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

II. Effects of the Endosperm Mutants, Waxy (*wx*), Shrunken (sh_2) and Sugary (su_1) on the Carbohydrate and Lipid Percentage[1]

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Summary. Mean percentages of sugars, water-soluble polysaccharides, starch, total carbohydrates and lipids were 40.1, 7.4, 28.6, 76.1, and 1.8 respectively. Differences among the mutants were found only for water-soluble polysaccharides with both *wx* and sh_2 decreasing the percentage a small but significant amount. In terms of the various carbohydrates measured, no correlation was found between the expression of these mutants in the pollen and the kernel.

Introduction

In a previous paper (Pfahler and Linskens 1970), the influence of *wx*, sh_2, and su_1 on the amino acid content and the fatty acid distribution in the pollen grain was reported. In general, these mutants were found to alter the level of several amino acids but did not change the fatty acid distribution. Apparently, these mutants when present in the pollen grain can alter some physiological processes associated with protein synthesis within the pollen grain itself.

Most studies of these mutants have emphasized their effect either on the physical appearance or the carbohydrate synthesis in the endosperm. The mutants sh_2 and su_1 produce a pronounced change in the physical appearance of the endosperm, while the endosperm of *wx* is normal in appearance but the starch lacks amylose (Neuffer *et al.* 1968). Biochemical analyses have indicated that these mutants produce major shifts in the percentage of sugars, water-soluble polysaccharides and starch in the kernel (Creech 1965). Few studies are available indicating the correlation between their effects on carbohydrate synthesis in the endosperm and other plant parts. However, it was found that the amylose content of the endosperm and pollen starch was not correlated, with one notable exception that no amylose was present in either when *wx* was present (Zuber *et al.* 1960).

The purpose of this study was to investigate the effect of these three endosperm mutants on the carbohydrate and lipid content of their pollen grains. A comparison between their effect on the carbohydrate content of the endosperm and the pollen grain was then made in an effort to determine possible differences in mechanisms of gene action.

Materials and Methods

The dry pollen used in this study was part of the same material used in a previous study (Pfahler and Linskens 1970) and thus for further information, this reference should be consulted. In presenting these results, the same terminology was continued with, as an example, *wx* (Table 1) indicating pollen containing 100% *wx* pollen grains and *Wx wx* (Table 1) indicating pollen containing 50% *wx* pollen grains and 50% *Wx* pollen grains. The use of groups (Table 1) was also continued so that comparisons between the sources in the same group are free from genetic background effects, while comparisons between groups would possibly be affected by the genetic background of the sources. The mutant su_1 was tested in two distinctly different genetic backgrounds (Groups C and D, Table 1).

The various carbohydrate percentages were determined by the procedure described in detail by Creech (1965). Lipid percentage was determined after extracting with a 2 parts chloroform: 1 part methanol medium (Folch *et al.* 1957). Before both analyses, the pollen grains were pulverized by grinding in a glass homogenizer with the appropriate extraction medium. For both analyses, extractions and estimates from each pollen source were made in duplicate.

An analysis of variance of each characteristic measured was performed including all nine pollen sources. 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

Considerable variation was obtained among the characteristics measured (Table 1). In terms of carbohydrates, sugars were the most abundant with a mean of 40.1%, while water-soluble polysaccharides were the least abundant with a mean of 7.4%. Starch was somewhat intermediate with a mean of 28.6%. Lipids were very low with a mean percentage of 1.8.

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Table 1. *Carbohydrate and lipid percentages of pollen obtained from various sources*

Pollen source	Group	Statistical test	Carbohydrate				Lipid	Carbohydrate plus lipid
			Sugar	Water-soluble polysaccharide	Starch	Total		
wx	A		36.5	6.0	28.3	70.8	2.8	73.6
Wx wx	A		41.9	7.4	29.3	78.6	1.5	80.0
sh_2	B		37.9	5.8	30.9	74.5	0.9	75.4
$Sh_2\ sh_2$	B		40.4	7.7	29.5	77.6	1.5	79.1
su_1	C		42.5	8.4	26.4	77.3	1.5	78.8
$Su_1\ su_1$	C		40.6	8.1	29.5	78.1	1.4	79.4
su_1	D		41.3	7.3	29.7	78.2	2.0	80.1
$Su_1\ su_1$	D		41.0	8.2	27.0	76.1	2.0	78.1
Normal	E		38.4	7.9	27.1	73.4	2.4	75.7
Mean			40.1	7.4	28.6	76.1	1.8	77.8
		F value	0.88	5.55**	1.20	0.65	0.73	0.74
		Minimum differences	.05 —	1.4	—	—	—	—
			.01 —	2.0	—	—	—	—

** $P = < .01$.

Significant differences between pollen sources were found for only one carbohydrate, water-soluble polysaccharides (Table 1). For this characteristic, the percentage between all pollen sources ranged from 5.8 to 8.4. An analysis of the differences between pollen sources in the same group indicated that both *wx* and sh_2 decreased water-soluble polysaccharides a significant amount, with a greater decrease observed with sh_2.

A comparison of the effects of these mutants on carbohydrate percentages in both the pollen and kernel is presented in Table 2. Considering the normal genotype, the pollen and kernels differ substantially in the distribution of the various carbohydrates, with pollen containing more sugar and the kernel more starch. As indicated earlier, the mutants tested produced significant differences only in the percentage of water-soluble polysaccharides in the pollen. However, some of these mutants produce a pronounced change in the carbohydrate distribution in the kernel. In general, *wx* and normal do not differ appreciably in the percentage or distribution of carbohydrates. However, when compared to normal, sh_2 kernels have a much higher percent sugar, a slightly higher water-soluble polysaccharide percentage and a much lower percentage of starch and total carbohydrates. The mutant su_1 produces an entirely different pattern in the kernel than sh_2. In comparison to normal, su_1 kernels have a slightly higher percentage of sugar, a much higher percentage of water-soluble polysaccharides, a much lower starch percentage and a slightly lower percentage of total carbohydrates. Apparently, not only is the carbohydrate distribution of the pollen and kernel greatly different, but these mutants can drastically alter carbohydrate synthesis in the kernel while having little or no effect in the pollen.

Discussion

Very limited information is available concerning the carbohydrate and lipid content of maize pollen grains. However, in comparison to other reports, the percentages found in this study are considerably different for carbohydrates but quite similar for lipids. Anderson and Kulp (1922) reported that the starch percentage ranged from 11.1 to 19.0 depending on whether yellow dent, flint, or popcorn was used as a pollen source. An equally broad range related to pollen source was found for sucrose and dextrose. For their study, dry, intact pollen grains were used and apparently were not ruptured during the analysis. The authors indicated that different extraction procedures greatly affected the percentages within the same pollen source and concluded that these differences were probably the result of differences in penetration through the pollen wall. In a more recent study using only one pollen source, Todd and Bretherick (1942) found 22.4% starch and 36.6% total carbohydrates. No indication was given whether their analysis involved intact pollen grains. However, since dry maize pollen grains are very difficult to disrupt, it is probable that the study of Todd and Bretherick (1942) also was done with intact pollen

Table 2. *The influence of wx, sh_2, and su_1 on the carbohydrate percentages in pollen and kernels*

Mutant	Location	Carbohydrate			
		Sugar	Water-soluble polysaccharide	Starch	Total
wx	Pollen	36.5	6.0	28.3	70.8
	Kernel*	3.3	2.2	69.0	74.5
sh_2	Pollen	37.9	5.8	30.9	74.5
	Kernel*	25.7	5.1	21.9	52.8
su_1	Pollen**	41.9	7.9	28.1	77.8
	Kernel*	8.3	24.2	35.4	69.6
Normal	Pollen	38.4	7.9	27.1	73.4
	Kernel*	3.0	2.2	73.4	78.6

* Adapted from Creech (1965). — ** Mean of su_1 from Groups C and D in Table 1.

grains. In the study reported here, sugars and indirectly total carbohydrates were, in general, the only carbohydrates with a considerably higher percentage than those cited. Since this study involved the analyses of ruptured pollen grains, possibly the increase in percentage was the result of extracting a greater amount from the ruptured grain. Another contributing factor could be that different methods of drying and storing the pollen after collection altered carbohydrate content.

The lack of correlation between the carbohydrate content of the pollen and kernel found in this study was probably related to differences in function. The pollen grain is a highly specialized, independent cell that is very active metabolically and is capable of rapid growth and cell wall synthesis under the proper conditions. The endosperm of the kernel which was, in large part, the portion measured in the study of Creech (1965), is a storage organ that supplies nutrients to a developing embryo for a relatively short period of time until the seedling is established as an independent entity.

In the study reported here, the effect of the mutants on the carbohydrate and lipid content of the pollen grain was found to be limited to relatively slight changes in the percentage of water-soluble polysaccharides. In the kernels, sh_2 and su_1 were shown to be responsible for major shifts in the distribution of carbohydrates (Creech 1965). Here again, this lack of correlation between the action of these mutants in the pollen grain and kernel is probably associated with function. Assuming that a certain energy level is necessary for the pollen grain to participate normally in fertilization, any mutation that would greatly disturb the carbohydrate and lipid content of the pollen grain would also decrease its fertilization ability. Therefore, such mutations would be rapidly eliminated from the population, and as a result, would probably not be recognized and isolated.

The results of this study indicate that certain of the mutants tested can alter the water-soluble polysaccharide percentage of the pollen grain, but in general, the carbohydrate and lipid content is not greatly affected. A previous study (Pfahler and Linskens 1970) has indicated that these mutants alter the amino acid pattern but do not change the fatty acid distribution. Apparently, the specific function of these mutants is associated in some manner with protein synthesis. However, an examination of the relationship between protein synthesis and energy sources in the form of carbohydrates and lipids may further elucidate the biochemical pathways influenced by these mutants on the pollen level.

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Zusammenfassung

Die Untersuchung des Kohlenhydrat- und Lipid-Gehaltes im Pollen der Endosperm-Mutanten waxy, shrunken und sugary vom Mais ergab einen gemittelten Gehalt an Zucker von 40,1%, wasserlöslichen Polysacchariden 7,4%, Stärke 28,6, Gesamtkohlenhydraten 76,1% und Lipiden 1,8%.

Unterschiede zwischen den Mutanten wurden lediglich hinsichtlich der wasserlöslichen Polysaccharide gefunden, und zwar bei *wx* und sh_2, die einen signifikant niedrigeren Gehalt aufwiesen.

Hinsichtlich der verschiedenen analysierten Kohlenhydrate wurde keine Korrelation zwischen der Expression der Mutanten im Pollen und in den Karyopsen gefunden.

Literature

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