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ABSENCE OF LONG CHAIN ALDEHYDES IN THE WAX OF THE *GLOSSY 11* MUTANT OF MAIZE

P. AVATO, G. BIANCHI and F. SALAMINI*†

Dipartimento di Chimica Organica, Università, 27100 Pavia, Italy; * Istituto Sperimentale per la Cerealicoltura, Sezione di Bergamo, 24100 Bergamo, Italy

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Key Word Index—*Zea mays*; maize; glossy 11 mutant; wax chemistry; biosynthesis.

Abstract—Wax from the *gl11* mutant of maize lacks aldehydes, which constitute 20% in the normal genotype. The absence of aldehydes is not associated with a block in the synthesis of alcohols. Moreover in contrast to the wild type, *gl11* wax is characterized by a higher content of C_{16} and C_{18} free acids, with a clear defect in the synthesis of C_{24}, C_{26} and C_{28} homologues. The results from this study are taken as evidence that the wild type elongation–decarboxylation I (EDI) pathway, leading to the synthesis of all the wax classes of compounds except esters, may be split into an early (EDIa) and a late (EDIb) group of reactions. Mutant *gl11* is apparently defective at the EDIa, governing the synthesis of C_{24}–C_{28} fatty acyl chains.

INTRODUCTION

The leaf surface of young maize seedlings is covered by a layer of epicuticular wax whose quantity decreases with plant age [1, 2]. It has been shown that wax production is under the control of at least 13 independent genes whose mutant alleles depress wax accumulation [3, 4]. Mutant seedlings can be visually distinguished from the wild type phenotype by the glossy appearance of their leaves.

So far, the chemistry of the epicuticular waxes from nine maize recessive mutants, namely *gl1* (*glossy-1*), *gl2*, *gl3*, *gl4*, *gl5*, *gl7*, *gl8*, *gl15* and *gl18*, and from an albino strain has been described [5–9]. The major effects of mutants *gl2* and *gl4* are a block of the elongation of long chain molecules at the C_{30}–C_{32} step, while *gl3* affects the step C_{28}–C_{30}. Mutants *gl1*, *gl8*, gl7 and *gl18* influence the synthesis of long chain wax components at a very early stage, and/or interfere with the supply of precursors. Moreover, mutant *gl15* is characterized by an abnormal synthesis of esterified primary alcohols of C_{16} and C_{18} chain lengths. In the albino strain wax composition resembles that of normal seedlings. In *gl5* waxes the main constituents are aldehydes (84%) in sharp contrast to normal wax in which alcohols predominate (63%). This last finding was interpreted as due to the existence in *gl5* of a metabolic block causing the accumulation of aldehydes, the substrates from which alcohols originate [7]. Esters are the wax components least affected in glossy and albino mutations.

This paper reports on the modification in the wax composition induced by *gl11* in maize. Although the wax of this mutant contains 27% free alcohols, aldehydes, the precursors of alcohols, are not found.

RESULTS AND DISCUSSION

As reported in Table 1, the total yield of wax appears reduced by *gl11* to 70% of that of the normal genotype. In the mutant the esters are the main class of compounds accounting for 66% of the total wax, while free alcohols, which are dominant (63%) in the wild type, only amount to 27%. Furthermore, *gl11* wax lacks aldehydes (Purpald test negative; [10]) a class of compounds which reach 20% in the normal genotype. Higher than normal amounts (5%) of free acids, moreover, are present in *gl11* wax. Tables 2 and 3 show the homologue distribution within each class of compounds. Alkanes from *gl11* are characterized by C_{29} (25%) as the dominant homologue which makes them different from those of normal maize where C_{31}, C_{29} and C_{27} are the major components (49, 29 and 13%, respectively). Furthermore, as reported for the wax

Table 1. Composition (%) of epicuticular waxes from the mutant *gl11* and from normal WF9 plants (*Gl*)

Components	*gl11*	*Gl*
Alkanes	2	1
Esters	66	16
Aldehydes	—	20
Alcohols	27	63
Acids	5	tr
Yield (mg/1000 g fr. wt)	404	580
Yield (% of *Gl*)	70	100

—, Not detected; tr, traces (≤ 0.5%).

†Present address: Max Peanck Institute, Egelspfad, D-5000 Köln-Vogelsang 30, West Germany.

Table 2. Composition (%) of alkanes, aldehydes, alcohols and acids from the mutant *gl11* and from normal WF9 plants (*Gl*)

Number of carbon atoms	Alkanes		Aldehydes		Alcohols		Acids	
	gl11	*Gl*	*gl11*	*Gl*	*gl11*	*Gl*	*gl11*	*Gl*
16							45	25
18							33	13
19								
20	9						3	4
21	5							
22	8						3	6
23	11	2						
24	7	tr			1		4	14
25	5	5						
26	4	tr			3	tr	4	22
27	13	13						
28	3	tr	—	1	2	tr	5	12
29	25	29						
30	1	tr	—	3	2	1	3	tr
31	9	49						
32	—	tr	—	96	92	99	—	4
33	—	2						

—, Not detected; tr, traces (≤ 0.5%).

of an albino mutant of maize [9] in *gl11* alkanes a large percentage of even carbon numbered chains is found (Table 2). As regards the alcohols, *n*-dotriacontanol is the dominant homologue as in normal wax (92 vs 99% in the normal). The free fatty acids of *gl11* comprise a wide range of homologues: 88% of the total is represented by C_{16} and C_{18} while in the wild type wax only 38% of total free acids is represented by these two compounds. The composition of the esters is shown in Table 3. Two groups of major homologues occur in normal maize seedlings namely C_{42}–C_{48} and C_{54}–C_{56}. In contrast, esters from mutant *gl11* are characterized by shorter components ranging from C_{42} to C_{48} (Table 3). The acid and alcohol moieties of the mutant esters clearly show a homologue composition very distinct from that of the normal genotype. Esterified alcohol C_{32}, which represented almost 100% in normal wax, amounts to only 13% of the total esterified alcohols in *gl11* where C_{24} and C_{30} are the dominant homologues. In the wild type esterified fatty acids range from C_{20} to C_{30} with C_{24} dominating, while in *gl11*, C_{20} is the predominant homologue accompanied by consistent amounts of C_{22}, C_{24} and C_{30}.

Previous studies on the chemical genetics of the waxes from glossy mutants of maize confirm the biosynthetic pathway proposed for these compounds by Kolattukudy [11, 12]. It was shown, in particular, that mutations interfere with epicuticular lipid synthesis by reducing the total amount of wax on the leaves and by affecting the relative percentages of the various classes of compounds, with relevant changes in their homologue composition [5–9]. Esters were the least affected by mutations with respect to other classes of compounds: in the mutant waxes they were always increased in percentage while their amount per plant showed only slight variations. This was taken as evidence that the long chain molecules of maize wax are synthesized by two distinct elongation–decarboxylation (ED) complexes: EDI governing the formation of the longest alkanes, aldehydes and alcohols, and EDII mainly responsible for the synthesis of the esters.

Results obtained with mutant *gl11* provide the opportunity for discussing further wax synthesis routes in maize plants. In mutant *gl11* the overall amount of wax is decreased by 30%, but the wax composition, as compared to the wild type, shows a strong accumulation of esters (66% of total wax). A derepression of EDII seems therefore to be activated by this mutation. Furthermore this is apparently accompanied by an imperfect functioning of EDI, the enzyme complex which in normal seedlings is responsible for the synthesis of the longest chains of aldehydes and alcohols. In fact, even to a certain extent more evident than in the mutants *gl2*, *gl4* and *gl3* which show metabolic blocks in the final steps of EDI

Table 3. Composition (%) of esters from the mutant *gl11* and from normal WF9 plants (*Gl*)

Number of carbon atoms	Esterified alcohols		Esterified acids		Number of carbon atoms	Esters	
	gl11	*Gl*	*gl11*	*Gl*		*gl11*	*Gl*
16	2		9		38		2
18	2		6		40	5	8
20	13		23	1	42	18	11
22	11		20	15	44	28	14
24	25		11	49	46	21	10
26	6		9	27	48	10	14
28	9		3	6	50	5	4
30	19	tr	18	2	52	3	4
32	13	100	1	tr	54	4	15
					56	4	15
					58	2	3
					60		tr

tr, Traces (≤ 0.5%).

[5, 6, 8], the esterified alcohols of *gl11* comprise all the intermediate homologues from C_{16} to C_{32}, while only the C_{32} alcohol is present in the wild type. A second dramatic variation in EDI function is observed in *gl11*: no aldehydes are released by the elongation complex, while this class of compounds is present in the wild type (20%). In this respect it must be mentioned that in the mutant *gl5* aldehydes represent 84% of the total wax [7], in striking contrast to the situation present in *gl11*. As regards free fatty alcohol accumulation, however, *gl11* is not dissimilar from the wild type: both genotypes accumulate mainly the C_{32} homologue (92 vs 99% of *Gl*), a fact that distinguishes *gl11* from other mutants defective in EDI complex.

A key observation which offers an explanation for the observed wax composition in *gl11* regards its abnormal high content of free C_{16} and C_{18} acids with the concomitant defect in the synthesis of C_{24}, C_{26} and C_{28} homologues. The hypothesis can be advanced that wildtype EDI may be split into an early (EDIa) and a late (EDIb) group of reactions. EDIa should govern the synthesis of C_{24}–C_{28} fatty acyl chains, the precursors of EDIb which uses these compounds for the production of C_{30}–C_{32} homologues. It may then be suggested that the mutant *gl11* is defective at the EDIa level: an abnormally low utilization of substrates should in fact result in an accumulation of C_{16}–C_{18} precursors, making them available in larger quantity for EDII. Therefore this situation, clearly observed in the study of an albino strain of maize [9], should stimulate ester synthesis.

The reduced availability of C_{24}–C_{28} molecules for EDIb may alone justify the absence of aldehydes in *gl11* waxes. These compounds, in fact, represent the intermediates in the biosynthesis of alcohols from fatty acids [13], being the two reductive steps catalysed respectively by an NADH-dependent acyl-CoA reductase and by a NADPH-dependent aldehyde reductase. In the case of *gl11*, absence of aldehydes is neither associated with a significant accumulation of free fatty acids, nor with an absolute block in alcohol synthesis. Nevertheless, the alcohol homologue distribution is not dissimilar from that of the normal genotype with C_{32} as the dominant chain. It seems reasonable then to suggest that in *gl11*, due to the limited availability of acyl substrates, the free alcohol molecules are under produced. Thus the amount of these precursors being under the saturation level of the aldehyde reductase, no aldehydes are formed.

This interpretation of the results obtained with the *gl11* mutant sustains the existence of two major groups of reactions in EDI: the first (EDIa) which leads to C_{24}–C_{28} chains, the second (EDIb) which further elongates these chains up to 32 carbon atoms (Fig. 1). Mutant *gl11* can then be regarded as affecting EDIa, while the previously described *gl2*, *gl3* and *gl4* [5, 6, 8], show defective steps in EDIb.

EXPERIMENTAL

The *gl11* mutant was obtained from the Maize Genetic Cooperative, Urbana, Illinois and backcrossed × 5 with the inbred WF9. The latter was used as the normal (*Gl*) genotype. The plants, grown in a greenhouse during May 1983, were collected at the fourth–fifth leaf stage of growth. The epicuticular waxes were extracted by dipping the seedlings in $CHCl_3$ for 30 sec and their composition analysed by TLC as previously described [9]. Individual wax classes were separated by CC and identified following published procedures [5, 9, 14, 15]. GC

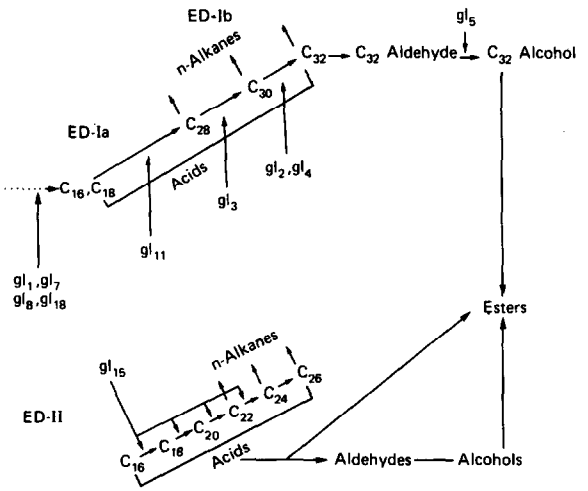

Fig. 1. Elongation–decarboxylation pathways in the biosynthesis of maize plant epicuticular wax constituents.

analysis of the purified fractions was carried out on an OV 1, 15 m, 0.1–0.15 μm film thickness, capillary column. Isothermal and programmed FID chromatograms were run under the appropriate conditions. Alkanes and aldehydes were analysed as such. Alcohols and free acids were converted into acetates and Me esters, respectively, as described in ref. [9]. Composition of the esters was determined by analysing them intact [16] and as the combined Me esters and alcohols acetates from their transesterification followed by acetylation [9].

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REFERENCES

1. Bianchi, A., and Marchesi, G. (1960) *Z. Vererbungsl.* **91**, 214.
2. Salamini, F. (1963) *Maydica* **8**, 67.
3. Bianchi, A. (1978) *Genetics and Breeding*, Urbana, Illinois, Sept. 8–12, 1975. John Wiley, New York.
4. Coe, E. H., and Neuffer, M. G. (1977) in *Corn and Corn Improvement* (Sprague, G. F., ed.) pp. 111–223. Am. Soc. Agronomy, Madison, U.S.A.
5. Bianchi, G., Avato, P. and Salamini, F. (1975) *Maydica* **20**, 165.
6. Bianchi, G., Avato, P. and Salamini, F. (1977) *Maydica* **22**, 9.
7. Bianchi, G., Avato, P. and Salamini, F. (1978) *Biochem. Genet.* **16**, 1015.
8. Bianchi, G., Avato, P. and Salamini, F. (1979) *Heredity* **42**, 391.
9. Bianchi, G., Avato, P. and Salamini, F. (1982) *Phytochemistry* **21**, 129.
10. Dupont Durst, H. and Gokel, G. W. (1978) *J. Chem. Educ.* **55**, 206.
11. Kolattukudy, P. E. (1968) *Science* **159**, 498.
12. Kolattukudy, P. E. (1970) *Ann. Rev. Plant Physiol.* **21**, 163.
13. Kolattukudy, P. E. (1971) *Arch. Biochem. Biophys.* **142**, 701.
14. Bianchi, G., and Salamini, F. (1975) *Maydica* **20**, 1.
15. Avato, P., Bianchi, G. and Mariani, G. (1984) *Phytochemistry* **23**, 2843.
16. Bianchi, G., Avato, P., and Salamini, F. (1984) *Cereal Chem.* **61**, 45.