9	14.4	33.0	8.49^{+}	14.4	
	Style	17.6	11.2	9.8	16.2	19.0	14.16^{++}	4.1	6.5
Glutamic acid	Pollen	11.9	14.6	10.0	11.7	14.9	1.36		
	Style	6.8	4.4	2.5	6.3	3.6	6.66^{+}	2.7	
Proline	Pollen	187.6	153.1	180.6	194.6	210.0	0.84		
	Style	0.0	0.0	0.0	0.0	0.0			
Alanine	Pollen	22.3	15.6	15.7	12.4	17.3	5.03		
	Style	27.0	22.5	23.4	21.6	19.9	3.96		
Valine	Pollen	3.1	1.8	3.1	3.8	3.2	0.88		
	Style	4.2	3.9	4.6	3.2	3.0	3.96		
Isoleucine	Pollen	0.3	0.4	0.3	1.3	0.4	0.78		
	Style	1.3	1.2	1.6	1.3	0.9	5.00		
Leucine	Pollen	0.3	0.6	0.5	1.4	0.5	0.88		
	Style	0.7	0.5	1.1	0.8	0.6	45.00^{++}	0.1	0.2
Tyrosine	Pollen	0.0	0.0	0.0	0.0	0.0			
	Style	14.8	15.7	18.1	7.4	10.2	34.77^{++}	2.8	4.4
Ethanolanine	Pollen	21.4	17.4	15.7	20.6	34.6	3.97		
	Style	2.3	2.4	1.9	4.0	3.6	14.73^{++}	0.9	1.4
α Aminobutyric acid	Pollen	3.6	2.8	5.1	3.2	5.3	1.68		
	Style	8.6	12.2	8.5	4.1	6.6	8.01^{+}	4.0	
NH_3	Pollen	15.4	16.8	17.1	18.7	16.6	0.36		
	Style	63.0	90.8	58.1	43.4	44.7	0.34		
Lysine	Pollen	1.3	2.2	2.9	0.4	2.5	50.00^{++}	0.5	0.9
	Style	0.3	0.4	0.3	0.4	0.3	1.25		
Histidine	Pollen	1.5	1.7	1.1	1.2	2.8	50.42^{++}	0.4	0.6
	Style	0.6	0.2	0.3	0.4	0.2	64.00^{++}	0.1	0.2

$^{+,++}$ F values significant at the 5 and 1% level respectively

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.

Amino acid content was determined by weighing 5 mg of pollen grains or 10 mg of styles in a centrifuge tube and then adding 2ml of the extraction medium (25 ml double distilled water: 1 ml thiodiglycol: 70 mg citric acid p.a.: 73 ml ethanol) and 150 μ moles norleucine as an internal standard. The mixture was centrifuged for 10 min at 20000 g and then the supernatant was removed. A second extraction with 2 ml of the medium was conducted on the residue with the same centrifugation schedule. The two 2 ml supernatants were combined with 10 ml chloroform and this mixture was centrifuged for 5 min at 3000 g. For the pollen, the supernatant from this centrifugation was partially dried to remove the alcoholic components and the dry residue was dissolved in 0.2 N sodium citrate (pH 2.2) for analysis in the amino acid analyzer (Jeol 6AH). For the style, a further purification of this supernatant was required to remove sugars and proteins. This was done by passing the supernatant through a sulfonacid column (Dowex 50 × 6, 25-50 mesh, 8% crosslinkages) to couple the amino acids to the resin. After the sugars and proteins were eluted, the amino acids were removed from the resin using 5N NH_4OH. Then the solution containing the amino acids was dried and the residue dissolved in 0.2 N sodium citrate (pH 2.2) for analysis.

For each amino acid, an analysis of variance including all genotypes was performed for the pollen and style analyses separately. The minimum differences for significance presented in Table 1 were obtained from Duncan's ranges using for p only the maximum number of means to be compared (Harter 1960). Linear regression techniques outlined by Snedecor (1956) were used to describe the relationship between the pollen and style for each amino acid.

Results

The amino acid contents of both pollen grains and styles are shown in Table 1. No tyrosine was present in the pollen grains of any genotype. For pollen grains, significant differences among genotypes were found for aspartic acid, threonine, serine, lysine and histidine with no consistent relationship between vigor and the content of these amino acids evident. No proline was found in the styles of any genotype. For styles,

Table 2. The relationship between the amino acid content of the pollen grains and styles as expressed by linear regression equations

Amino acid	Linear regression equation	t value
Aspartic acid	y=5.76+0.093x	0.309
Threonine	y=20.63-0.585x	4.423+
Serine	y=16.00-0.038x	0.180
Glutamic acid	y=4.95-0.018x	0.231
Alanine	y=14.40+0.509x	1.685
Valine	y=4.68-0.299x	0.060
Isoleucine	y=1.26-0.003x	0.009
Leucine	y=0.71+0.052x	1.690
Ethanolanine	y=1.24+0.073x	1.304
α Aminobutyric acid	y=10.67-0.067x	0.286
$\overline{NH_3}$	y=15.99-5.906x	0.680
Lysine	y=0.39-0.028x	1.037
Histidine	y=0.53-0.114x	0.919

+ b value in the regression equation significant from zero at the 5% level

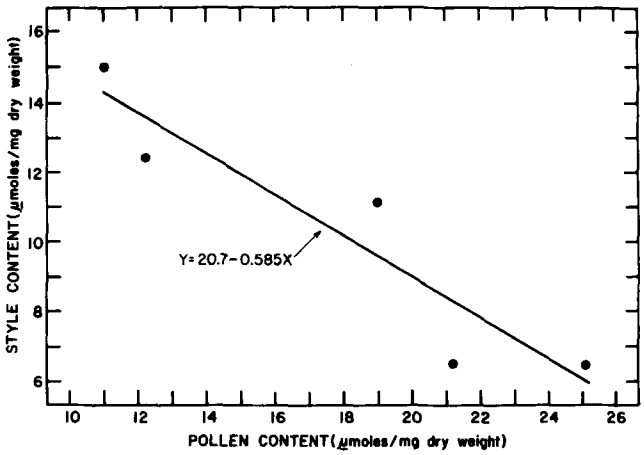

Fig. 1. The influence of genotype on the threonine content of the pollen grains and styles as indicated by linear regression

significant differences among genotypes were found for threonine, serine, glutamic acid, leucine, tyrosine, ethanolanine, α aminobutyric acid and histidine. Vigor was associated with the amount of certain amino acids in the styles. In comparison to the three hybrids as a group, the two inbreds as a group had a lower content of tyrosine and α aminobutyric acid and a higher content of ethanolanine.

The linear regression equations characterizing the relationship between the amino acid content of the pollen grains and styles as influenced by genotype are presented in Table 2. With the exception of threonine, the b values were small and not significant from zero. The significant inverse relationship for threonine is illustrated graphically in Fig. 1. In general, the observed data fit the linear regression line within reasonable limits with every 1 μ mole/mg dry weight decrease in style content accompanied by a 0.585 μ mole/mg dry weight increase in pollen grain content.

Discussion

In the genetic analysis of the pollen grain-style interaction, the fact that different ploidy levels are involved must be considered. Genetically, the pollen grain is haploid and thus, the effect of all alleles in the genome is expressed. Since heterosis is a major factor influencing the expression of quantitative characters in maize, the sporophyte producing the pollen grains can manifest heterosis while the pollen grains technically cannot. On the other hand, styles are genetically diploid and as a result, the effect of certain alleles can be altered depending on the type of gene action involved. Also, styles can exhibit heterotic effects. Therefore, the genetic effects in the pollen grain-style interaction include not only classical genetic elements and patterns but also haploid and diploid components with possible heterotic expression.

Considerable diversity in the amino acid content of the pollen grains was found in this study. A high level of proline was present with no significant genotypic effect detected. Other studies with maize pollen grains have shown that certain endosperm mutants (Pfahler and Linskens 1970) and pollen storage interacting with endosperm mutants (Linskens and Pfahler 1973) alter proline a small but statistically significant amount. The presence of extremely high levels of proline in the pollen grains of many species led to the postulation that proline was involved in fundamental reactions in the sexual process (Bathurst 1954; Britikov et al. 1964). Proline was found to be an important source of nitrogen in plant metabolism (Britikov 1975; Britikov et al. 1970; Linskens and Schrauwen 1969). In this study no tyrosine was found in the pollen grains of any genotype. Apparently, tyrosine is not necessary to maintain the viability of the pollen grain when independent, and if necessary for germination and tube growth, adequate amounts

must be supplied by the style. The content of certain amino acids was influenced by pollen genotype but no pronounced and consistent effect was related to the vigor of the pollen source. Certain endosperm mutants (Pfahler and Linskens 1970) and pollen storage interacting with certain endosperm mutants (Linskens and Pfahler 1973) were shown to alter certain amino acids. Information on the effect of pollen source vigor on biochemical constituents is extremely limited. Ash percentage of maize pollen grains was found to be closely associated with pollen source vigor (Pfahler and Linskens 1974).

Substantial variation in the amino acid content of the styles was found. No proline was present in any genotype indicating that if proline is necessary for pollen germination and tube growth, it must be contributed by the pollen grain.

Significant differences between style genotypes for certain amino acids were found with a relationship between the vigor of the style genotype present for three amino acids. No information is available regarding the effect of genotype or vigor on the amino acid content of the styles. However, ash percentage of maize styles was found to be related to vigor level (Pfahler and Linskens 1974).

The scarcity of information about the genetic influence on the biochemical constituents and pathways in pollen grains and styles and the complexity of the pollen grain-style interaction makes analysis of the mechanisms associated with differential male transmission extremely difficult. Differential male transmission by the pollen grains from the hybrids used in this study has been established (Pfahler 1965, 1967). Possibly these differences result from various levels of certain amino acids in the pollen grains and styles so that a form of amino acid complementation occurs. If this is the case, the transmission of a pollen grain will be altered by its level of certain amino acids in relation to the level of certain amino acids in the style. The negative relationship found in this study with threonine suggests that a mechanism of this nature may be a contributing factor in differential male transmission. However, much more knowledge must accumulate before a complete and meaningful explanation is possible.

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Prof. Dr. H.F. Linskens
Department of Botany
University of Nijmegen
Toernooiveld
Nijmegen (the Netherlands)

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