GLOSSY MUTANTS OF MAIZE

IX. CHEMISTRY OF GLOSSY 4, GLOSSY 8, GLOSSY 15 AND GLOSSY 18 SURFACE WAXES*

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SUMMARY

Modifications of chemical composition of surface waxes are induced by the recessive alleles of the *gl-4*, *gl-8*, *gl-15* and *gl-18* loci. Mutant *gl-4* blocks the elongation of long chain wax molecules at the C_{30} — C_{32} step. *gl-8* and *gl-18* mimic the action of *gl-1* and *gl-7*, and may affect a very early stage of the biosynthetic pathway leading to long chain compounds or, alternatively interfere with the supply of wax precursors. *gl-15* shows both an unusual spectrum of n-alkanes, in that the percentage of even chain compounds is higher than normal, and an abnormal synthesis of esterified primary alcohols of C_{18} and C_{18} chain lengths.

1. INTRODUCTION

THE leaf surface of maize seedlings is covered by epicuticular waxes (Bianchi and Marchesi, 1960; Lorenzoni and Salamini, 1975). Coe and Neuffer (1977) list 13 independent genes interfering with wax synthesis and accumulation. The modifications of wax chemical composition induced by the recessive alleles at the *gl-1*, *gl-2*, *gl-3*, *gl-5* and *gl-7* loci have been described previously (Bianchi *et al.*, 1975, 1977, 1978). In this paper the results of a study of the chemical composition of the waxes produced by seedlings homozygous for the mutant alleles *gl-4*, *gl-8*, *gl-15* and *gl-18* are reported.

2. MATERIALS AND METHODS

The four mutants were obtained from the Maize Genetics Cooperative, Urbana, Illinois and backcrossed four times to the inbred WF9. The latter was then considered the normal *Gl* type. Mutant and normal genotypes were grown in a greenhouse during May 1977. Plants were collected at the fourth-fifth leaf stage of growth. Waxes were extracted by dipping the seedlings in chloroform for 45-60 sec. and fractionated by column chromatography to give n-alkanes, aldehydes, alcohols and esters (Bianchi *et al.*, 1975). The esters were converted to methyl esters and alcohols by refluxing with 10 per cent methanolic hydrogen chloride and benzene for 24 hours. Gas-liquid chromatography (GLC) analysis of the homologous terms within each class of compounds was carried out as described (Bianchi *et al.*, 1977).

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TABLE 1

Composition of waxes of normal Gl, and mutant gl-4, gl-8, gl-15 and gl-18 seedlings

Components	Percentage					mg/1000 g fresh weight				
	Gl	gl-4	gl-8	gl-15	gl-18	Gl	gl-4	gl-8	gl-15	gl-18
Alkanes	1.4	0.2	1.1	4.9	2.4	8.1	0.5	2.0	21.6	8.8
Aldehydes	20.4	15.7	11.5	19.9	4.3	118.3	40.1	20.0	88.8	16.1
Alcohols	62.7	41.9	49.4	55.0	56.6	363.7	107.2	86.8	242.8	209.4
Esters	15.5	42.2	38.0	20.2	36.7	89.9	108.1	66.7	89.3	135.6
Total	—	—	—	—	—	580.0	255.9	175.5	441.7	369.9
% of normal	—	—	—	—	—	100	44.1	30.3	76.2	63.8

3. RESULTS

The composition of normal and mutant waxes is reported in table 1. The four mutants have higher percentages of esters than the normal genotype. However, if the results are expressed as mg of esters per 1000 g of fresh weight, their quantities appear similar to the normal, except for *gl-18* which accumulates more esters than the others. The percentages of aldehydes and free alcohols are considerably reduced in *gl-4* and *gl-18*. The same pattern is found also on a fresh weight basis for *gl-4*, *gl-8* and *gl-18*. The alkane fraction of *gl-4* is lower (0.2 per cent) while it is higher (4.9 per cent) in the case of *gl-15*.

The percent distribution of homologous chains of wax classes is shown in table 2. The dominant chain lengths of the n-alkanes, aldehydes and alcohols of mutant *gl-4* are shorter by two carbon units compared with the same classes of compounds in the normal plant. In *gl-4*, the C_{30} chains (C_{29} in the alkane fraction) prevail while the normal plant synthesises almost entirely the C_{32} homologues (C_{31} for the alkanes).

Mutations *gl-8* and *gl-18* differ less from the normal genotype. The long molecules in the waxes extracted from these mutations are those found in normal wax. The two mutations, however, reduce drastically the overall amounts of all the compounds synthesised except esters (table 1). This result suggests that *gl-8* and *gl-18* both show an early partial block in the synthesis of long chain molecules; alternatively an abnormally low supply of precursors may cause the reduced rate of wax synthesis in these mutations. *gl-15* shows minor although peculiar modifications in wax composition. As in the normal genotype the C_{32} chain constitutes 100 per cent of the aldehydes and free alcohols of this mutant. The spectrum of n-alkanes of *gl-15* is unusual in two respects; firstly there is a considerable increase in short chain homologues, secondly the percentage of even chain components is exceptionally high (21.7 per cent). The esterified primary alcohols show an unusual composition (table 2) having more than 50 per cent cetyl (C_{16}) and stearyl (C_{18}) alcohols and 39 per cent of hentriacontanol, while the intermediate chains (C_{20} to C_{30}), which are the dominant constituents of the other three mutants, are present in small amounts.

4. DISCUSSION

The four glossy mutants studied, clearly interfere with epicuticular wax synthesis. Mutant *gl-4* blocks the elongation of long chain molecules at the

TABLE 2
Relative composition (%) of fractions from normal maize (GI) and from gl-4, gl-8, gl-15, gl-18

[illegible]

FIG. 1.—Wax biosynthesis of plant surface waxes (Kolattukudy, 1969, 1970).

FIG. 2.—Wax biosynthesis of maize surface waxes. This pathway derives from that of fig. 1 together with the evidence provided by the mutation studies. In the scheme the major blocks induced by nine independent mutations are reported.

$C_{30} \rightarrow C_{32}$ step, resembling the action of the recessive allele at the *gl-2* locus (Bianchi *et al.*, 1975). A mutation interfering with the last elongation step, as in *gl-2* and *gl-4* of maize, has also been described for *Brassica oleracea* (Macy and Barber, 1970; Macey, 1974). The kind of metabolic modification brought about by *gl-8* and *gl-18* is not as clearly definable, and resembles that of *gl-1* and *gl-7*. The biosynthetic pathway leading to long chain compounds appears affected at a very early stage or, alternatively, the supply of precursors is abnormally low (Bianchi *et al.*, 1977).

The most plausible biosynthetic pathway for wax synthesis is represented by the scheme of fig. 1, Kolattukudy (1969; 1970) and verified for normal maize by Bianchi and Salamini (1975). Further work on wax synthesis in maize (Bianchi *et al.*, 1975, 1977, 1978) indicates the existence of two elongation-decarboxylation complexes: ED-I, related to n-alkane, aldehyde and free alcohol synthesis, and ED-II responsible mainly for ester synthesis. The results discussed in this paper support the modified wax biosynthetic scheme shown in fig. 2. In the scheme are indicated the major effects of mutations, those described in this paper together with those found for the others previously studied. According to this *gl-15* is effective somewhere between C_{18} and C_{22} of ED-II. As a result there is an accumulation of alcohols of 16 or 18 carbon atoms which are promptly incorporated into esters. Moreover, the ED-II complex releases, or does not accept many, short chain fatty acids, and these are therefore converted to n-alkanes by decarboxylation. In addition, ED-II of *gl-15* appears to have lost its strict specificity towards the synthesis of odd numbered n-alkanes, and more even chain n-alkanes are produced according to well documented biochemical mechanisms (Kolattukudy, 1976).

5. REFERENCES

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