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Flavan-4-ol Concentration in Leaf Tissues of Grain Mold Susceptible and Resistant Sorghum Plants at Different Stages of Leaf Development†

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Fully opened youngest leaves from sorghum [*Sorghum bicolor* (L.) Moench] germ plasm accessions, exhibiting both resistance and susceptibility to the grain mold complex, were collected at 56, 63, and 70 days after emergence (DAE) of plants. They were extracted in methanol followed by acidified methanol and analyzed for flavan-4-ols. Methanol and acidified methanol extracts of leaves of mold-resistant accessions contained at least 3-fold higher concentrations of flavan-4-ols than susceptible accessions at 56, 63, and 70 DAE. The concentration of flavan-4-ols was monitored in the flag leaves of mold-resistant accessions that had no testa at 77, 84, 91, and 98 DAE, and it decreased sharply at or after 77 DAE. The estimation of concentration of flavan-4-ols in sorghum leaves, therefore offers scope for screening sorghum accessions for their grain mold resistance.

INTRODUCTION

Bate-Smith (1969) reported the presence of luteoforol in the seed coat of sorghum cultivars which was characterized by the production of a blue-rose color when treated with mineral acid, but luteoforol was not detected in the fully developed leaves of sorghum. Watterson and Butler (1983) reported the presence of an unusual leucoanthocyanidin in the leaves of 12 lines of sorghum [*Sorghum bicolor* (L.) Moench] which they identified as apiforol, but none of the leaves contained proanthocyanidin. Haskins and Gorz (1986) studied the inheritance of leucoanthocyanidin in sorghum leaves, using two forage sorghums, and reported that a single allelic pair was primarily responsible for the control of leucoanthocyanidin. Among the cereals, sorghum polyphenols have been widely investigated, and a recent review (Butler, 1989) describes in detail the chemistry and the role of sorghum polyphenols.

Bandyopadhyay et al. (1988) screened 7132 accessions of sorghum germ plasm for their resistance to grain mold and identified 156 resistant accessions on the basis of their threshed grain mold rating (TGMR) scores. However, identification of biochemical compounds that are associated with grain mold resistance would facilitate the screening of sorghum germ plasm accessions for this purpose. In an experiment conducted in a greenhouse in the United States, Jambunathan et al. (1986) reported that leaves of mold-resistant sorghum cultivars have a much higher concentration of flavan-4-ols than mold-susceptible cultivars. The objective of the present study was to estimate the concentration of flavan-4-ols in the leaves of grain mold susceptible and resistant sorghum plants that were grown under semiarid tropical field conditions. We also monitored the changes in flavan-4-ol concentration in the leaves of these plants at different stages of leaf development.

EXPERIMENTAL PROCEDURES

Ten sorghum accessions were grown in a randomized complete block design with three replications during the 1985 rainy season on a Vertisol at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The resistance and susceptibility of these 10 sorghum accessions to grain mold were identified by screening a world collection of sorghum germ plasm accessions (Bandyopadhyay et al., 1988). Details of the sorghum accessions, planting plan, and other cultural practices were described earlier (Jambunathan et al., 1990).

Collection of Leaf Tissues. Fully expanded and youngest leaves were harvested at 56, 63, and 70 days after emergence (DAE). This represented flag leaves in all the accessions except in the case of three late-maturing accessions (more than 70 days for 50% flowering stage), where the leaves below the flag leaves were collected. From each replication, three plants were chosen randomly from the six rows that were grown. The period of collection was based on our earlier observations (Jambunathan et al., 1986). To monitor the concentration of flavan-4-ols in the three mold-resistant sorghum accessions beyond 70 DAE, flavan-4-ols were estimated in the flag leaves that were collected at 77, 84, 91, and 98 DAE as well. The leaves collected from one replication were wiped to remove foreign matter, and midribs were removed. Leaf blades were cut into small pieces (approximately 2–5 mm^2 area) by using a pair of scissors. The cut leaves were mixed well and assumed to represent one sorghum accession from one replicate of the randomized complete block design. A similar operation was carried out with all the replications. About 1.5 g of fresh leaves was accurately weighed into a 30-mL polyethylene centrifuge tube (Sorvall), and 10 mL of methanol (Me) was added; the mixture was homogenized in a homogenizer (Kinematica GmbH, Kriens-Luzean, Switzerland) using the probe PT 20 ST at setting 6 for 30 s. The homogenate was centrifuged in a table-top Sorvall centrifuge (Sorvall Superspeed Centrifuge SS-3 automatic) at 3000*g* for 5 min, and the supernatant was saved. To the residue was added 15 mL of Me, and the suspension was homogenized and centrifuged as described above. The two Me extracts were pooled for analysis. To the residue was added 10 mL of methanol containing 1% (v/v) concentrated HCl (H^+/Me), and the suspension was homogenized for 30 s and centrifuged. The supernatant was saved, and the residue was re-extracted similarly with 15 mL of H^+/Me. The two supernatants of H^+/Me extracts were pooled together for analysis. The moisture content in the fresh leaf tissue was determined by drying a subsample at 110 °C for 16 h.

Flavan-4-ol concentration in Me and H^+/Me extract was estimated by using poly(vinylpyrrolidone) (PVP) according to the method of Watterson and Butler (1983). The method in brief is as follows: To 0.5 g of PVP in a screw-top test tube was added 0.5 mL of leaf extract, and the mixture was vortexed and allowed to stand for 5 min. To remove chlorophyll, 10 mL of Me was added, and the mixture was vortexed and centrifu[illegible] speed in a table-top centrifuge. The supernantant was d

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Table I. Description of Sorghum Accessions, Days to 50% Flowering, Grain Color, and Munsell Color Coding

group[a]	accession	days to 50% flowering	grain color	
CRT+ (colored, resistant, with testa)	IS 2825	51	reddish brown	2.5 YR/3/3
	IS 9363	61	reddish brown	2.5 YR/4/4
	IS 18759	52	reddish brown	2.5 YR/3/4
CST- (colored, susceptible, without testa)	IS 402	49	reddish yellow	5 YR/6/8
	IS 417	55	reddish yellow	5 YR/6/8
CRT- (colored, resistant, without testa)	IS 14375	72	red	2.5 YR/4/8
	IS 14380	71	red	2.5 YR/5/6
	IS 14384	71	red	2.5 YR/4/8
WST+ (white, susceptible, with testa)	IS 2433	54	white	10 YR/8/1
	IS 2516	50	white	10 YR/8/1

[a] Based on phenotypic grain color, reaction to mold, and presence or absence of testa. [b] Munsell color coding denotes the hue (first value), olor (second value), and chroma (third value), respectively.

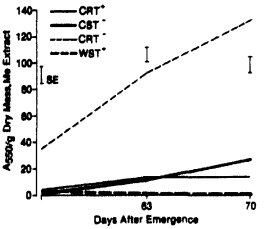

Figure 1. Concentration of flavan-4-ols in methanol (Me) extracts of leaves of mold-resistant and mold-susceptible sorghum accessions at different days after emergence of the plant, ICRISAT Center, rainy season 1985. Each value represents the mean of three field replicates, and each analysis was carried out at least in duplicate. SE, standard error representing means of groups.

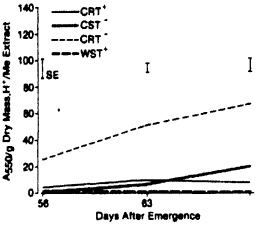

Figure 2. Concentration of flavan-4-ols in acidified methanol (H^+/Me) extracts of leaves of mold-resistant and mold-susceptible sorghum accessions at different days after emergence of the plant ICRISAT Center, rainy season 1985. Each value represents the mean of three field replicates, and each analysis was carried out at least in duplicate. SE, standard error representing means of groups.

and the PVP was washed similarly two more times. Then 7 mL of 30% HCl/70% water-saturated butanol (v/v) was added to the PVP residue, and the mixture was slowly rotated on a test tube mixer (Stuart Tube Rotator TR-2) for 1 h at room temperature. After centrifugation, the absorbance of the supernatant was measured at 550 nm in a Spectronic 21 spectrophotometer. To prevent the reagent fumes from escaping into the laboratory, this operation was carried out inside a fume hood. The absorbance of each sample was expressed as A_{550} g^{-1} of leaf tissue on a dry mass basis. Analysis was replicated, and each analysis was conducted in duplicate. The mean values (A_{550} g^{-1} of leaf tissue) obtained for the three field replications of each accession are reported on a dry mass basis.

RESULTS AND DISCUSSION

The description of grains of these 10 sorghum accessions for their grain color, reactions to grain mold, Munsell (*Munsell Soil Color Charts*, 1973) color coding, and grouping into CRT+, CST-, CRT-, and WST+ based on the presence or absence of testa is given in Table I.

The mean values of flavan-4-ol concentration in Me extract of leaves of sorghum accession groups at different stages of growth are shown in Figure 1. The concentration of flavan-4-ols in the groups CRT- and CST- showed a sharp increase between 56 and 70 DAE. The value of the CRT- at 56 DAE was the highest (35.3) among all the groups and increased more than 3-fold (131.6) at 70 DAE as compared with other groups. In the case of CRT+ mold-resistant sorghums, the grains of which have testa and tannin, the difference between the 56 and 70 DAE values was negligible but the value at 70 DAE was much less (10.8) as compared with that of the CRT- group. The magnitude of increase between the 56 and 70 DAE values of the CST- group was the highest among all the groups due to the fact that, at 56 DAE, the value was extremely low (0.6) as compared with the 70 DAE value (27.1). Among the four groups, WST+ mold-susceptible accessions, the grains of which have testa, exhibited extremely low values both at 56 DAE (1.4) and at 70 DAE (1.0).

The mean values of flavan-4-ol concentration in acidified methanol extracts of leaves of different groups are shown in Figure 2. In general, they followed the pattern shown in Figure 1, although the absolute values for all the groups were much lower than that of methanol extracts.

To estimate the magnitude of differences in the flavan-4-ol concentration, all the values were expressed as ratios in relation to the CRT- group (Table II). In the case of methanol extract, at all stages, the concentration of flavan-4-ols in the CRT- group was at least 3-fold higher than that in the CRT+ group. Also, the ratio CRT-/CRT+

Table II. Concentration of Flavan-4-ols in Methanol and Acidified Methanol Extracts of Leaves of the CRT- Group Expressed as Ratios in Comparison with Other Groups[a]

	methanol			H^+/methanol		
ratio	56 DAE	63 DAE	70 DAE	56 DAE	63 DAE	70 DAE
CRT-/CRT+	3.0[a]	6.6[a]	12.2[a]	3.7[a]	5.1[a]	8.1[a]
CRT-/CST-	59.9[b]	8.2[a]	4.9[a]	72.6[b]	8.0[a]	3.4[a]
CRT-/WST+	25.6[c]	75.1[b]	131.6[b]	30.6[c]	70.1[b]	61.4[b]

[a] Based on phenotypic grain color, reaction to grain mold, and presence or absence of testa. The ratios having the same superscript and shown under each DAE are not significantly different from each other (Waller–Duncan K ratio test).

Table III. Concentration of Flavan-4-ols in the Mold-Resistant Sorghum (CRT⁻) Leaves at Different Stages of Plant Growth

days after emergence[b]	flavan-4-ols (A_{480} g^{-1} of dry mass)[a]			
	solvent	IS 14375	IS 14380	IS 14384
56	Me	50.5 ± 2.12	25.6 ± 6.96	29.9 ± 2.16
	H⁺/Me	35.2 ± 7.18	18.8 ± 2.44	22.2 ± 5.13
63	Me	118.7 ± 14.34	62.3 ± 16.31	22.2 ± 18.55
	H⁺/Me	67.3 ± 7.45	39.8 ± 13.22	46.3 ± 4.01
70	Me	161.4 ± 17.86	107.2 ± 16.23	126.1 ± 11.33
	H⁺/Me	76.6 ± 2.71	56.0 ± 9.65	69.8 ± 13.87
77	Me	365.6 ± 20.22	215.1 ± 2.83	264.6 ± 5.66
	H⁺/Me	38.5 ± 8.02	43.5 ± 3.09	29.8 ± 1.19
84	Me	101.4 ± 2.81	77.5 ± 14.03	145.4 ± 10.43
	H⁺/Me	39.5 ± 0.81	35.5 ± 2.58	47.9 ± 6.96
91	Me	66.2 ± 10.7	58.3 ± 1.49	81.4 ± 10.01
	H⁺/Me	28.1 ± 1.04	22.8 ± 2.87	27.0 ± 2.77
98	Me	54.0 ± 13.99	58.8 ± 4.59	86.9 ± 14.62
	H⁺/Me	21.3 ± 2.86	23.1 ± 2.21	30.7 ± 3.29

[a] Means of three replicates. [b] 56–70 DAE, youngest fully opened leaves. 77–98 DAE, flag leaves.

showed a 4-fold increase between 56 and 70 DAE. This was due to a sharp increase in concentration of flavan-4-ols in the CRT⁻ group, while there was a negligible variation in the CRT⁺ group during that period. The CRT⁻/CST⁻ ratio was very high (59.9) at 56 DAE due to a very low value of the CST⁻ as compared with CRT⁻, as shown in Figure 1. However, the ratios at 63 and 70 DAE were reduced to 8.2 and 4.9, respectively, because of a sharp increase in the levels of flavan-4-ols in the CST⁻ (11.2 at 63 DAE and 27.1 at 70 DAE, Figure 1), while the increase in the CRT⁻ group was relatively small during the same period (91.6 at 63 DAE and 131.6 at 70 DAE). The reason for the increase in the ratio of CRT⁻/WST⁺ from 25.6 at 56 DAE to 131.6 at 70 DAE was a rapid increase of flavan-4-ols in the CRT⁻ (35.3–131.6) and a decrease in the case of WST⁺ (1.4 to 1.0) during the same period. This could also be due to the cumulative effect caused by the three variables that are evident between these two groups.

The CRT⁻/CRT⁺ ratio of the acidified methanol extract showed more than a 2-fold increase from 56 to 70 DAE, as the increase in the concentration of flavan-4-ols in the CRT⁻ group was higher (from 25.4 to 67.5) than in the CRT⁺ group (from 6.8 to 8.3) during that period. The ratio of CRT⁻/CST⁻ decreased drastically from 72.6 to 3.4 as there was a sharp increase in CST⁻ values from 0.4 to 20.0 (50-fold increase) in comparison to the CRT⁻ values during the same period. The ratio CRT⁻/WST⁺ increased from 30.6 to 61.4 due to a small increase in the WST⁺ values (from 0.8 to 1.1) and a larger increase (from 25.4 to 67.5) in the CRT⁻ values between 56 and 70 DAE.

We used the Waller–Duncan K ratio test (Shane, 1990) to find out if the ratios obtained at 56, 63, and 70 DAE were different from each other. The data obtained for Me and H⁺/Me extracts differed significantly from each other at 56 DAE (Table II). The ratio CRT⁻/WST⁺ was also significantly different from others at all three stages in both Me and H⁺/Me extracts.

The concentration of flavan-4-ols in the three mold-resistant accessions IS 14375, IS 14380, and IS 14384 was estimated at 56, 63, 70, 77, 84, 91 and 98 DAE. The concentration of flavan-4-ols in Me extract was highest at 77 DAE, and in the case of H⁺/Me extract, it was highest at 70 DAE in these mold-resistant accessions (Table III). Between 77 and 98 DAE, the mean flavan-4-ol value in Me extract of three mold-resistant accessions showed a drastic reduction, and at 98 DAE, it was only 24% of the corresponding value at 77 DAE. Similarly, in the case of H⁺/Me extract, the mean value at 98 DAE was only 37% of the value at 70 DAE.

CONCLUSIONS

The data obtained from sorghum crops grown under tropical conditions during the rainy season showed that leaves of mold-resistant accessions had a much higher concentration of flavan-4-ols than mold-susceptible accessions. This observation was recorded both in methanol and in acidified methanol extracts of leaves. This confirmed the observation reported from an earlier study (Jambunathan et al., 1986). In the mold-resistant accessions, the concentration of flavan-4-ols in the leaves increased continuously from 56 to 77 DAE but declined rather steeply after 77 days. This indicated that the highest concentration of flavan-4-ols in the leaf was found around the flowering period (77 DAE) for the CRT⁻ group. The role of grain color or testa can be better explained only when data from groups such as WST⁻, WRT⁻, and CST⁺ become available. However, the data from the four sorghum groups and the three resistant accessions that did not have testa suggest the higher concentrations of flavan-4-ols in leaves may give an indication of grain mold resistance. This procedure, therefore, can be used as a preliminary screening method to test large numbers of germ plasm accessions and breeding lines, especially photoperiod-sensitive lines for which there is no screening methodology developed at present.

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