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Effects of Growth Conditions on Grain Molding and Phenols in Sorghum Caryopsis*

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Phenolic compounds (PC) and phenolic acids (PA) were quantified in mature caryopses of sorghum grown in wet or dry environments. Seventeen cultivars varying in pericarp color and presence of a pigmented testa exhibited different levels of resistance to molding in the wet environment. Sorghum caryopses with a white pericarp contained lower levels of free PC (14 μg/caryopsis) than those with a red pericarp (41 μg/caryopsis) when grown in the dry environment. This difference diminished under humid conditions. Cultivars with a pigmented testa were more resistant to grain molding, contained higher levels of free PC (151 μg/caryopsis), and had a softer endosperm texture than cultivars without a pigmented testa. In cultivars without a pigmented testa, higher levels of free PC and free PA, especially free p-coumaric, ferulic, and caffeic acids, were observed in mold-susceptible cultivars. A scatter plot of free PC vs. free p-coumaric acid indicated that mold-susceptibility was related to higher levels of p-coumaric acid, regardless of environment.

Introduction

During development, grain of *Sorghum bicolor* [L.] Moench is exposed to sunshine, rain, diseases and pests. Grain molding is a serious fungal disease complex because it decreases grain yield and quality[1]. Many cultivars in the world collection of sorghum germplasm were screened for grain mold resistance and 156 accessions exhibited some level of resistance[2,3]. All resistant cultivars had a red pericarp and most had a pigmented testa layer containing condensed tannins (oligomers of flavan-3-ols). However, 14 resistant cultivars lacked the testa layer and condensed tannins. Jambunathan *et al.*[4] observed that mold-resistant cultivars contained high levels of flavan-4-ols and/or condensed tannins. Mold resistance was also correlated with higher levels of phenolic acids in mature caryopses[5]. Thus, phenolic compounds appear to be important for resistance to grain molding.

Many phenolic compounds in plants inhibit microbial growth[6] and are present in

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Abbreviations used: PC = phenolic compounds; PA = phenolic acids. ICRISAT = International Crops Research Institute for the Semi-Arid Tropics.

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sorghum caryopses[5] and glumes[7]. These compounds may aid in sorghum mold resistance before and after grain maturity. This study was conducted to determine the effects of environmental conditions on grain molding and the concentration of phenolic compounds (PC) and phenolic acids (PA) in mature caryopses of various cultivars of sorghum. These data will then be correlated with kernel characteristics.

Experimental

Samples

Seventeen sorghum cultivars varying in pericarp color and presence of a pigmented testa were selected because they exhibited different levels of resistance to molding (Table I). Sorghum cultivars were grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India during the 1984 rainy season (moldy) and during the 1984–1985 post-rainy season (control) (Table II). At anthesis, 20 panicles from plots in a replicated, randomized block designed trial were tagged. The tagged panicles were harvested 54 days later, i.e. about 2 weeks after physiological maturity. The rainy season treatment was watered after anthesis using an overhead sprinkler system for 1 h each morning and evening on days when it did not rain. The control treatment was watered using the furrow method when needed.

Individual panicles were threshed manually and the grain was visually evaluated for pericarp thickness, endosperm texture and extent of molding. The scale for pericarp thickness was: 1 = thin, pearly; 2 = thin; 3 = intermediate; 4 = thick, chalky and 5 = very thick. The scale for endosperm texture was: 1 = corneous; 2 = mostly corneous; 3 = intermediate; 4 = mostly floury; and 5 = floury. The scale for grain molding was: 1 = no visible mold; 2 = 1 10% of grain surface molded; 3 = 11–25% of area molded; 4 = 26–50% of area molded; and, 5 = > 50% of grain surface. Threshed grain was composited, and sun-dried to about 12% moisture. The grain was treated with phosphine (3 g/100 g grain for 3 days) before shipping to Texas A&M University via the USDA Plant Germplasm Quarantine Center (Beltsville, MD 20705).

Chemical analyses

To quantify free and bound PC and PA, sixty caryopses (about 1·2 g) were added to 1% HCl in methanol (30 ml) at 25 °C and homogenized (Polytron, PT10 probe, Brinkman Instruments) for 30 s at the highest speed[5]. The sample was agitated for 1 h and centrifuged for 10 min at 2000 × *g*. An aliquot (20 ml) of the supernatant was rotoevaporated (< 30 °C) to dryness and 4 M NaOH (10 ml) was added to hydrolyze the soluble phenolic compounds at 25 °C. After 14 h in *vacuo*, the hydrolyzate was acidified to pH 2 with 6 M HCl. Phenols were extracted with ethylacetate (3 × 20 ml), rotoevaporated (< 30 °C) to dryness, dissolved in methanol (5 ml) and filtered (0·45 μm). Free PC were quantified using the Folin-Ciocalteu method[8]. Free PA were separated on a C18–10 μm column (Econoshere column, Alltech Associates, Deerfield, IL) and detected at 260 and 340 nm. A multistep gradient from 98% water containing 2% acetic acid to 90% methanol containing 10% n-butanol eluted free PA at 1·5 ml/min. Bound PA and bound PC in the residue remaining after acidic methanol extraction were hydrolyzed and solubilized using 4 M NaOH at 25 °C and processed as described above.

The data were analyzed statistically using ANOVA and correlation procedures[9].

Results and Discussion

Physical properties

Grain that developed and matured in relatively hot and dry conditions (control, Table II) had no visible mold. Grain that developed and matured in warm and wet or humid

TABLE I Characteristics of sorghum cultivars varying in resistance to grain molding

Class[a]	Cultivar	Pericarp thickness[b]	Endosperm texture[b]	Mold rating[b]	1000 kernel weight (g)	
					Control	Moldy
W/S	CSH1	5	4	4 7	27 8	32 2
W/S	IS18758	3	3	4 5	28 5	28 2
W/S	SPV104	2	4	5 0	31 2	34 9
W/S	SPV351	2	3	4 5	23 0	20 7
					27 6	29 0
W/MR	IS14332	4	2	3 4	18 1	16 8
R/S	IS402	4	3	4 4	33 0	31 2
R/S	IS417	3	3	4 3	25 7	27 9
					29 4	29 6
R/R	IS14375	2	3	2 1	18 1	18 7
R/R	IS14380	2	2	2 2	16 8	17 4
R/R	IS14384	3	3	2 1	18 8	18 2
R/R	IS14390	4	2	2 1	16 8	18 1
R/R	IS21599	3	3	2 1		15 6
					17 6	17 6
R(+)/R	IS14387	4	3	2 0	17 6	18 6
R(+)/R	IS20620	4	4	2 3	21 9	20 9
R(+)/R	IS21454	3	3	2 1	18 0	17 8
R(+)/R	IS2867	2	4	2 0	22 5	20 3
R(+)/R	IS8545	3	4	2 0	25 0	26 1
					21 0	20 7
LSD[c]		—		0 4	0 6	0 8

[a] Pericarp color/mold resistance white (W) or red (R) pericarp, presence of a pigmented testa (+) and susceptible (S), resistant (R) or moderately resistant (MR) to grain molding

[b] Lower values reflect a thinner pericarp, more corneous endosperm and a less moldy caryopsis (rainy season samples)

[c] Least significant difference ($P = 0\ 05$)

TABLE II Environmental conditions during grain development to produce clean (control) and moldy sorghum grain[a]

	Post-rainy season (control)	Rainy season (moldy)
Relative Humidity		
Daily minimum (%)	8–40	25–98
Daily maximum (%)	9–95	70–99
Days with > 85%	7	34
Temperature		
Daily minimum (°C)	14 2–25 0	15 0 23 6
Daily maximum (°C)	30 0–38 0	24 6–32 0

[a] The rainy season had 14 days of rainfall (183 5 mm) and 40 days of overhead irrigation during the post flowering period No rain or water fell on the control treatment during grain development

conditions (moldy, Table II) had various levels of molding and kernel deterioration (Table I). Similar results were reported earlier[1,10,11].

All mold-resistant cultivars in this study had a red pericarp color. Cultivars lacking a pigmented testa had an intermediate or mostly corneous endosperm texture. Cultivars with a pigmented testa had a floury or intermediate endosperm texture. Pericarp thickness, however, did not correspond to mold-resistance in this study.

Mold-susceptibility was observed in cultivars with a white or red pericarp color and intermediate or mostly floury endosperm texture. Glueck and Rooney[10] reported that cultivars with a thin pericarp and a hard endosperm texture had more mold resistance. However, most cultivars that contain a pigmented testa are mold resistant even though they had a softer endosperm texture[3,5,10].

Kernel size of mold-susceptible cultivars was significantly greater than mold-resistant cultivars without a pigmented testa (Table I). Mold deterioration of susceptible grain did not consistently affect kernel weight. Forbes *et al.*[11] observed that the caryopsis weight of moldy grain was 5–40 % less than the weight of clean (control) caryopses when grown under identical conditions. Apparently, seasonal variations and agronomic conditions affected kernel weight.

Phenolic compounds and phenolic acids

Levels of PC in the caryopsis changed with pericarp color, pigmented testa and environmental conditions (Table III). Caryopses with a white pericarp had lower levels of free PC (14 μg/caryopsis) than those with a red pericarp (41 μg/caryopsis). Caryopses with a red pericarp and pigmented testa had on average 150 μg/caryopsis of free PC, significantly more than other cultivars.

The warm and moist environment tended to increase levels of free PC in white pericarp sorghums and to decrease levels in red pericarp sorghums. The very high levels of free PC in cultivars with a pigmented testa were not consistently altered by warm and moist conditions during caryopsis development.

Levels of free PA in caryopses corresponded to changes in mold-susceptibility and presence of a pigmented testa (Table IV). Susceptible cultivars contained 8·1 μg/caryopsis of free PA while resistant cultivars without a pigmented testa contained 3·4 μg/caryopsis of free PA. Cultivars with a pigmented testa contained significantly more PA (11·2 μg/caryopsis) than did other cultivars. Exposure to warm and moist conditions during kernel development did not consistently change the levels of free PA. Bound PA levels in caryopses were not consistently modified by any independent variable. However, all cultivars contained less free than bound PA. Hahn *et al.*[5] also observed this for all sorghum cultivars.

Major PA (> 1 μg/caryopsis) in sorghum caryopses were [retention time (min) in parentheses]: protocatechuic (9), gentisic (12), caffeic (18), p-coumaric (25), salicylic (27), ferulic (29) sinapic (30) and cinnamic acids (42). Other PA (< 1 μg/caryopsis) present were gallic (6), p-hydroxybenzoic (14), vanillic (17), and syringic (20). Warm and moist conditions during kernel development caused significant increases in free caffeic acid in susceptible cultivars and decreases in bound cinnamic acid in all cultivars (Table V). Average levels of selected free PA in susceptible cultivars in warm and moist and control

TABLE III. Free and bound phenolic compounds (μg/caryopsis) in control and moldy sorghum caryopses

Class[a]	Cultivar	Free phenolic compounds Control	Free phenolic compounds Moldy	Bound phenolic compounds Control	Bound phenolic compounds Moldy
W/S	CSH1	10·4	31·0	16·8	16·1
W/S	IS18758	16·1	20·6	26·6	39·7
W/S	SPV104	12·4	40·1	31·2	43·1
W/S	SPV351	17·2	17·9	37·0	15·6
		14·2	27·7	27·9	28·6
W/MR	IS14332	14·5	19·4	28·0	11·1
R/S	IS402	47·8	39·4	34·2	34·0
R/S	IS417	38·4	31·5	24·6	22·4
		43·1	35·4	29·4	28·2
R/R	IS14375	41·4	21·8	10·1	15·1
R/R	IS14380	37·6	21·4	13·5	18·4
R/R	IS14384	42·7	18·7	16·4	16·0
R/R	IS14390	39·7	19·9	12·0	12·4
R/R	IS21599	—	26·2	—	19·9
		40·4	21·6	13·0	16·4
R(+)/R	IS14387	144·6	139·0	73·5	32·9
R(+)/R	IS20620	190·9	194·7	97·1	59·5
R(+)/R	IS21454	113·6	103·8	70·4	26·5
R(+)/R	IS2867	121·6	160·0	108·4	70·0
R(+)/R	IS8545	180·5	142·0	84·3	85·3
		150·2	147·9	86·7	54·8
LSD[b]		7·8	12·4	9·8	10·3

[a] Pericarp color/mold resistance – See Table I for details.
[b] Least significant difference ($P = 0{\cdot}05$).

environments were: p-coumaric (0·76 vs. 0·14 μg/caryopsis), ferulic (1·74 vs. 0·18 μg/caryopsis), and caffeic (1·52 vs. 0·33 μg/caryopsis, respectively).

Correlations of mold ratings with PC and PA data of cultivars lacking a pigmented testa revealed useful information. These cultivars appear to have a different biochemical mechanism than cultivars with a pigmented testa and tannins. Increased levels of free p-coumaric, ferulic and caffeic acids had correlation coefficients with mold ratings of 0·68, 0·76, and 0·54 (Table VI). Other correlation coefficients were significant ($P < 0{\cdot}05$) but smaller than 0·54. Hence, mold-susceptible cultivars generally had higher levels of these specific free PA.

Factorial analysis of free PC and PA data of cultivars lacking a pigmented testa was utilized to cluster cultivars into mold-susceptible and resistant groups (graphs not shown). Phenols that contributed significantly to each orthogonal axis were subsequently plotted (Fig. 1). The scatter plot of free PC vs. free p-coumaric acid separated mold-susceptible and mold-resistant cultivars, regardless of environmental conditions.

TABLE IV. Sum of free and bound phenolic acids (μg/caryopsis) in control and moldy sorghum caryopses[a]

Class[b]	Cultivar	Free phenolic acids Control	Free phenolic acids Moldy	Bound phenolic acids Control	Bound phenolic acids Moldy
W/S	CSH1	4·8	8·4	39·6	100·0
W/S	IS18758	4·4	5·4	53·2	73·6
W/S	SPV104	15·7	12·5	50·1	55·3
W/S	SPV351	4·1	2·7	64·1	45·1
		7·2	7·2	52·2	68·5
W/MR	IS14332	1·7	3·5	61·7	23·7
R/S	IS402	12·5	9·3	56·8	33·9
R/S	IS417	7·0	6·1	38·9	49·1
		9·2	7·7	47·8	41·5
R/R	IS14375	3·3	2·6	38·4	53·4
R/R	IS14380	5·2	4·5	34·9	40·1
R/R	IS14384	5·4	4·1	38·5	49·6
R/R	IS14390	2·8	2·3	43·8	49·0
R/R	IS21599	—	5·6	—	49·3
		4·2	3·8	38·9	48·3
R(+)/R	IS14387	6·4	5·8	67·5	102·5
R(+)/R	IS20620	15·3	40·2	44·1	99·8
R(+)/R	IS21454	6·0	6·3	66·1	80·8
R(+)/R	IS2867	11·7	10·0	58·0	58·0
R(+)/R	IS8545	16·8	5·2	59·0	96·1
		11·2	13·15	58·9	87·4
LSD[c]		2·0	8·3	31·2	33·5

[a] Total of identified and unidentified phenolic acids.
[b] Pericarp color/mold resistance. See Table I for details.
[c] Least significant difference ($P = 0{\cdot}05$).

Therefore, chemical analysis of phenols in sorghum caryopses can classify (predict) the mold-susceptibility of the cultivar.

Discussion

Changes in PA caused by the warm and moist treatment probably resulted from fungal metabolism as well as secondary metabolism in the plant. Levels of individual PA changed dramatically during maturation[11] which suggests that PA are involved in the plants' defensive mechanism against fungi. Fungi metabolize host plant 'defensive' compounds during their colonization of the pericarp and interior tissues in immature[12] and mature caryopses[1,10,13].

Jambunathan *et al.*[4] reported that mature grain of resistant cultivars contained more flavan-4-ols and leucoanthocyanidins than susceptible cultivars. However, neither compound was measured in cultivars with a white pericarp; even though several white pericarp cultivars exhibited molding resistance[5,11]. Thus, flavan-4-ols and leuco-

TABLE V Major free and bound phenolic acids in control and moldy sorghum caryopses[a]

Class[b]	Free phenolic acids[c]	Bound phenolic acids[c]
	Control	*Control*
W/S	Sal	Cou Sal Fer Cin
R/S	—	Cou Sal Fer Cin
W/R	—	Sal Fer Cin
R/R	—	Sal Fer Cin
R(+)/R	Cou Sal	Pro Gen Cou Sal Fer
	Weathered	*Weathered*
W/S	Caf	Caf Cou Sal Fer
R/S	Caf Sal	Caf Cou Sal Fer
W/R	—	Sal Fer
R/R	—	Sal Fer
R(+)/R	Cou Sal Fer	Pro Gen Caf Cou Sal Fer Sin

[a] Phenolic acids present at > 1 μg/caryopsis
[b] Pericarp color/mold resistance.- See Table 1 for details
[c] Abbreviations for phenolic acids Sal = salicylic, Cou = p-coumaric, Sin = sinapic, Fer = ferulic, Cin cinnamic, Pro = protocatechuic, Gen = gentissic, Caf = caffeic

TABLE VI. Correlation coefficients between mold rating of sorghum caryopsis and their free and bound phenolic constituents

Phenolic constituent	Free phenolic constituents			Bound phenolic constituents		
	Control	Moldy	Combined	Control	Moldy	Combined
Phenolic compounds	−0·61*	0·52*	−0·14	0·68*	0·46*	0·56*
Phenolic acids						
Total	0·50*	0·61*	0·55*	0·33*	0·29*	0·29*
Gentisic	0·52*	0·23	0·38*	0·25	0·32*	0·29*
p-Hydroxybenzoic	0·22	0·25	0·19	0·30*	0·32*	0·29*
Vanillic	0·63*	0·40*	0·51*	0·54*	0·42*	0·48*
Caffeic	0·55*	0·60*	0·54*	0·31*	0·53*	0·38*
Syringic	−0·19	0·33*	0·08	0·41*	0·31*	0·24*
p-Coumaric	0·65*	0·76*	0·68*	0·65*	0·50*	0·45*
Salicylic	0·28	0·24	0·25*	0·14	0·09	0·13
Ferulic	0·76*	0·78*	0·76*	0·29	0·51*	0·40*
Sinapic	−0·01	0·35*	0·16	0·25	0·40*	0·31*
Cinnamic	−0·47*	0·37*	−0·09	0·35*	0·15	0·16

* $P \leqslant 0.05$, $n = 44$ (control), 48 (moldy), and 92 (combined)

anthocyanidins may be associated with resistance to molding, but other compounds and kernel characteristics are also involved[10,13].

Most caryopses have fungi in the pericarp[2], even if the surface of the caryopsis is without visible mold growth. Hence, PC and PA compositions of visually moldy grain are probably different from 'sterile' caryopses.

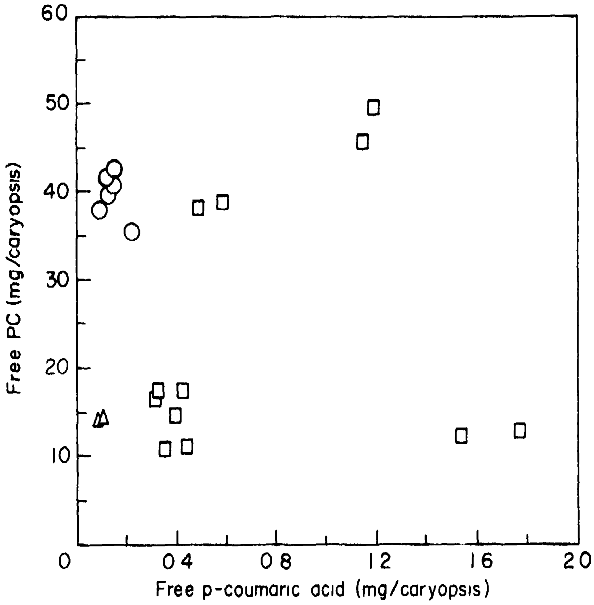

FIGURE 1. Grouping of sorghum cultivars into susceptible (□), moderately resistant (△) and resistant (○) to grain molding in a scatter plot of free phenolic compounds (PC) vs. free p-coumaric acid in the caryopsis.

Chemical analyses of PC and PA in mature caryopses of sorghum were analyzed statistically to cluster cultivars correctly into mold-resistance and mold-susceptible groups. Hence, PA composition of mature grain can determine which cultivars are mold-resistant, regardless of environmental conditions during caryopsis development. Verification of this is the objective of current projects at ICRISAT and Texas Agricultural Experiment Station.

Pericarp color and thickness did not correspond to mold resistance in this study. A more corneous endosperm texture usually corresponds to mold-resistance[10]. However, the presence of pigmented testa is more influential than a corneous endosperm or a thin pericarp in limiting fungal deterioration of sorghum[3,10,13].

The host plant response to fungal infection and colonization during caryopsis development was monitored only in mature grain in this study. Hammerschmidt and Nicholson[14] reported that the resistance of corn to *Colletotrichum graminicola* was due to both the amount of PC and the plant's speed of the response after infection. A similar study with immature caryopses and *Fusarium moniliforme* is currently being investigated[13].

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