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Notes

A RAPID COLORIMETRIC METHOD FOR EPICUTICULAR WAX CONTENT OF SORGHUM LEAVES[1]

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ABSTRACT

Epicuticular wax deposition on sorghum [(*Sorghum bicolor*) L. Moench] leaf blades imparts drought resistance. Screening of a large number of sorghum genotypes for increased leaf epicuticular wax content, utilizing the available gravimetric method, is impractical. A more rapid colorimetric method was developed and evaluated against the current gravimetric method. The colorimetric method is based on the color change produced due to the reaction of wax with acidic $K_2Cr_2O_7$ reagent. Wax content determined by the colorimetric method was highly correlated (r = 0.984) with that determined by the gravimetric method. Leaf epicuticular wax content in 11 field-grown grain sorghum cultivars did not vary between growth stage #4 (tip of flag leaf showing) and #7 (soft dough), at which stages comparable wax readings were obtained among the four top leaf blades. A CK 60 'bloomless' (bmbm) genotype had only half of the epicuticular wax content of the CK 60 'normal' (BmBm) genotype. Ten cultivars, all of the BmBm genotype, significantly differed in epicuticular wax content, ranging from 1.14 to 1.99 (SE = 0.006) mg/dm² of leaf.

***Additional index words:* *Sorghum bicolor* (L.) Moench, Breeding methods, Selection, Drought resistance, Leaves.**

PRESENCE of the characteristic waxy bloom on sorghum [(*Sorghum bicolor*) L. Moench] leaf sheaths is recognized in most cultivated sorghums. It is controlled by one gene (1), with the recessive being 'bloomless.' The difference between 'normal' and 'bloomless' sorghum is apparent on the leaf sheath as well as the leaf blade (4). The 'normal' genotype has more epicuticular wax deposited on the leaf blade in the form of a thick amorphous layer, covered by scattered flakes of wax.

Excessive deposition of epicuticular wax in sorghum was found to increase leaf reflectance of visible and near infra-red radiation (4), decrease net radiation in the field, and decrease cuticular transpiration (3). Epicuticular wax is, therefore, an effective component of drought resistance (avoidance mechanism) in sorghum.

The extent of variation in leaf epicuticular wax content between various cultivated sorghums is unknown. Furthermore, any attempt to survey this variation or improve epicuticular wax content by selection will be limited by the available methodology. The current gravimetric method (7) is too slow for routine selection work.

This research was carried out in order to develop a faster and more efficient method for epicuticular wax analysis and to explore variability among sorghum genotypes in epicuticular wax content.

MATERIALS AND METHODS

Eleven sorghum [(*Sorghum bicolor*) L. Moench] cultivars were grown in the field at Bet Dagan, Israel, in 1975. These cultivars (Table 1) represent a range of cultivated sorghums. They have been described elsewhere (2). Two of these cultivars were near isogenic 'normal' and 'bloomless' Combine Kafir-60 (CK 60).

Planting was done under dryland conditions. Each cultivar was planted in eight rows, 1 m apart and 16 m in length. No rainfall occurred during the growing season, and plants grew on stored soil moisture.

Leaf blade samples were taken for wax analysis on four dates: stages #4 (tip of flag leaf showing) through #7 (soft dough) (10). At each date separate samples were taken from leaves 1 through 4 (leaf 1 = uppermost fully expanded leaf). Five samples were taken for each cultivar × date × leaf combination. Each sample was subdivided into two subsamples which were used for gravimetric and colorimetric analysis.

Gravimetric analysis. The procedure followed that of Silva Fernandes et al. (1). Four leaf blades were immersed, one at a time, each for 15 sec, in 100 ml redistilled chloroform. The extract was filtered and evaporated in vacuo at 35 C. The residue was weighed after additional drying for 24 hours at room temperature. The amount of wax was calculated against leaf area (both leaf surfaces) of sample, as determined by linear measurements (9).

Colorimetric analysis. The development of the method was based on the color change produced due to the reaction of wax with acidic $K_2Cr_2O_7$ (5). The reagent was prepared by mixing 40 ml deionized water with 20 g powdered potassium bichromate. The resulting slurry was mixed vigorously with 1 liter concentrated sulfuric acid and heated (below boiling) until a clear solution was obtained.

The individual sample consisted of 10 sorghum leaf discs, having a total area (both surfaces) of 30.8 cm². Each sample was immersed in 15 ml redistilled chloroform for 15 sec. The extract was filtered and evaporated on a boiling water bath, until the smell of chloroform could not be detected. After adding 5 ml of reagent, samples were placed in boiling water for 30 min. After cooling, 12 ml of deionized water was added. Several minutes were allowed for color development and cooling and then the optical density of the sample was read at 590 nm.

Standard wax solutions were prepared from carnauba wax [found to be very similar to sorghum grain wax (6)], carbowax-3000 (polyethylene glycol-3000) and sorghum wax collected from leaf sheaths of test plants. Waxes were dissolved in redistilled

Table 1. Ranking of 11 sorghum cultivars in leaf blade epicuticular wax content as determined by colorimetric and gravimetric methods of analysis.

Colorimetric analysis		Gravimetric analysis	
Cultivar	mg dm^{-2} ± SE	Cultivar	mg dm^{-2} ± SE
KS 9	1.99 ± 0.09*	KS 9	1.54 ± 0.16
Hegari	1.89 ± 0.26	Hegari	1.34 ± 0.19
Feterita	1.75 ± 0.23	Feterita	1.27 ± 0.25
Kafir	1.61 ± 0.18	Shallu	1.14 ± 0.11
CK 60	1.41 ± 0.12	Kafir	1.11 ± 0.13
Shallu	1.38 ± 0.16	CK 60	1.05 ± 0.12
RS 610	1.35 ± 0.10	RS 610	1.02 ± 0.12
Milo	1.26 ± 0.22	Milo	0.93 ± 0.21
1136-1	1.20 ± 0.11	Durra	0.89 ± 0.09
Durra	1.11 ± 0.11	1136-1	0.76 ± 0.14
CK 60 bloomless	0.58 ± 0.07	CK 60 bloomless	0.25 ± 0.04

* Cultivars joined by the same line are not significantly different (at P = 0.05) according to Duncan's multiple range test

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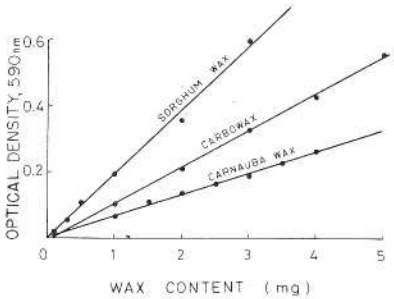

Fig. 1. Standard curves for sorghum leaf sheath wax, carbowax-3000 and carnauba wax.

chloroform and 15 ml aliquots containing a range of concentrations were prepared. These aliquots were carried through the above analytic procedures.

The resulting standard curves, for all waxes used, were linear throughout the concentrations used (Fig. 1). Sorghum leaf sheath wax was used as the standard in the remainder of this study.

As a further test of the method, "Cut-rite" brand wax paper was cut into pieces of various sizes and treated as leaf samples according to the above procedure. The resulting relationship between absorbance at 590 nm and wax paper area extracted was linear ($r = 0.992$).

RESULTS AND DISCUSSION

The gravimetric method required larger leaf samples, a larger volume of chloroform and required a much longer period of time to evaporate the chloroform extract, compared with the colorimetric method. The number of samples which could be processed per day was at least 10 times greater with the colorimetric method.

No significant differences in epicuticular wax content by the gravimetric or colorimetric method were revealed between leaf samples taken at various plant development stages. Similarly, no differences were found between the various leaves sampled during one period within a cultivar. Comparative evaluation of sorghum cultivars for epicuticular wax content may therefore be performed between growth stages #4 (tip of flag leaf showing) and #7 (soft dough) using any of the top four fully expanded leaves.

Results were pooled and analyzed for the various cultivars (Table 1). Conditions of water stress, as may have developed in this dryland study, were found to promote epicuticular wax content (8). Furthermore, it is suspected that under conditions of intensive rainfall or sprinkler irrigation, some of the loose flakes of epicuticular wax (4) may wash off the leaf. In this study, no rainfall occurred during the growing season. Thus, the levels of epicuticular wax observed in this study may be higher than levels found with the same cultivars grown in a more humid environment.

Very significant differences were revealed in epicuticular wax content among cultivars. Ranking of cultivars, in this respect, was very close for both methods of analysis. The two methods compared very well, with a correlation coefficient of 0.984. Two of the cultivars tested were nearly isogenic lines of CK 60, differing in waxy bloom formation over the leaf sheaths. Transition from 'normal' (BmBm) to 'bloomless' (bmbm) genotype caused a reduction to nearly one-half of the leaf blade epicuticular wax content (from 1.26 to 0.58 mg/dm^2).

Judging by the appearance of waxy bloom on the leaf sheaths, all the entries in this test, except 'bloomless', were of the 'normal' genotype. Appreciable and significant variation in leaf blade epicuticular wax content was found among the various 'normal' genotypes. Additional genetic factors may therefore be involved in the control of leaf blade epicuticular wax content. This variation should allow the genetic improvement of epicuticular wax content as a component of drought resistance in cultivated sorghums.

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