Theoretical and Applied Genetics 43, 49–53 (1973)

Biochemical Composition of Maize (*Zea mays* L.) Pollen

III. Effects of Allele × Storage Interactions at the Waxy(*wx*), Sugary (su_1) and Shrunken (sh_2) Loci on the Amino Acid Content[1]

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Summary. Pollen grains containing either the *Wx*, *wx*, Su_1, su_1, Sh_2 or sh_2 alleles were stored at 0, 1, 2, 3, 4 and 5 days at 2 °C. After each storage period, a portion of pollen from each genotype was analyzed for free amino acid content. Over all genotypes, storage significantly altered the content of all 16 amino acids measured. With increasing storage, a relatively consistent increase in aspartic acid, isoleucine, leucine, phenylalanine, ethanolanine, α aminobutyric acid, NH_3 and lysine was found. A relatively consistent decrease in glutamic acid, proline, glycine and alanine occurred with increasing storage. No consistent response to storage was obtained with threonine-serine, valine, histidine and the unknown. Apparently, storage or stage of viability loss has a pronounced effect on amino acid metabolism in maize pollen grains. The experiment was designed so that comparisons free of genetic background effects could be made between alleles at each locus. Significant allele × storage interactions at each locus were found as follows: at the waxy locus, aspartic acid, glycine, alanine and ethanolanine; at the sugary locus, aspartic acid, alanine, ethanolanine and α aminobutyric acid; and at the shrunken locus, aspartic acid, alanine, valine, leucine and ethanolanine. Amino acid metabolism is apparently influenced by the action of the alleles at these loci. The differences between the loci in the amino acids affected indicate the different areas of amino acid metabolism are influenced by each locus.

Introduction

Knowledge concerning the genetics, physiology and germination of maize pollen grains is limited. Maize pollen grains retain viability, in terms of fertilization ability, for only short periods of time even under the most optimum storage conditions (Johri and Vasil 1961; Jones and Newell 1948; Linskens 1967; Walden 1967). A recent study indicated that maize pollen grains stored at 2 °C with the relative humidity approaching 100% showed some *in vitro* germination after 2–3 days of storage and possessed some fertilization ability after 4–5 days of storage (Pfahler and Linskens 1972). The mechanism associated with this rapid loss in viability is not known, but it has been reported that maize pollen grains are in a highly active metabolic state at anthesis (Goss 1968) and thus biochemical changes within the pollen grain must be occurring during this period of viability loss. Differences in the amino acid content and distribution of fresh maize pollen grains have been reported as a result of the action of the alleles at the waxy, sugary and shrunken loci (Pfahler and Linskens 1970). Apparently, pollen genotype influences amino acid metabolism. A recent study also indicated that alleles at these 3 loci affected the *in vitro* germination and fertilization ability of pollen grains stored for various periods at 2 °C with the relative humidity approaching 100% (Pfahler and Linskens 1972). Therefore, pollen grains containing the alleles at these loci have altered amino acid content and distribution at the time of anthesis and also display different viabilities in terms of *in vitro* germination and fertilization ability.

More information on the relationships between pollen genotype, viability loss and biochemical composition is needed. This study was conducted to investigate the effect of various storage periods (stages in viability loss) on the amino acid content of pollen grains containing the dominant and recessive alleles at the waxy, sugary and shrunken loci.

Materials 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. The crossing scheme to obtain these genotypes and their genetic relationships are presented in a previous paper (Pfahler and Linskens 1972). The genetic relationships will be summarized here. The homozygous dominant and 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 reasonably free from genetic background effects.

Large quantities of pollen grains from at least 50 plants of each genotype were collected by the method of Pfahler (1965). Immediately after collection, a portion from each genotype was removed and dried in a desiccator at 30 °C. The remainder was placed in an open container and stored at 2 °C with the relative humidity above the surface of the pollen grains approaching 100%. At 1, 2, 3, 4 and 5 days, a portion of the pollen grains from each genotype was removed and dried in the same manner described above. Therefore, 6 storage periods, 0, 1, 2, 3, 4 and 5 days were represented.

[1] Journal Series Paper No. 4425, Florida Agricultural Experiment Station.

Table 1. *Storage effects on the amino acid content (μ moles/mg dry pollen) of pollen grains*

Amino acid	(Storage days at 20 °C)						F value and significance level	Minimum differences	
	0	1	2	3	4	5		.05	.01
Aspartic acid	4.12+	5.86	7.39	8.72	9.40	10.20	337.87**	0.43	0.56
Threonine-serine	33.19	34.36	33.27	35.32	37.25	35.67	6.80**	1.75	2.32
Glutamic acid	7.36	6.04	4.41	4.03	3.46	3.95	47.67**	0.70	0.93
Proline	208.31	198.26	192.97	196.22	190.07	191.08	6.05**	9.08	11.99
Glycine	1.22	1.23	1.12	1.08	0.86	1.06	2.78*	0.24	
Alanine	12.57	12.58	11.75	10.90	9.99	8.77	79.84**	0.55	0.73
Valine	1.19	1.13	1.17	1.24	1.20	1.21	2.74*	0.08	
Isoleucine	0.14	0.21	0.32	0.38	0.38	0.33	80.13**	0.04	0.05
Leucine	0.41	0.47	0.59	0.69	0.70	0.64	34.27**	0.07	0.09
Phenylalanine	0.59	0.69	0.83	0.91	0.89	0.83	16.89**	0.10	0.13
Ethanolanine	2.60	3.16	3.11	3.76	4.71	8.61	180.68**	0.49	0.65
α Aminobutyric acid	2.66	4.43	7.05	7.81	7.43	6.08	47.28**	0.94	1.25
NH_3	5.81	5.87	7.89	9.19	7.67	7.30	9.00**	1.35	1.79
Lysine	0.78	0.78	0.87	0.92	0.92	0.83	5.56**	0.08	0.11
Histidine	1.23	1.21	1.20	1.32	1.41	1.27	3.47*	0.15	
Unknown++	5.90	5.84	5.69	6.07	6.31	5.87	2.67*	0.55	

* $.01 < P < .05$. — ** $P < .01$. — + Each value represents the mean of 12 measurements, 2 from each of the 6 pollen sources. — ++ Glycine amide, β amino-n-butyric acid, α aminocapyryrllic acid, OH lysine, methyl histidine, anserine, 5-hydroxytrytophan, kyneurenine, 6-hydroxytrytophan or homocarnosine.

The extraction method for the amino acid analysis is described in a previous paper (Pfahler and Linskens 1970). The amino acid content was determined on an automatic amino acid analyzer. Duplicate extractions and determinations from each genotype at each storage period were made.

An analysis of variance was performed for each amino acid including all 6 genotypes. The F values in Table 1 are associated with the main effect, storage from these analyses.

A second analysis of variance was performed for each amino acid including only the homozygous dominant and homozygous recessive genotypes at each locus. The F values in Table 2 are associated with the allele × storage interactions from these analyses.

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

Results

Significant differences between storage periods were found for all 16 amino acids measured (Table 1). With increasing storage, a relatively consistent increase in aspartic acid, isoleucine, leucine, phenylalanine, ethanolanine, α aminobutyric acid, lysine and NH_3 was obtained while glutamic acid, proline, alanine and glycine decreased consistently. No consistent response to increasing storage was obtained with threonine-serine, valine, histidine and the unknown. Based on the magnitude of the F values, storage affected aspartic acid, the most and the unknown, the least. Very large F values were associated with glutamin acid, alanine, isoleucine, leucine, phenylalanine, ethanolanine and α aminobutyric acid indicating that storage also had a pronounced effect on these amino acids.

The effect of storage on the amino acid content of pollen grains containing the alleles at the various loci was quite pronounced for some amino acids. The F values and significance levels associated with the allele × storage interactions are presented in Table 2. Significant F values were found as follows: at the waxy locus, aspartic acid, glycine, alanine and ethanol anine; at the sugary locus, aspartic acid, alanine, ethanolanine and α aminobutyric acid; and at the shrunken locus, aspartic acid, alaninc, valine, leucine and ethanolanine. A differential response to storage was found at all loci for aspartic acid, alanine and ethanolanine, while differences between loci were ob-

Table 2. *F values and significance levels of the allele × storage interactions for each amino acid at each locus*

Amino acid	Locus		
	Waxy	Sugary	Shrunken
Aspartic acid	6.04**	19.54**	15.80**
Threonine-serine	2.64	1.01	0.90
Glutamic acid	1.11	2.34	1.36
Proline	0.54	2.32	2.09
Glycine	4.02*	0.96	0.06
Alanine	6.54**	4.77*	10.94**
Valine	1.52	1.72	4.21*
Isoleucine	0.89	1.72	1.50
Leucine	0.42	0.56	3.48*
Phenylalanine	0.84	0.90	1.25
Ethanolanine	11.36**	12.98**	12.56 **
α Aminobutyric acid	2.02	6.73**	1.30
NH_3	2.66	1.81	0.87
Lysine	0.26	0.24	1.53
Histidine	2.39	0.90	0.89
Unknown+	1.96	2.73	0.71

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

tained for glycine, valine, leucine and α aminobutyric acid.

The means associated with the significant allele × storage interactions at the waxy locus are presented in Table 3. At 0 day of storage, the differences between *Wx* and *wx* were not significant for aspartic acid, glycine, alanine and ethanolanine. With increasing storage, the aspartic acid content of *Wx* increased considerably less than that of *wx*. With increasing storage, no distinct pattern emerged between *Wx* and *wx* for glycine content. However, an increase was obtained for both *Wx* and *wx* at 1 day of storage. With increasing storage, the alanine content of *Wx* decreased more rapidly and uniformly than that of *wx*. With increasing storage, the ethanolanine content of *Wx* increased much more rapidly than that of *wx*.

The means associated with the significant allele ×storage interactions at the sugary locus are presented in Table 4. At 0 day of storage, the aspartic acid content of Su_1 was significantly higher than that of su_1 while no significant differences between Su_1 and su_1 were found for alanine, ethanolanine or α aminobutyric acid. With increasing storage, the aspartic acid content of Su_1 increased more rapidly than that of su_1. With increasing storage, the alanine content of Su_1 decreased uniformly while that of su_1 increased from 0 to 1 day of storage and then with further increases in storage, decreased slightly. With increasing storage, the ethanolanine content of Su_1

Table 3. *Storage effects on the amino acid content (μ moles/mg dry pollen) of pollen grains containing the dominant (Wx) and recessive (wx) alleles at the waxy locus. Only those amino acids showing significant allele × storage interactions in Table 2 are presented*

Amino acid	Allele	Storage (days at 2 °C)					
		0	1	2	3	4	5
Aspartic acid*	*Wx*	4.23	5.69	7.90	10.08	10.42	8.69
	wx	4.51	6.64	8.93	10.35	12.11	12.62
Glycine*	*Wx*	1.43	1.76	1.09	1.23	1.06	1.26
	wx	1.21	1.44	1.20	0.92	1.03	1.06
Alanine*	*Wx*	12.39	11.38	10.21	8.08	7.45	8.14
	wx	12.04	12.36	12.10	11.30	10.00	8.91
Ethanolanine*	*Wx*	2.95	3.75	3.76	6.15	6.36	11.17
	wx	2.16	2.83	2.77	2.13	2.48	5.88

* Each value represents the mean of 2 measurements. Minimum differences for significance between any 2 means within each amino acid at the .05 and .01 level respectively: aspartic acid = 1.25 and 1.77; glycine = 0.46 and 0.65; alanine = 1.18 and 1.67; and ethanolanine = = 1.58 and 2.24.

Table 4. *Storage effects on the amino acid content (μ moles/mg dry pollen) of pollen grains containing the dominant (Su_1) and recessive (su_1) alleles at the sugary locus. Only those amino acids showing significant allele × storage interactions in Table 2 are presented*

Amino acid	Allele	Storage (days at 2 °C)					
		0	1	2	3	4	5
Aspartic acid*	Su_1	4.13	5.99	7.35	8.56	10.68	12.01
	su_1	3.25	4.45	5.86	6.20	6.21	7.98
Alanine*	Su_1	13.33	12.92	12.34	12.41	9.90	7.34
	su_1	12.26	14.00	11.43	11.21	11.25	10.37
Ethanolanine*	Su_1	2.96	4.26	4.31	5.00	7.02	11.08
	su_1	2.61	2.71	2.54	3.65	6.25	5.98
Aminobutyric acid*	Su_1	3.02	5.68	8.69	10.96	8.49	5.42
	su_1	1.70	4.10	5.53	6.71	8.16	8.00

* Each value represents the mean of 2 measurements. Minimum differences for significance between any 2 means within each amino acid at the .05 and .01 level respectively: aspartic acid = 0.83 and 1.17; alanine = 1.93 and 2.73; ethanolanine = 1.16 and 1.65; and aminobutyric acid = 2.24 and 3.17.

Table 5. *Storage effects on the amino acid content (μ moles/mg dry pollen) of pollen grains containing the dominant (Sh_2) and recessive (sh_2) alleles at the shrunken locus. Only those amino acids showing significant allele × storage interactions in Table 2 are presented*

Amino acid	Allele	Storage (days at 2 °C)					
		0	1	2	3	4	5
Aspartic acid*	Sh_2	4.57	6.44	8.11	9.63	11.98	12.80
	sh_2	3.75	5.91	6.18	7.54	6.55	8.65
Alanine*	Sh_2	13.13	13.79	12.53	11.84	9.47	9.28
	sh_2	12.35	11.84	11.99	10.57	11.41	9.50
Valine*	Sh_2	1.23	1.10	1.27	1.47	1.45	1.34
	sh_2	0.84	0.91	0.99	1.02	0.95	1.10
Leucine*	Sh_2	0.44	0.46	0.70	0.88	0.81	0.82
	sh_2	0.34	0.44	0.55	0.62	0.58	0.65
Ethanolanine*	Sh_2	2.72	2.83	2.70	3.19	4.12	8.99
	sh_2	2.00	2.21	2.89	2.48	3.51	4.70

* Each value represents the mean of 2 measurements. Minimum differences for significance between any 2 means within each amino acid at the .05 and .01 level respectively: aspartic acid = 1.26 and 1.78; alanine = 1.13 and 1.60; valine = 0.18 and 0.25; leucine = 0.11 and 0.16; and ethanolanine = 1.11 and 1.57.

increased sharply while that of su_1 increased slightly. With increasing storage, the α aminobutyric acid content of Su_1 increased sharply reaching a maximum at 3 days of storage then with further storage, decreased sharply. With increasing storage, the α aminobutyric acid content of su_1 increased uniformly until 4 days of storage with no further change at 5 days of storage.

The means associated with the allele $\times$ storage interactions at the shrunken locus are presented in Table 5. At 0 day of storage, no significant differences between Sh_2 and sh_2 were obtained for aspartic acid, alanine, leucine or ethanolanine but the valine content of Sh_2 was significantly higher than that of sh_2. With increasing storage, the aspartic acid content of Sh_2 increased sharply while that of sh_2 increased slightly. The alanine content of Sh_2 remained relatively constant through 2 days of storage and then decreased while that of sh_2 showed a relatively small decline from 0 to 4 days of storage and with 5 days of storage declined. With increasing storage, no consistent change in valine content was found with Sh_2 but sh_2 increased slightly. With increasing storage the leucine content of Sh_2 increased more rapidly than that of sh_2. The ethanolanine content of Sh_2 increased slightly from 1 through 4 days of storage and increased sharply from 4 to 5 days of storage. The same pattern was observed with sh_2 but the increase from 4 to 5 days of storage was much smaller.

Discussion

Very limited information is available regarding the biochemical changes occurring in maize pollen grains between anthesis and pollination. Rapid viability loss has been observed even under the most optimum storage conditions (Johri and Vasil 1961; Jones and Newell 1948; Linskens 1967; Walden 1967) suggesting that the pollen grains are in a highly active metabolic state. Most studies agree that low temperature and high humidity extend viability (Goss 1968). Although the exact mechanism associated with viability loss is not known, Knowlton (1922) suggested from his studies that viability of maize pollen grains is associated with desiccation and not with exhaustion of stored food or loss of enzyme activity. He speculated that some other factors such as protein precipitation were the primary cause. The amino acid content of maize pollen grains immediately after anthesis has been reported (Nielson et al. 1955; Pfahler and Linskens 1970; Ray Sarkar et al. 1949; Vinson 1927). The high concentration of proline produced speculation that proline acts as a substrate for respiration, a source of nitrogen and assumes a role in pollen tube formation at germination (Britikov et al. 1965). Therefore, changes in proline content might indicate changes in viability. In this study, proline content decreased with increasing storage but the decrease, as a percentage of 0 day of storage, was relatively small. Apparently, proline content cannot be used as an accurate measure of viability. The content of other amino acids showed greater changes as a percentage of 0 day of storage and this could be a more accurate indicator of viability.

The specific mechanisms associated with these changes in amino acid content as a result of storage are not known. Proteolytic enzyme activity has been detected in maize pollen grains (Paton 1921). The presence of transaminases has been established particularly involving glutamic-aspartic and glutamic-alanine conversions (Sawada 1960). Apparently, amino acid metabolism continues during storage. A study with germinating pollen grains demonstrated that with tube development, changes in the amino acids occurred (Pozsar 1960). The study reported here indicated that the amino acid content was altered even during storage with no pollen tube development.

Little knowledge is available concerning the association between the amino acid content, *in vitro* germination capacity and fertilization ability. A recent study indicated that maize pollen grains stored at 2 °C showed some *in vitro* germination capacity after 2—3 days of storage and possessed some fertilization ability after 4—5 days of storage (Pfahler and Linskens 1972). The results presented here indicated that consistent changes in the content of some amino acids occurred with increasing storage. This suggests that no threshold level of most amino acids is associated with *in vitro* germination capacity or fertilization ability. Apparently, the silk provides a factor(s) that induce germination of a pollen grain which would not germinate on an *in vitro* medium.

This study indicated that a differential response to storage as a result of the alleles at various loci was found for some amino acids. Most studies with alleles at these loci have involved their effect on the appearance or carbohydrate characteristics of the endosperm. The relationship between the action of these alleles in the endosperm and pollen grain has not been examined adequately. With the exception of *wx*, it was found that amylose content of the endosperm and pollen starch was not correlated (Zuber et al. 1960). A recent study has indicated that little or no relationship exists between various carbohydrate characteristics of the endosperm and pollen grain as a result of the action of these alleles (Pfahler and Linskens 1971). Apparently, the alleles do not have the same action in different organs or tissues of the plant even in terms of regulating reactions which appear to be identical.

Very little information is available concerning the enzymatic activity of the alleles at these loci, although all these alleles are associated with extreme alterations in the carbohydrate characteristics of the endosperm. A lack of uridine diphosphate glucose-starch glycosyl transferase activity has been associated with the *wx* allele, while a lack of adenosine diphos-

phate glucose pyrophosphorylase activity has been related to the sh_2 allele (Nelson 1967). It was concluded from examining the relationship between alleles, reactions involved, and end products that starch synthesis involves many alternate pathways. Thus genetic alterations in starch synthesis could occur by altering one of many reactions in these pathways. Probably, the effect of these alleles on amino acid content and indirectly on amino acid metabolism is similar.

Acknowledgement

We thank drs. J. A. M. Schrauwen for preparing the samples, Mr. M. G. J. Buys for supervising the automatic analyzer, Mr. M. van der Donk for calculating the analytical results, and Mr. H. S. Anspach for technical assistance.

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Received May 13, 1972

Communicated by H. F. Linskens

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