Flavan-4-ols Concentration in Mold-Susceptible and Mold-Resistant Sorghum at Different Stages of Grain Development

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Sorghum [*Sorghum bicolor* (L.) Moench] accessions exhibiting contrasting reactions to the grain mold complex were grown in two consecutive rainy seasons at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India. Methanol and acidified methanol extracts of grains harvested at different grain development stages were analyzed for flavan-4-ols. The concentrations of flavan-4-ols in mold-resistant grains were at least 2-fold higher than in mold-susceptible grains in both extracts at or after 30 days of flowering. Concentration of flavan-4-ols in mature, grain sorghums could, therefore, be an indicator of their potential resistance or susceptibility to grain mold, and this method could be an important tool in screening sorghum cultivars for such characteristics.

Polyphenols are secondary plant metabolites, and their presence has been reported in several plant species (Haslam, 1981). Among cereals, sorghum polyphenols have been widely investigated and a recent review (Butler, 1988) describes in detail the chemistry and the role of sorghum polyphenols. Phenolic compounds in sorghum caryopses are reported to improve resistance to insects, fungi, and other pathogens (Dreyer et al., 1981; Butler, 1988). However, the physiological function of these compounds has not yet been fully understood although attempts have been made to elucidate their role (Subramanian et al., 1983; Woodhead, 1981). Harris and Burns (1973) reported that sorghum grain tannin content was significantly and negatively correlated with preharvest seed-molding indices. We have reported earlier (Jambunathan et al., 1986) that mature grains of mold-resistant sorghum cultivars have much higher concentration of flavan-4-ols than mold-susceptible cultivars. However, this experiment was conducted in a greenhouse in the United States. The objective of the present study was to confirm our earlier observation by estimating flavan-4-ols concentration in developing and mature grains of sorghum cultivars grown under semiarid tropical field conditions.

EXPERIMENTAL SECTION

Agronomy. Ten sorghum accessions were grown during the 1984 and 1985 rainy seasons on a Vertisol at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. They were planted in a randomized complete block design with three replicates. Each plot was comprised of six 4-m-long rows. A basal fertilizer dose of 56 kg of N and 56 kg of P_2O_5 ha^{-1} was applied. Seeds were sown in June before the onset of the southwest monsoon on ridges spaced 0.75 m apart. Seedlings were thinned 14 days after emergence to maintain five plants per meter row length. The crop was top-dressed with 47 kg of N ha^{-1}, 21 days after emergence. To promote grain mold, wet and humid conditions were created from flowering to 14 days beyond grain maturity by providing overhead sprinkler irrigation for 1 h twice daily (mornings and evenings) on all rain-free days (Bandyopadhyay and Mughogho, 1988).

Grain Samples. Developing sorghum panicles were tagged at 50% flowering (Bandyopadhyay et al., 1988) and were harvested at 10, 20, 30, 40, and 50 days after flowering (DAF) in 1984 while in 1985 they were tagged similarly and harvested at 14, 21, 30, 40, and 50 DAF. At each time of sampling, three panicles from the same replicate were collected and freeze-dried soon thereafter. Grains from freeze-dried panicles were removed, and about 5 g was ground in a Udy cyclone mill (U.D. Corp., Boulder, CO) to pass through a 0.4-mm screen. The sorghum meal was defatted with *n*-hexane and air-dried, and a 250-mg sample was extracted with 5 mL of methanol (Me) for 30 min in a screw-capped test tube. After centrifugation, the residue was reextracted with Me (5 mL) and the two Me extracts were combined for analysis. The residue was further extracted twice with methanol (5 mL) containing 1% (v/v) concentrated HCl (H^+/Me), and these two extracts were pooled together for analysis of flavan-4-ols in the Me and H^+/Me extracts according to Butler (1982). Analysis of samples from each replication was carried out in duplicate, and mean values are reported on a dry weight basis.

Reaction to Grain Mold. The resistance and susceptibility of the sorghum accessions to grain mold were confirmed by evaluating them under field conditions for at least the previous 2 years, and they were grouped on the basis of grain color and their reactions to mold. For visual mold evaluation, 10 panicles from each plot were harvested 14 days after physiological maturity (black layer formation; 40 DAF) and threshed individually. About 35 g of grain samples of each panicle was spread uniformly in 90-mm-diameter Petri dishes and scored visually for threshed grain mold rating (TGMR) on a 1–5 scale, where 1 denoted no visible mold and 5 denoted severe mold with more than 50% of the grain surface molded (Bandyopadhyay et al., 1988). TGMR values of 10 panicles of each entry in each replications were averaged to obtain the ratings for each accession.

Ergosterol was determined in mature grains according to Naewbanij et al. (1984), using a Shimadzu LC-6A high-performance liquid chromatograph. Ergosterol (Sigma) was used as the reference standard. Three replicates of each sample were analyzed, and mean values are reported.

RESULTS AND DISCUSSION

The 10 accessions selected were identified because of their varied reactions to grain mold caused by a complex range of unspecialized fungi including *Fusarium moniliforme* (Sheld.), *Curvularia lunata* (Wakker) Boedijn, and *Phoma sorghina* (Sacc.) Boerma et al. Data in Table I show the identities of the 10 accessions, their grain colors, and the corresponding color codings according to the reference Munsell color chart (*Munsell Soil Color Charts*, 1973). On the basis of color and for the convenience of referring to these sorghum accessions in the text, we have divided them into four groups according to white (W) or colored (C) pericarp color, resistance (R) or susceptibility (S) to grain mold, and presence (T^+) or absence (T^-) of testa (Table I). Values for 100-grain mass and mold-resistance assessed by TGMR and ergosterol methods in 1984 and 1985 are shown in Table II. The 100-grain mass of the CRT^- group was less than 2 g in both 1984 and 1985 while group WST^+ showed the highest grain mass for both years. The mean TGMR values showed wide variation from 1.8 to 5.0 among the 10 acces-

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Table I. Description of Sorghum Accessions, Grain Color, and Munsell Color Coding

group[a]	accession	grain color	Munsell color coding[b]
CRT^+ (colored, resistant, with testa)	IS 2825	reddish brown	2.5 YR/3/3
	IS 9353	reddish brown	2.5 YR/4/4
	IS 18759	reddish brown	2.5 YR/3/4
CST^- (colored, susceptible, without testa)	IS 402	reddish yellow	5 YR/6/8
	IS 417	reddish yellow	5 YR/6/8
CRT^- (colored, resistant, without testa)	IS 14375	red	2.5 YR/4/8
	IS 14380	red	2.5 YR/5/6
	IS 14384	red	2.5 YR/4/8
WST^+ (white, susceptible, with testa)	IS 2433	white	10 YR/8/1
	IS 2516	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 number for the color, chroma, and hue, respectively.

sions in 1984 and from 1.6 to 5.0 in 1985. The values of resistant lines were 2 or less, while the susceptible lines had values of 5 or close to 5.

Ergosterol is considered to be the predominant sterol component of nearly all fungi, and its concentration has been reported to correlate well with fungal invasion in sorghum, wheat, and corn (Seitz et al., 1977). In 1984, all the susceptible accessions contained more than 20 $\mu g \cdot g^{-1}$ of ergosterol while resistant ones had values of 10 $\mu g \cdot g^{-1}$ or lower except in the case of IS 2825, which had 11 $\mu g \cdot g^{-1}$. In 1985, the susceptible accessions had more than 13 $\mu g \cdot g^{-1}$ while resistant accessions had less than 7 $\mu g \cdot g^{-1}$ of ergosterol. The correlation coefficient between TGMR and ergosterol concentration for the data obtained in 1984 and 1985 was positive and significant ($r = 0.85$, $P < 0.01$, $n = 20$), indicating agreement between TGMR and ergosterol values.

The accumulation of flavan-4-ols in Me extracts of grains at different stages of development in mold-resistant and mold-susceptible accessions in 1984 and 1985 is shown in Figures 1 and 2. In 1984, mean flavan-4-ols values obtained at different maturity stages for the four groups of accessions were distinctly different from each other (Figure 1). However, there was a sharp decline in flavan-4-ols values in all the groups except in WST^+ between 20 and 40 DAF while a slight decrease in values was observed thereafter until 50 DAF.

In 1985, the pattern obtained was not as clearly defined as that of 1984 (Figure 2). The mold-susceptible group CST^- had a very high initial value (27.6) but later showed a 76% decrease at 30 DAF. A similar but lower magnitude of reduction (51%) in flavan-4-ols value was obtained for the CRT^- group between 14 and 21 DAF. However, the flavan-4-ols value of the CRT^- group increased after this stage while other groups exhibited either a reduction or relatively small increase in their values. It was noticed that, in all the groups, the values at 30 DAF were lower than those obtained at 14 DAF and this observation was similar to the data obtained in 1984.

The changes in the concentration of flavan-4-ols in H^+/Me extracts of sorghum at different stages of maturation in 1984 and 1985 are shown in Figures 3 and 4. Acidified methanol extracts different phenolic compounds from group II sorghum grains than methanol by itself, though the cause of these differences is not known. There was a sharp decrease in flavan-4-ols values in all groups of accessions except WST^+ between 10 and 40 DAF in 1984 and between 14 and 40 DAF in 1985. The percentage decrease in values from 10 to 40 DAF for samples in all the groups obtained in 1984 was as follows, except in the case of WST^+ where an increase of 43% was observed: CRT^+, 76%; CST^-, 89%; CRT^-, 86%. In samples obtained in 1985, the percentage decrease in values from 14 to 40 DAF was as follows: CRT^+, 64%; CST^-, 88%; CRT^-, 80%; WST^+, 43%. The values did not show any major change in any of the groups after 40 DAF in both years. Interestingly, it was observed that, until 40 DAF, the CRT^- group had higher values than the CRT^+ group in both 1984 and 1985.

The correlation coefficient between flavan-4-ols levels in Me extracts and ergosterol concentration for the combined data obtained on mature grains in 1984 and 1985 was negative and significant ($r = -0.66$, $P < 0.01$, $n = 20$). The correlation between flavan-4-ols in H^+/Me extracts and ergosterol concentration for 1984 and 1985 was also negative and significant ($r = -0.72$, $P < 0.01$, $n = 20$). Total flavan-4-ols in Me and H^+/Me extracts also exhibited a negative and significant correlation ($r = -0.70$, $P < 0.01$, $n = 20$) with ergosterol data obtained for the mature grain samples in 1984 and 1985. All these data indicated that, in sorghum samples, a higher concentration of flavan-4-ols is associated with lower fungal mass.

The reasons for the sharp decline between the initial values and the values at 40 DAF are not clear at present. Flavan-4-ols may be an intermediate in the synthesis of other polyphenolic compounds or may be translocated to other remaining panicle parts or to the glumes not analyzed in the present study.

To estimate the differences in the magnitude of concentrations of flavan-4-ols in the CRT^- group in relation to other groups at different times of sampling, the flavan-4-ols values were expressed as ratios. Flavan-4-ols values of Me extracts obtained in 1984 and 1985 and expressed as ratios are shown in Table III. The CRT^-/CRT^+ ratios in 1984 were less than 1 throughout the maturity period. This indicated that flavan-4-ols values of the CRT^+ group were always higher than those of the CRT^- group. This is also evident from Figure 1. This can be expected as the accessions of the CRT^+ group generally have tannin in the testa, which may produce materials that test positive in the assay for flavan-4-ols (Butler, 1988). The CRT^-/CST^- ratios were close to 1 at 10 and 20 DAF. At or after 30 DAF, the values were more than 2 until maturity, indicating that mold-resistant sorghum accessions had more than twice the amount of flavan-4-ols as compared to mold-susceptible sorghum accessions. The ratios of CRT^-/WST^+ showed very high values in 1984 for the various grain development stages because the values of WST^+ were almost negligible at all stages of maturity.

The values of CRT^-/CRT^+ ratios in 1985 were similar to those in 1984 in that they were less than 1 except at early grain development stages. While flavan-4-ols could be associated with mold resistance in groups without testa (CRT^-), both tannins and flavan-4-ols may be imparting mold-resistant characteristics in the case of sorghum accessions with testa (CRT^+), and similar observations have been reported (Harris and Burns, 1973; Nicholson et al., 1987). We do not have any experimental method to separate the effect of tannins and flavan-4-ols on grain mold susceptibility or resistance. Therefore, at this point we can only attribute the resistance of the CRT^+ group to the presence of both flavan-4-ols and tannins until confirmation using isogenic lines with and without the presence of testa. As in 1984, the CRT^-/CST^- values were lower in the initial stages of maturity. However, at or after 30 DAF, the ratios were above 2, indicating that mold-resistant accessions without testa (CRT^-) had at

Table II. Sorghum Accessions and Their Hundred-Grain Mass, Threshed Grain Mold Ratings (TGMR), and Ergosterol Concentrations

group[a]	accession	100-grain mass,[b] g		TGMR[c]		ergosterol,[d] $\mu g \cdot g^{-1}$	
		1984	1985	1984	1985	1984	1985
CRT^+	IS 2825	2.5 ± 0.22	2.7 ± 0.21	2.0 ± 0	2.0 ± 0	11.0 ± 0.17	2.3 ± 0.35
	IS 9353	2.4 ± 0.23	2.4 ± 0.04	2.0 ± 0	1.9 ± 0.03	6.5 ± 0.51	3.2 ± 0.41
	IS 18759	2.6 ± 0.32	2.7 ± 0.08	2.0 ± 0.03	2.0 ± 0.03	7.2 ± 0.62	3.5 ± 0.33
CST^-	IS 402	3.1 ± 0.19	2.9 ± 0.20	4.8 ± 0.07	4.9 ± 0.03	21.5 ± 1.62	14.3 ± 1.51
	IS 417	3.2 ± 0.20	3.2 ± 0.22	4.9 ± 0.03	4.7 ± 0.09	34.5 ± 2.71	13.7 ± 2.00
CRT^-	IS 14375	1.7 ± 0.02	1.9 ± 0.04	1.8 ± 0.07	1.6 ± 0.13	10.0 ± 0.04	3.4 ± 0.95
	IS 14380	1.7 ± 0.08	1.9 ± 0.11	1.9 ± 0.03	2.0 ± 0.00	8.7 ± 0.02	6.1 ± 1.02
	IS 14384	1.8 ± 0.05	1.9 ± 0.16	2.0 ± 0.05	1.7 ± 0.09	5.3 ± 0.02	2.6 ± 0.78
WST^+	IS 2433	3.2 ± 0.21	3.4 ± 0.31	5.0 ± 0	5.0 ± 0	25.8 ± 2.19	24.8 ± 2.66
	IS 2516	4.8 ± 0.48	5.1 ± 0.38	5.0 ± 0	5.0 ± 0	28.1 ± 3.13	13.9 ± 3.33

[a] As in Table I. [b] Means ± SE of six determinations including field replicates. [c] 1 = no mold, 5 = more than 50% of grain surface molded; means ± SE of 30 observations. [d] Means ± SE of three replications obtained on mature samples.

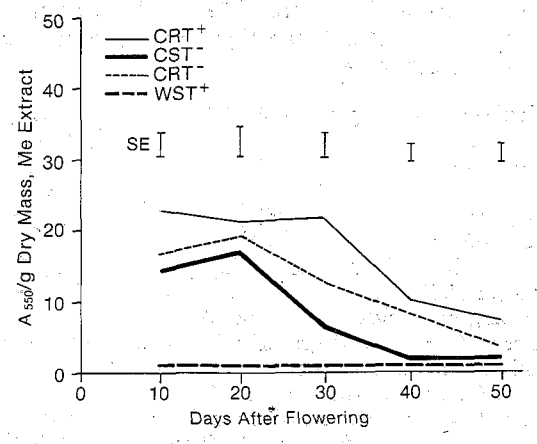

Figure 1. Concentrations of flavan-4-ols in methanol extracts of developing grains of mold-resistant and -susceptible sorghum accessions, ICRISAT Center, rainy season 1984. Each value represents the mean of three field replicates, and each analysis was carried out at least in duplicate. SE = standard error representing means of groups.

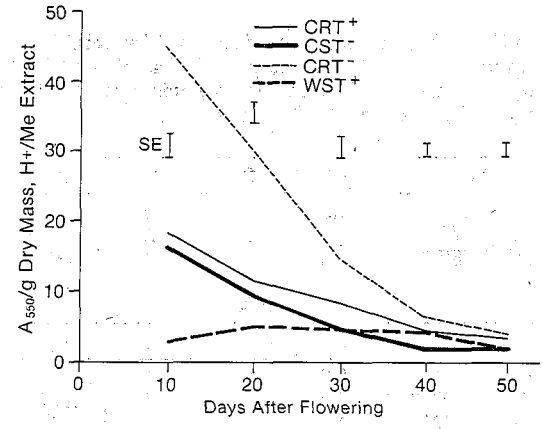

Figure 3. Concentrations of flavan-4-ols in acidified methanol extracts of developing grains of mold-resistant and -susceptible sorghum accessions, ICRISAT Center, rainy season 1984. Each value represents the mean of three field replicates, and each analysis was carried out at least in duplicate. SE = standard error representing means of groups.

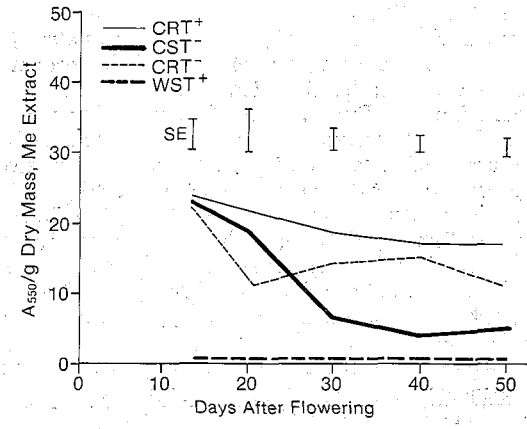

Figure 2. Concentrations of flavan-4-ols in methanol extracts of developing grains of mold-resistant and -susceptible sorghum accessions, ICRISAT Center, rainy season 1985. Each value represents the mean of three field replicates, and each analysis was carried out at least in duplicate. SE = standard error representing means of groups.

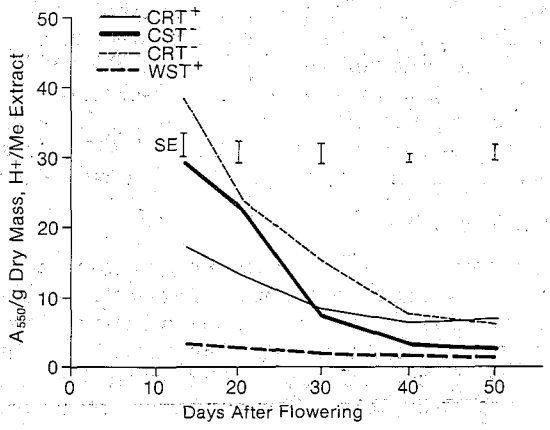

Figure 4. Concentrations of flavan-4-ols in acidified methanol extracts of developing grains of mold-resistant and -susceptible sorghum accessions, ICRISAT Center, rainy season 1985. Each value represents the mean of three field replicates, and each analysis was carried out at least in duplicate. SE = standard error representing means of groups.

least twice the concentration of flavan-4-ols as compared to mold-susceptible (CST^-) accessions. The CRT^-/WST^+ ratios in 1985 again exhibited the highest values as compared to other groups but were much lower in magnitude when compared to similar values of 1984.

The values of the CRT^-/CRT^+ group in the H^+/Me extract in 1984 were more than 1 at all stages (Table IV). This indicated that H^+/Me extracts of mold-resistant sorghum without testa contained higher concentrations of flavan-4-ols than mold-resistant sorghum with testa at all grain development stages. This observation was different from those observed with Me extract where the CRT^-/CRT^+ ratios were less than 1 throughout the different maturity stages. The CRT^-/CST^- ratios showed values of 2 and above, indicating a higher concentration of H^+/Me-extractable flavan-4-ols at all levels of maturity in the CRT^- group. The ratio of CRT^-/WST^+ of H^+/Me extracts was 9% or less of the CRT^-/WST^+ ratio

Table III. Flavan-4-ol Concentrations in Methanol Extracts of Developing Grains of the CRT⁻ Group Expressed as Ratios in Comparison with Other Groups[a] (Days after 50% Flowering)

1984					
ratio	10	20	30	40	50
CRT⁻/CRT⁺	0.7	0.9	0.6	0.8	0.9
CRT⁻/CST⁻	1.3	1.0	2.2	5.1	5.4
CRT⁻/WST⁺	167.4	190.1	125.0	80.8	34.91
1985					
ratio	14	21	30	40	50
CRT⁻/CRT⁺	1.0	0.5	0.8	0.9	0.6
CRT⁻/CST⁻	0.8	0.6	2.1	3.9	2.2
CRT⁻/WST⁺	37.0	21.1	20.5	19.1	18.0

[a] As in Table I.

Table IV. Flavan-4-ols Concentrations in Acidified Methanol Extracts of Developing Grains of the CRT⁻ Group Expressed as Ratios in Comparison with Other Groups[a] (Days after 50% Flowering)

1984					
ratio	10	20	30	40	50
CRT⁻/CRT⁺	2.5	2.7	1.8	1.4	1.2
CRT⁻/CST⁻	2.7	3.3	3.2	3.7	2.4
CRT⁻/WST⁺	15.5	5.9	3.3	1.6	2.1
1985					
ratrio	14	21	30	40	50
CRT⁻/CRT⁺	2.2	1.9	1.9	1.2	0.9
CRT⁻/CST⁻	1.3	1.1	2.1	2.3	2.4
CRT⁻/WST⁺	11.9	9.4	8.4	4.1	5.3

[a] As in Table I.

in the Me extract. This is because the sorghums of the WST⁺ group are reported to belong to group II, which have tannins (and flavan-4-ols) extractable only in H⁺/Me (Butler, 1988). As these values were much higher than those obtained with Me, the CRT⁻/WST⁺ ratios were reduced considerably. The observation of ratios obtained in 1985 with H⁺/Me extracts was similar to those obtained in 1984 except that CRT⁻/CST⁻ values were less than 2 in the early stages of grain development.

CONCLUSIONS

Our results showed that colored, mold-resistant sorghum accessions without testa had at least a 2-fold higher concentration of flavan-4-ols than mold-susceptible accessions without testa in both 1984 and 1985. This was observed in both Me and H⁺/Me extracts of all grains obtained at 30 DAF. This confirmed the observation reported from an earlier study (Jambunathan et al., 1986).

This study showed that there is a correlation between flavan-4-ols and ergosterol, which is an indicator of fungal invasion in grains. The fungal invasion generally occurs around 30 DAF. In the present study, grains at very early stage of maturity, i.e., 10 and 14 DAF, contained the highest flavan-4-ols concentrations followed by a drastic decrease with increased maturity. As grain development proceeds, a constant population of flavan-4-ol molecules would appear to decrease as other compounds are synthesized. They could degrade, be converted, or be incorporated into other molecules (i.e., tannins). The role of flavan-4-ols in the physiological processes of seed development is not known. Whether the flavan-4-ols are transported as such within the various plant parts or degraded and resynthesized is not clear at present.

It is evident that the concentrations of flavan-4-ols in mature sorghum seed should give an indication about the expected reaction of the sorghum cultivars to grain mold in the field. Therefore, this procedure could be a very important aid in screening cultivars for grain mold resistance or susceptibility. Additional information is needed to understand tannin biosynthesis and its control mechanism in plants. Therefore, elucidation of the role of flavan-4-ols and its implication in grain mold resistance needs further basic research. Use of radioactive isotopes and testing of extracts of grains on mold-causing organisms may give additional insight in this area.

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