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EPICUTICULAR WAXES OF *SORGHUM* AND SOME COMPOSITIONAL CHANGES WITH PLANT AGE

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Abstract—Epicuticular wax from mature plants of *Sorghum bicolor* SD-102 was compared with that from panicles and seedlings of the same variety at the fourth–fifth leaf stage of growth. The composition of wax from SD-102 panicles was quite different from that of mature leaf blades and sheaths. Free fatty alcohols were the dominant class of wax from SD-102 seedlings and C_{32} was the major homologue of alcohols and aldehydes. For comparison purposes, the epicuticular waxes from whole plants of two other *S. bicolor* varieties, Alliance A and Martin A, grown up to the tasseling stage, have been analysed. The major wax components were free fatty acids. The typical chain lengths of aldehydes, free alcohols and free fatty acids were C_{28} and C_{30}. *p*-Hydroxybenzaldehyde was not a wax component of the studied varieties of sorghum.

INTRODUCTION

The present paper reports on the variation of *Sorghum bicolor* SD-102 epicuticular wax composition with plant age. In order to have comparison elements, the waxes from whole mature plants of two other *S. bicolor* varieties, namely Martin A and Alliance A, were also analysed.

RESULTS AND DISCUSSION

In a previous study qualitative and quantitative analyses of waxes from leaf blades and sheaths of mature plants of Alliance A and SD-102 sorghum lines were reported [1, 2]. Free fatty acids were the major wax class of compounds of the leaf sheaths of both varieties and of SD-102 leaf blades. Esters were the largest wax constituent (44%) of Alliance A leaf blades [1, 2].

In the present work analyses were performed on wax samples isolated from whole mature plants of Martin A and Alliance A. The waxes from both lines had free fatty acids as the dominant class (Table 1). Esters and alcohols of Alliance A were also present in substantial quantities (21 and 15%, respectively), while Martin A variety appeared to be characterized by alcohols (16%) and alkanes (10%) as other important wax components (Table 1). Inspection of the data available in the literature on other sorghum varieties revealed wax composition patterns different from ours [3, 4]. In particular, in a study on the wax of *S. bicolor* Redbine 60 bloom, alkanes have been reported as the dominant class (62%) followed by alcohols (31%), while free acids represented only 7% [3]. As shown in Table 2, Alliance A and Martin A wax classes did not exhibit any marked difference in chain length distribution. Homologues C_{27}, C_{29} and C_{31} were the major alkanes, while aldehydes, alcohols and free fatty acids were characterized by the two chains C_{28} and C_{30} (Table 2). The presence of C_{28} and C_{30} as the most important homologues of aldehydes, alcohols and free fatty acids is peculiar to sorghum wax as already shown for waxes of blades, sheaths [1] and grain [5] of the sorghum variety SD-102. Results reported in Table 5 indicate that C_{28} and C_{30} are the dominant homologues also of aldehydes, alcohols and free fatty acids of SD-102 panicle wax. While the presence of the same dominant chain lengths in waxes from different parts of the plant is of considerable importance from a biosynthetic point of view, the occurrence of two major homologues in sorghum is in agreement with those found for other panicoid grasses. It is known [6] that waxes of panicoid grasses usually contain a range of chain lengths, or else C_{32} as the major chain, in contrast to those of festucoid species. Thus C_{28} is the dominant chain in wheat and related species [7, 8], C_{26} in oat, rye and barley [9–11] and C_{32} in corn [12].

Esters from Alliance A and Martin A showed wide chain length ranges (Table 3). The esterified alcohols ranged mainly from C_{22} to C_{28}, while acids comprised C_{20} to C_{24} as major homologues. GC analysis of intact esters (Table 3) showed that the most important chain lengths were in the range C_{42}–C_{52} in good agreement with data from mass spectrometric analysis. Moreover, ester fractions contained triterpenes which accounted for 44% and 37% in Alliance A and Martin A sorghum varieties,

Table 1. Chemical composition of epicuticular waxes from mature sorghum plants

	%	
Components	Martin A	Alliance A
Alkanes	10	7
Esters	8	21
Aldehydes	8	7
Alcohols	16	15
Acids	58	50

Table 2. Composition (%) of alkanes, aldehydes, alcohols and acids from mature sorghum plants

Chain length	Alkanes		Aldehydes		Alcohols		Acids	
	Martin A	Alliance A	Martin A	Alliance A	Martin A	Alliance A	Martin A	Alliance A
16							1	—
18							tr	—
20							tr	—
22					1	tr	1	1
24	—	tr	tr	—	1	1	1	2
25	1	2	—	—	—	tr	—	—
26	tr	1	2	5	7	5	2	2
27	13	14	1	1	—	1	—	—
28	2	3	43	40	73	57	57	54
29	54	50	2	2	—	3	—	—
30	1	2	39	47	16	24	36	39
31	18	18	tr	—	—	3	—	—
32	tr	tr	11	5	2	6	2	2
33	7	7	2	—				
34	—	—						
35	4	3						

—, Not detected; tr, trace, ≤ 0.5%.

Table 3. Composition (%) of esters from mature sorghum plants

Chain length	Esterified alcohols		Esterified acids		Chain length	Esters	
	Martin A*	Alliance A†	Martin A	Alliance A		Martin A	Alliance A
18	—	2	—	5	40	—	2
20	2	6	26	35	42	5	17
22	24	38	32	33	44	15	21
24	27	20	27	27	46	17	16
25	—	—	—	—	48	12	9
26	24	12	8	tr	50	16	16
27	—	—	—	—	52	15	19
28	21	22	7	tr	54	4	tr
29	—	—			56	9	tr
30	2	—			58	7	tr

—, Not detected; tr, trace, ≤ 0.5%.
*Ester fraction contained 37% of triterpene alcohols.
†Ester fraction contained 44% of triterpene alcohols.

respectively (Table 3). Work on triterpenes composition is in progress.

It is well documented that both the quality and quantity of surface lipids are influenced by photoperiod [13, 14], water stress and drought [15], temperature [13, 17] and age [4, 7, 16–19]. As a time study seemed worthwhile for sorghum, we chose to compare the waxes from seedlings and mature plants of the SD-102 variety [1] presenting also the composition of the panicle wax whose data serves for comparison purposes.

Wax samples from seedlings and mature plants contained the same components (Table 4), nevertheless their relative proportions were quite different. In seedlings free alcohols were the largest class (45%), whereas they accounted only for 7 and 2% in mature leaf blades and sheaths, respectively [1]. As alcohols increased, free fatty acids, which were the dominant wax class from mature

Table 4. Chemical composition of epicuticular waxes from different parts of SD-102 sorghum variety

Components	%			
	S	P	B*	Sh*
Alkanes	9	7	1	1
Esters	21	4	19	4
Aldehydes	15	7	19	4
Alcohols	45	42	6	1
Acids	10	40	55	90

*Data from ref. [1].
S, Seedlings; P, panicles; B, blades; Sh, Sheaths.

plants, decreased to the value of 10%. SD-102 seedlings wax was also characterized by a large quantity of esters (21%) and aldehydes (15%), in this case resembling SD-102 mature leaf blades [1].

According to the generally accepted scheme for wax biosynthesis [20], the foregoing results can be explained assuming that, in mature plants of sorghum, wax synthesis has reached a stage where decarboxylation to alkanes and reduction processes giving rise to aldehydes and alcohols are not very active. Consequently, the elongated acyl chains accumulate to give rise to free fatty acids. In contrast, at the fourth–fifth leaf seedlings stage maximum deposition of wax has not yet been reached and wax components formed by a very efficient reductive system dominate. Similar results were found when waxes from whole corn seedlings and mature plants were compared [21]. Thus, free alcohols amounted to 63% of the total wax from corn seedlings, whereas they accounted only for 14% in the leaves of mature plants. Furthermore, while free fatty acids were present only in trace amounts on young leaves, they represented 14% of the wax from mature corn plants [21].

Table 5 shows the homologue distribution within each class of compounds of the wax from SD-102 sorghum seedlings compared with those of the panicle. Alkanes, acids and esters of seedlings exhibited the same homologue distribution as those from mature leaves. The only relevant difference was found in aldehyde and alcohol chain length distributions which showed C_{32} as the dominant chain (Table 5). Previous studies [7, 9, 18] reported that homologue composition changes on plant ageing and frequently an increase of chain length was observed in wax from older leaves. Thus, in contrast with our finding, a recent report on sorghum epicuticular wax showed that hydrocarbon chain length increased with plant age from 7 to 52 days [4]. However, our results seem well correlated with earlier observations on corn wax [21–23].

While alkanes, acids and esters of corn seedlings comprise relatively higher amounts of short chain homologues than in the same classes of compounds from mature leaves, in contrast, aldehydes and alcohols from young plants showed only a dominant C_{32} chain length [21]. Furthermore, in a study dealing with the composition of waxes from yellow and green segments of developing corn leaves [22], it was found that the chain length distribution of alkanes and esters from the yellow tissue were distinguishable from those of green tissue by prominent amounts of shorter chains. However, the homologue distribution of aldehydes and primary alcohols from the two types of tissue were similar, with C_{32} as the major homologue [22].

Waxes from SD-102 seedlings and panicles and from Alliance A and Martin A mature plants were also checked for the possible presence of *p*-hydroxybenzaldehyde in connection with recent studies [24–26] in which this substance is reported to be a free component of sorghum wax, or to arise from dhurrin on wax extraction [26]. *p*-Hydroxybenzaldehyde was not found as a component of any of the waxes isolated from the sorghum varieties we analysed.

EXPERIMENTAL

Plant material and growth conditions. Martin A and Alliance A sorghum plants grown in field conditions near Rome were harvested at the stage of full panicle exsertion and whole plants were used for wax recovery. SD-102 sorghum seedlings were collected at the fourth–fifth leaf stage of growth. Some SD-102 plants were grown to the tasseling stage at which time panicles were also extracted for epicuticular wax.

Isolation and analysis of waxes. Surface lipids were extracted

Table 5. Composition (%) of wax fractions from SD-102 sorghum seedlings (S) and panicles (P)

Chain length	Alkanes		Aldehydes		Alcohols		Acids		Esterified alcohols		Esterified acids	
	S	P	S	P	S	P	S	P	S*	P†	S	P
16							tr	—	—	—	6	—
18							1	—	5	tr	9	—
20					—	tr	tr	tr	7	2	49	1
22	—	tr			—	tr	1	tr	58	3	23	10
23	1	2			—	—	—	tr	—	—	—	—
24	tr	tr	tr	tr	—	4	4	1	4	8	13	17
25	2	5	—	—	—	tr	—	tr	—	—	—	—
26	1	1	3	2	1	43	3	2	9	7	tr	32
27	12	13	—	4	tr	tr	—	tr	—	—	—	—
28	2	3	19	41	23	43	63	61	14	39	tr	22
29	43	66	—	3	tr	tr	—	2	—	—	—	—
30	2	1	35	48	13	10	23	34	2	35	—	18
31	26	9	—	—	—	—	—	—	—	—		
32	1	tr	43	2	63	tr	5	—	1	6		
33	6	tr										
34	tr	—										
35	4	—										

—, Not detected; tr, trace, ≤ 0.5%.

*Ester fraction contained 16% of triterpene alcohols.

†Ester fraction contained 17% of unknown peaks.

using a 45–60 sec immersion of the aerial plant organs in $CHCl_3$. Evapn of the solvent on a rotary evaporator yielded the crude wax samples. Composition of the waxes was checked by TLC using CCl_4, $CHCl_3$ and $CHCl_3$ containing 1% EtOH as solvent systems. Detection of wax components was carried out as previously described [23]. Individual lipid classes were separated by gradient elution CC on silica gel 60/H (Merck). CCl_4 eluted in order *n*-alkanes, esters and aldehydes. $CHCl_3$ afforded alcohols and $CHCl_3$ containing 1% HOAc gave the free fatty acids. The fractions obtained were analysed for homologue content by GC on a dual FID instrument using glass columns (2 m × 4 mm) packed with 1% OV-1. Isothermal and temp prog chromatograms were run from 160–280° as required. Alkanes and aldehydes were analysed as such. Acetates and Me esters derivatives were prepared from alcohols and free fatty acids, respectively [23]. Esters were subjected to transesterification followed by acetylation and the combined acid and alcohol acetates analysed [23]. Composition of the intact esters was also determined by capillary GC as previously described [21]. Authentic samples of each class of compounds were used as ref. standards. IR and MS of individual wax classes were obtained as already published [5].

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