Planta 148, 328–331 (1980)

Planta

Cuticular Wax of Wheat

The Effects of Chromosomal Deficiencies on the Biosynthesis of Wax Components

Giorgio Bianchi[1], Elisabetta Lupotto[1], Basilio Borghi[2], and Maria Corbellini[2]

[1] Istituto di Chimica Organica, Viale Taramelli, 10, I-27100 Pavia, and

[2] Istituto Sperimentale per la Cerealicoltura, Sezione di S. Angelo Lodigiano, I-20079 S. Angelo Lodigiano (Milano), Italy

Abstract. n-Alkanes, esters, aldehydes, free alcohols, β-diketones and hydroxy-β-diketones were found to be the lipid components of the cuticular waxes of common wheat Chinese Spring (*Triticum aestivum* L.). The ditelosomic lines 7A-L and 7D-S showed a dramatic decrease in the amount of β-diketones and hydroxy β-diketones which are reduced to traces. The homologue composition within each class of compounds has also been determined for all three of the lines of wheat. The effects of chromosomal deficiencies have been demonstrated. Chromatographic techniques and mass spectrometry have been used for the separation and identification of the substances which compose the waxes. This study has provided further evidence of the role of genes situated on well defined chromosomes in determining the nature of classes of compounds composing wax and governing the homologous composition within each class of substances.

Key words: Chromosomal deficiencies – Cuticular waxes – *Triticum* – Waxes.

Introduction

The amount of wax and the chain-length composition of the lipid classes which compose the waxes of numerous wheat varieties were previously studied (Tulloch, 1973; Bianchi and Corbellini, 1977). The present paper reports on an attempt to correlate the wax production and composition of wheat varieties with their chromosomal constitution. It is, in fact, commonly accepted that the formation and deposition of the various lipid classes found in several plant cuticular waxes are controlled by genes located on different chromosomes (Newton-Barber and Netting, 1968; von Wettstein-Knowles, 1972, 1974, 1976; Bianchi et al., 1977, 1978). We have carried out a comparative study on the composition of waxes of the wheat variety Chinese Spring and of the ditelosomic lines 7A-L and 7D-S (Sears, 1954). The latter two lines have been chosen from a group of 29 lines whose n-alkane composition has been analyzed previously (Bianchi et al., 1979).

Materials and Methods

Plants of the wheat variety Chinese Spring (*Triticum aestivum*) and of the ditelosomic lines were kindly supplied by Dr. Law (Cambridge, England) and were field grown in the Po Valley near Milan during 1976. As wax yield and composition are dependent on the stage of development of the plant (Tulloch, 1973; Bianchi and Corbellini, 1977), the waxy material was obtained from plants at the same growth stage, following a standard procedure for all the three lines. The plants (ca. 90 culms) were collected at the stage of maximum wax production when the flag leaf was completed and the spike was emerging from the sheath (late boot stage). Wax extraction was effected by dipping the shoots into chloroform for approximately 60 s. Evaporation of the solvent under reduced pressure on the rotary evaporator gave the desired wax samples. The wax class composition was determined by thin-layer chromatography (TLC). The TLC plates were 0.25 mm layer of silica gel G (Merck) activated at 120° C for two hours and developed either in carbon tetrachloride, cyclohexane, or benzene. Spots were detected by spraying with CrO_3 2% in H_2SO_4 1:1 followed by charring at 120° C. β-Diketones were also easily detected under UV light (254 nm) using silica gel GF_{254} (Merck). Column separations were made by gradient elution on silica gel H (Merck). Columns 5·50 cm, packed with 400–500 g silica gel H prewashed with CCl_4 were loaded with ca. 150 mg of crude wax material dissolved in the same solvent. The eluted fractions were 10 ml, collected by a fraction collector and checked by TLC one by one.

The following solvents were used in succession: carbon tetrachloride to give n-alkanes, esters, aldehydes, and β-diketones (the latter two classes of compounds were often unresolved; they were resolved on preparative TLC plates with benzene as eluent) and chloroform to elute alcohols and hydroxy β-diketones in order. In the case of 7A-L wax, unidentified material was eluted as the last fraction (eluent chloroform). A Carlo Erba Model Fractovap 2 400 T gas chromatograph with flame ionization detectors was used for gas chromatographic investigations. GLC analyses were carried out by using a 2 m·3 mm glass column packed with 1% OV 1 on Chromosorb W, 100–120 mesh. Isothermal, and pro-

0032-0935/80/0148/0328/$01.00

Table 1. Percentage composition[a] of waxes isolated from wheat variety Chinese Spring and 7A-L and 7D-S ditelosomic lines

Components	Chinese spring	7A-L	7D-S
n-Alkane	10.0±0.4	10.6±0.5	8.7±0.4
Esters	16.7±0.6	20.8±0.8	18.0±0.8
Aldehydes	8.7±0.4	13.3±1.1	9.6±0.5
Free alcohols	50.5±0.9	50.3±1.2	63.2±1.0
β-Diketones	11.8±0.4	5.0±0.3	0.5±0.1
Hydroxy β-diketones	2.3±0.2	traces	traces

[a] Calculated from the weights of compounds obtained by 2–3 separate silica gel chromatography analyses. The range in values used to obtain the data is indicated by ±

grammed chromatograms were run using column temperatures from 160 to 280° C as required, while nitrogen, hydrogen, and air streams were adjusted to yield optimum sensitivity. Combined acids and alcohols of esters were determined by acid methanolysis (Bianchi and Corbellini, 1977). Authentic samples of each class of compounds were used as standards for TLC and GLC. The data reported refer to results of at least two replicates. Infrared spectra were obtained using KBr discs with a Perkin Elmer Model 257. Ultraviolet spectra of a 1% ($E^{1\%}_{1\,cm}$) solution of the wax in *iso*-octane were recorded with a Perkin Elmer Model 135. The mass spectra were run on a Du Pont 21492 B mass spectrometer at 70 eV of filament energy, an ion source temperature of 230° C, an accelerating voltage of 1,400 V and using a direct inlet system at 100° C.

Results and Discussion

The classes of compounds which constitute the waxes of Chinese Spring and of the two ditelosomic lines 7A-L and 7D-S are given in Table 1.

The effects of the deficiency of one arm of chromosomes 7A and 7D, respectively, appear at the wax composition level of these lines: β-diketones are reduced to less than 5% in 7A-L and 0.5% in 7D-S; hydroxy β-diketones have practically disappeared in both the ditelosomic lines.

The spectra of the n-alkanes of the three lines differ from each other (Table 2). The long chain homologues, as usual, predominate over the short chains. However, in Chinese Spring the group C_{14}–C_{20} represents 7.2% of the total, while these chains are found only in traces in the other lines. In the case of 7A-L n-alkanes, while the chain length range is C_{21} to C_{33}, 24.1% of this class of compounds is made up by even chains, namely C_{22} 5.0%, C_{24} 8.1%, C_{26} 7.0%, and C_{28} 4.0%. The 7D-S n-alkanes showed the normal chain-length pattern found for almost all wheat waxes with C_{27}, C_{29}, and C_{31} major homologues. The structure of all the alkane homologues was assured by Co-GLC and GLC-MS spectrometry.

Table 2. Homologue composition of wax fractions of wheat variety Chinese Spring (C.S.) and 7A-L and 7D-S ditelosomic lines

Number of C atoms	n-Alkanes			Aldehydes			Free primary alcohols			β-Diketones; hydroxy β-diketones			Esterified acids			Esterified alcohols		
	C.S.	7A-L	7D-S	C.S.	7A-L	7D-S	C.S.	7A-L	7D-S	C.S.	7A-L	7D-S	C.S.[a]	7A-L[b]	7D-S[c]	C.S.[a]	7A-L[b]	7D-S[c]
14	0.4	tr.	tr.										0.5	0.4	tr.	tr.		tr.
16	3.7	tr.	tr.										14.9	10.1	11.6	tr.		tr.
18	2.2	tr.	tr.										8.3	6.7	10.6	0.9		0.8
20	0.9	tr.	tr.										41.4	11.2	26.6	4.0	tr.	0.8
21	–	2.3	tr.										–	0.5	–	–	–	–
22	tr.	5.0	tr.			3.0	tr.	0.3					26.8	52.5	37.0	13.4	5.0	5.8
23	0.4	9.7	0.8			–	tr.	–					–	0.5	–	–	1.7	–
24	tr.	8.1	tr.	1.1		1.9	tr.	0.7	1.5				7.4	6.4	8.5	13.4	7.3	7.7
25	0.7	10.8	1.5	–		–	–	–	–				–	–	–	–	–	–
26	–	7.0	0.1	2.5	tr.	4.3	1.6	1.6	2.1				0.7	4.8	3.7	9.8	5.0	3.8
27	15.2	14.3	6.2	–	–	–	–	–	–				–	–	–	–	–	–
28	tr.	4.0	0.2	80.6	65.4	73.9	92.8	95.9	95.6				tr.	6.9	2.0	58.5	81.0	81.1
29	51.5	25.0	58.2	–	–	–	–	–	–									
30	–	tr.	0.1	15.8	28.4	15.4	–	0.5	0.5									
31	23.7	10.6	29.2	–	–	–		–		100[e]	100[e]	100[e]						
32	–	tr.	tr.	tr.	6.2	1.5		0.4										
33	1.3	3.2	3.7															
Unident.							5.6[d]	0.6[d]	0.3[d]									

[a] Two unidentified peaks, between ester C_{24} and alcohol C_{26}, and ester C_{26} and alcohol C_{28}, amounting to 2.1% are present in the gas-chromatogram

[b] See [a]; unidentified 3% between alcohol C_{26} and ester C_{28}

[c] See [a]; unidentified 1.5%. Three peaks between esters-alcohols C_{16}–C_{18}, C_{24}–C_{26} and C_{26}–C_{28}, respectively

[d] Unidentified peaks between C_{26} and C_{28}

[e] See Results and Discussion

How can these results be accounted for? The biosynthesis of epicuticular wax alkanes is considered to be governed by the so-called elongation-decarboxylation mechanism (Kolattukudy, 1976), according to which palmitic acid C_{16} (with varying amounts of myristic acid C_{14} and stearic acid C_{18}) is elongated by addition of 2-carbon units from malonly-CoA until the appropriate chain length is reached; then the fatty acid undergoes decarboxylation to give the corresponding alkane. This mechanism of n-alkanes biosynthesis fully explains the formation of the odd-carbon hydrocarbons but requires revision in order to rationalize the formation of even-carbon homologues. In addition to the two possible explanations for the formation of even-carbon n-alkanes widely accepted in this area of biochemistry, i.e., (i) the precursor odd-carbon fatty acid may be produced by incorporation of propionate rather than acetate in the initial condensation step, (ii) α-oxidation of the even-carbon fatty acid followed by decarboxylation; we advanced a tentative reaction path whose mechanism involves a complete reduction of the even-carbon chain acid substrate to the final stage of n-alkane, without loss of carbon dioxide. Why this phenomenon is confined to the parent variety Chinese Spring and its ditelosomic line 7A-L, although in two different ranges of carbon chain lengths, remains to be determined. However, in the case of 7A-L, it might be suggested that the increase in even-carbon alkanes is a consequence of a less effective decarboxylation process controlled by genes situated somewhere on the missing short arm of chromosome 7A. As a consequence, the accumulated acids have a greater chance of being reduced to even-carbon chain alkanes.

The percentage composition of aldehydes and free primary alcohols reveals that the homologue composition within each class are rather similar. Provided that the biosynthetic path aldehyde-alcohol is correct, the data in Table 2 indicate a high degree of chain-length specificity of the enzymes performing the reduction process. In fact n-octacosanol is the major component of all the alcoholic fractions and only very minor amounts of longer and shorter chain homologues were found in the 7A-L and 7D-S lines.

As in free alcohols, n-octacosanol is the major esterified primary alcohol. However, significant amounts of other homologues, namely C_{22}, C_{24}, and C_{26} are present in the esters of all three lines of wheat. This fact suggests that either the esterification process is very active and part of the precursor chains of octacosanol are trapped by acids to give esters, or the esterified alcohols are produced in a synthetic pool distinct from that responsible for the production of free primary alcohols. This hypothesis has already been advanced for the waxes of maize mutants (Bianchi et al., 1977). The percentage composition of esterified acids shows that the major components are even chains in the range of C_{16}–C_{24}. The mass spectra of esters show that the dominant chains are C_{42}, C_{44}, C_{46}, and C_{48}. On the basis of these data, it seems plausible to assume that the esterification enzymatic system requires suitable chain length to accomodate the active synthetic sites of the enzyme(s). Small amounts of odd-carbon chains are also present in the ester fractions. The ion patterns fitting C_{13} and C_{15} alcohol chains found in the mass spectra, indicate that the odd-carbon esters originate from esterification of even carbon acids with the two odd-carbon chain alcohols.

The β-diketone fractions showed spectroscopic data (IR, UV and mass spectrum) identical to those reported previously for the hentriancontane-14, 16-dione found in Demar 4 (Bianchi and Corbellini, 1977). As far as the hydroxy β-diketones of the Chinese Spring is concerned, mass spectrometry of the crude material revealed that they consist of a mixture of 25-hydroxy ($\geqq 90\%$) and 26-hydroxy ($\leqq 10\%$) hentriacontane-14,16-diones (Tulloch, 1976). A single crystallization of this material from ethyl acetate, gave pure 25-hydroxyhentriacontane-14,16-dione. Further evidence in favor of this structural attribution is represented by the higher Rf (TLC) of these compounds compared with the 8- and 9-hydroxy isomers, as already reported in the literature (Tulloch and Hoffman, 1971). Hitherto, these isomers have only been found in tetraploid wheats and not in hexaploid wheats. Furthermore, the mass spectrum of this material shows peaks which might be tentatively attributed to oxo β-diketones.

While considerable data are now available in the literature (Bianchi and Corbellini, 1977) regarding the chemical composition of epicuticular waxes of several wheat lines, very little is known about the biochemical genetics of wheat waxy material. In the only paper dealing with chemical genetics of β-diketone formation, Newton-Barber and Netting (1968) reported that gene(s) on chromosome 6B were involved in the production of β-dicarbonyl compounds.

The availability of numerous ditelosomic lines of Chinese Spring permits one to study further the direct connection between genes and lipid components of wax; such a connection was shown for the 7A-L line.

The authors gratefully acknowledge the CNR (Rome) for financial aid. Contract 78-01465-06.

References

Bianchi, G., Avato, P., Salamini, F.: Glossy mutants of maize. VII. Chemistry of *glossy 1, glossy 3* and *glossy 7* epicuticular waxes. Maydica **22**, 9–17 (1977)

Bianchi, G., Avato, P., Salamini, F.: Glossy mutants of maize. VIII. Accumulation of fatty aldehydes in surface waxes of *gl 5* maize seedlings. Biochem. Genet. **16**, 1015–1021 (1978)

Bianchi, G., Corbellini, M.: Epicuticular wax of *Triticum aestivum* Demar 4. Phytochemistry **16**, 943–945 (1977)

Bianchi, G., Lupotto, E., Borghi, B., Corbellini, M.: Epicuticular wax of wheat. Alkane composition of 29 ditelosomic lines. Wheat Inf. Serv. **49**, 5–9 (1979)

Bianchi, G., Lupotto, E., Corbellini, M.: Composition of epicuticular waxes of *Triticum aestivum* Demar 4 from different parts of the plant: a revision. Agrochimica **23**, 96–102 (1979)

Kolattukudy, P.E.: Chemistry and Biochemistry of Natural Waxes. Amsterdam, Oxford, New York: Elsevier 1976

Newton-Barber, H., Netting, A.G.: Chemical genetics of β-diketone formation in wheat. Phytochemistry **7**, 2089–2093 (1968)

Sears, E.R.: The aneuploids of common wheat. Mont. Agric. Exp. Stn. Res. Bull. **572**, p. 59 (1954)

Tulloch, A., Hoffman, L.L.: Leaf wax of durum wheat. Phytochemistry **10**, 871–876 (1971)

Tulloch, A.: Composition of leaf surface waxes of *Triticum* species: variation with age and tissue. Phytochemistry **12**, 2225–2232 (1973)

Tulloch, A.: Epicuticular wax of *Agropyron smithii* leaves. Phytochemistry **15**, 1153–1156 (1976)

Von Wettstein-Knowles, P.: Genetic control of β-diketone and hydroxy β-diketone synthesis in epicuticular waxes of barley. Planta **106**, 113–130 (1972)

Von Wettstein-Knowles, P.: Gene mutation in barley inhibiting the production and use of C_{26} chains in epicuticular wax formation. FEBS Lett. **42**, 187–191 (1974)

Von Wettstein-Knowles; P.: Biosynthetic relationship between β-diketones and esterified alkan-2-ols deduced from epicuticular wax of barley mutants. Mol. Gen. Genet. **144**, 43–48 (1976)

Received 4 July; accepted 8 November 1979