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ROLE OF PIGMENTS AND TANNINS IN THE REACTION OF TAN AND RED NEAR-ISOGENIC SORGHUM LINES TO LEAF DISEASES

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ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench) plant pigments have been associated with resistance to leaf diseases and grain deterioration. Four near-isogenic pairs of tan and non-tan (red) sorghum lines were assayed for their phenolic content and evaluated for their reaction to leaf diseases along with six other sorghum lines. Leaves, stems, sheaths, glumes and seeds of mature plants were assayed for tannins, pigments, and precursors of these pigments. No tannins were detected in the various plant tissues although other genotypes can have high levels of these compounds in seed coats. The red plants (especially the glumes and sheath) were found to accumulate 3-deoxyanthocyanidins, the major pigments in sorghum, but these were absent in the tan plants. The glumes and sheath of tan plants accumulated apigenin. Near-isogenic pairs were tested for differential reactions to anthracnose (*Colletotrichum graminicola* Ces.) at Tifton, Georgia and to rust (*Puccinia purpurea* Cooke) at Isabella, Puerto Rico during the 1991 growing season. Tan and red isogenic pairs, which differ significantly in phenolic composition, did not show significant differences in their disease reaction. The six other sorghum lines which showed marked differences in their reaction to leaf diseases also showed differences in their phenolic composition. However, for the phenols that were analyzed, there was no correlation with resistance or susceptibility. It is proposed that tannins, pigments, and pigment precursors are not responsible for disease resistance in these genotypes.

***Key Words*: *Colletotrichum lindemuthianum*, leaf diseases, *Puccinia sorghi*, resistance, *Sorghum bicolor*, tannins**

RÉSUMÉ

La pigmentation chez le sorgho est associée à la résistance contre les maladies des feuilles et à la dégénérescence des graines. Nous avons déterminé le contenu en phénols et la réaction contre des maladies des feuilles chez quatre paires de lignées presque isogéniques tan et non-tan (rouge) et chez six autres lignées de sorgho. Les tannins, pigments et précurseurs de ces pigments ont été analysés dans les feuilles, les tiges, les glumes et les graines des plantes adultes. Nous n'avons pas détecté de tannins dans les différents tissus, malgré qu'on en trouve beaucoup dans le tégument de la graine d'autre génotypes. Les plantes rouges, mais pas les plantes tan, accumulent de la 3-deoxyanthocyanidin, le pigment principal du sorgho, surtout dans les glumes et les tiges. Les glumes et tiges des plantes tan accumulent de l'apigenin. Pendant la saison 1992, nous avons comparé la réaction des lignées presque-isogénique contre l'anthracnose (*Colletotrichum*

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***graminocola* Ces.) à Tifton (Georgia) et contre la rouille (*Puccinia purpurea* Cooke) à Isabella (Puerto Rico). Les paires isogéniques tan et rouge, qui sont significativement différentes pour leur contenu en phénols, n'ont pas manifesté de réaction différente après inoculation des différentes maladies. Les six autres lignées de sorgho, qui ont une réaction différente vis-à-vis des maladies des feuilles, présentent aussi différentes quantités de phénols. Pour les phénols analysés, nous n'avons pas trouvé une corrélation avec la résistance ou avec la susceptibilté. Nous arrivons donc à la conclusion que les tannins, les pigments et les précurseurs de pigment n'ont pas de fonction dans la résistance contre les maladies des feuilles chez ces génotypes.**

***Mots Clés*: *Collectotrichum lindemuthianum*, maladies des feuilles, *Puccinia sorghi*, résistance, *Sorghum bicolor*, tannins**

INTRODUCTION

Three plant colours (tan, red, and purple) reflective of differential pigment composition are recognized in sorghum. Sorghum plant pigments (3-deoxyanthocyanidins) have been implicated in resistance to leaf diseases and grain deterioration (Snyder *et al.*, 1991). Other sorghum polyphenols like flavan-4-ols and tannins have also been associated with grain mould resistance (Harris and Burns, 1973; Jambuna Thau *et al*; 1986). Sorghum produces the antimicrobial phytoalexin (3-deoxyanthocyanidins) in response to attempted infection by *Colletotrichum graminicola* Ces. and it has been suggested that these compounds are essential components in its defense mechanism (Nicholson *et al.*, 1987). Recently, it was proposed that the accumulation of 3-deoxyanthocyanidin pigments in juvenile sorghum leaves can be used as an aid to breeding for anthracnose resistance (Tenkouano *et al.*, 1993). The basis of the relative resistance tan sorghums to leaf diseases is not clear.

The objective of this study was to test if pigment and/or phenolic compounds in tan, red, and purple sorghum plants can be correlated to resistance to leaf diseases. We examined tan and red near-isogenic sorghum lines along with resistant and susceptible lines for their reaction to rust (*Puccinia purpurea* Cooke) and anthracnose (*Colletotrichum graminicola* Ces). Stems, leaves, sheath, glumes, and seeds of mature plants were analyzed for flavan-4-ols, tannins, and 3-deoxyanthocyanidin pigments.

MATERIALS AND METHODS

Sources of seeds and standards. The sorghum genotypes used in the study are listed in Table 1. Four near-isogenic pairs of tan and red sorghum lines (P-87001T and P-87001R through P87004T and P-87001R) were identified in individual progeny rows of a segregating (F_5) population of a cross (TAM 428 x M-62641). The other non-isogenic lines are breeding entries and introductions accumulated in our breeding programme for their differential disease reaction and food quality characteristics. Sample seeds for all entries were obtained from experimental plots grown at Purdue University Agronomy Research Centre.

TABLE 1. The source of seeds and the designations used in the study

Designation	Type of cultivar	Source
P-87001T	Breeding line	Purdue University
P-87001R	Breeding line	Purdue University
P-87002T	Breeding line	Purdue University
P-87002R	Breeding line	Purdue University
P-87003T	Breeding line	Purdue University
P-87003R	Breeding line	Purdue University
P-87004T	Breeding line	Purdue University
P-87004R	Breeding line	Purdue University
CMS 180R	Breeding line	Embrapa, Brazil
Feterita Ajebsido	Land race	Sudan
P-954035	Breeding line	Purdue University
M-66341	Breeding line	ICRISAT
B-Var 1	Parental line	ICRISAT
TAM 428	Parental line	Texas A & M University

Luteolinidin and apigenin were purchased from Plantech (Reading, UK). Apigeninidin was prepared as described by Schutt and Netzly (1991). All other reagents were HPLC grade.

Disease ratings. The field trials were done at Isabella, Puerto Rico and at Tifton, Georgia, during the 1991 growing season. Both locations have high natural infection levels for both rust (Isabella) and anthracnose (Isabella and Tifton), respectively. Infection levels were rated on the scale of where a rating of 1 denotes no symptoms or a few lesions

of hypersensitive reaction and 5 denotes heavy infection with leasions covering most of the leaves.

Chemical analysis. Stems, leaf blades, sheath, glumes and seeds were dried at 60°C for 72 hr and ground in a Cyclotec 1093 sample mill (Hoganas, Sweden). Each ground sample (0.5 g) was extracted with 10 ml ethyl acetate. After filtering, the residue was extracted with 10 ml 1% HCl in methanol. The ethyl acetate extract was analyzed for flavonoids by HPLC as described below. The methanol extracts (1 ml) were analyzed for tannins and flavan-4-ols as described by Watterson and Butley (1983).

Total pigments were determined by reading the absorbance of the methanol extracts at either 485 or 495 nm on a Gilford Response spectrophotometer (Ciba Corning Diagnostics Corp., Medfield, MA). The leaf, sheath, and glume methanol extracts were first extracted with an equal volume of n-hexane to remove chlorophyll before being analyzed for pigments.

HPLC analysis was done on the ethyl acetate and methanol extracts and standards in order to determine the major compounds in the tan and red plants. Separation was done on a Waters Bondpack C_{18} analytical (3.9x300 mm)column connected to a Varian 5000 LC system equipped with a Hewlett Packard HP1040A photodiode array detector. Samples (0.05 mL) were eluted at a flow rate of 1 ml/min. The solvents were water (A) and 10% acetic acid in methanol (B). The samples were eluted with a linear gradient from 0 to 40% B in 20 min, followed by another linear gradient to 100% in 10 min. The column was held at 100% B for 10 min before being brought to 0% B in 10 min. Eluting compounds were monitored at 280, 320 and 495 nm.

RESULTS

There were no significant differences between the tan and red near-isogenic pairs in their reaction to rust and anthracnose (Table 2). At Isabela (PR) all near-isogenic pairs were resistant to both rust and anthracnose. However, these pairs were susceptible when subjected to a highly virulent strain of anthracnose found at Tifton, Georgia. Significant differences were observed between the resistant and susceptible non-isogenic lines grown under similar conditions regardless of plant colour (Table 3).

Insignificant levels of flavan-4-ols or tannins were detected in the tan and red plants of the near-isogenic sorghum lines (Table 4). However, there were significant differences between the tan and red plants in the amount of pigments they accumulated. In some tissues, up to a 20 fold difference in pigment production was observed. Similarly, no significant differences in flavan-4-ols and tannin production were observed between the resistant and susceptible non-isogenic lines (Table 5). Although there were differences in the amounts of pigments produced by the resistant

TABLE 2. Differential reactions of near-isogenic sorghum lines to rust (*Puccinia purpurea* Cooke) and anthracnose (*Colletotrichum graminicola* Ces.) at Isabella (Puerto Rico) and at Tifton (Georgia, USA) during the 1991–1992 growing season

		Leaf disease ratings[a]		
		(Puerto Rico)		(Georgia)
Designation	Plant colour	Rust	Anthracnose	Anthracnose
P-87001T	Tan	1.0	2.0	4.0
P-87001R	Red	1.0	2.0	5.0
P-87002T	Tan	1.0	2.5	5.0
P-87002R	Red	2.0	3.0	5.0
P-87003T	Tan	1.0	2.0	5.0
P-87003R	Red	1.0	2.5	5.0
P-87004T	Tan	1.0	2.0	5.0
P-87004R	Red	1.0	2.0	5.0
SRN 39	Tan	1.0	2.0	1.0
Shanqui Red	Red	4.0	3.0	4.0

[a]1 = Highly resistant; 2 = Intermediate; 3 = Highly susceptible

TABLE 3. Differential reactions of sorghum lines to rust (*Puccinia purpurea* Cooke) and anthracnose (*Colletotrichum graminicola* Ces.) at Isabella (Puerto Rico) and at Tifton (Georgia) during the 1991–1992 growing season

		Leaf disease ratings[a]		
Designation	Plant colour	(Puerto Rico) Rust	Anthracnose[b]	(Georgia) Anthracnose
CMS 180R	Purple	1.0	1.5	1.5
B-Var-1	Tan	1.0	1.5	3.0
TAM 428	Red	1.0	1.5	5.0
P-954305	Red	4.0	2.0	5.0
Feterita Ajebsido	Purple	4.0	3.0	5.0
M-66341	Tan	1.0	4.0	5.0
SRN 39	Tan	1.0	2.0	1.0
Shanqui Red	Red	4.0	3.0	4.0

[a]1 = Highly resistant; 1–2.9 = Intermediate; and ≥ 3 = Highly susceptible
[b]Mean for Puerto Rico and Georgia

TABLE 4. Chemical analysis of near-isogenic sorghum lines[a]

Designation (Plant colour)	Tissue	Flavan-4-ols ($A_{550/g}$)	Proanthocyanidins ($A_{550/g}$)	Pigments (mg/g)
P-87001T	Seeds	1	0	0.0
(Tan)	Sheath	0	1	0.2
	Glumes	1	0	0.3
	Leaves	0	1	0.2
	Stems	0	0	0.1
	Seedlings	0	1	0.5
P-87001R	Seeds	0	0	0.0
(Red)	Sheath	2	1	4.4
	Glumes	1	1	2.8
	Leaves	0	1	0.7
	Stems	1	0	0.4
	Seedlings	0	2	0.5
BR-64[a]	Seeds	2	44	
(High tannin)	Leaves	8	0	
P 954035[a]	Seeds	7	1	
(Red)	Leaves	47	0	
P-87002T	Seeds	0	0	0.0
(Tan)	Sheath	1	2	0.2
	Glumes	2	0	0.7
	Leaves	0	1	0.2
	Stems	0	0	0.1
	Seedlings	0	1	0.3
P-87002R	Seeds	0	0	0.1
(Red)	Sheath	2	1	7.0
	Glumes	1	1	12.9
	Leaves	0	1	1.3
	Stems	1	0	0.7
	Seedlings	0	2	0.5

TABLE 4 Contd.

Designation (Plant colour)	Tissue	Flavan-4-ols ($A_{550/g}$)	Proanthocyanidins ($A_{550/g}$)	Pigments (mg/g)
BR-64[a] (High tannin)	Seeds	2	44	
	Leaves	8	0	
P 954035[a] (Red)	Seeds	7	1	
	Leaves	47	0	
P-87003T (Tan)	Seeds	0	0	0.0
	Sheath	1	2	0.4
	Glumes	1	0	0.5
	Leaves	0	1	0.2
	Stems	0	1	0.2
	Seedlings	0	1	0.7
P-87003R (Red)	Seeds	0	0	0.1
	Sheath	2	7	8.5
	Glumes	2	2	8.9
	Leaves	0	2	1.5
	Stems	0	1	0.6
	Seedlings	0	1	0.7
BR-64[a] (High tannin)	Seeds	2	44	
	Leaves	8	0	
P 954035[a] (Red)	Seeds	7	1	
	Leaves	47	0	
P-87004T (Tan)	Seeds	0	0	0.0
	Sheath	1	2	0.6
	Glumes	1	0	0.4
	Leaves	0	1	0.3
	Stems	0	0	0.2
	Seedlings	0	0	0.6
P-87004R (Red)	Seeds	0	0	0.1
	Sheath	1	7	8.8
	Glumes	2	2	6.6
	Leaves	0	2	1.5
	Stems	0	1	0.8
	Seedlings	0	0	0.6
BR-64[a] (High tannin)	Seeds	2	44	
	Leaves	8	0	
P 954035[a] (Red)	Seeds	7	1	
	Leaves	47	0	

[a]Watterson and Butler (1983)

and susceptible non-isogenic lines, this did not correlate with resistance or susceptibility (Table 4).

The pigments in the red plants were identified by comparing the retention times (Rt) and spectra with that of the standards. The major pigments observed were the 3-deoxyanthocyanidins, apigeninidin (Rt of 18.8 min) and luteolinidin (Rt of 20.0 min) (Fig. 1 and 2). A third peak with a similar spectrum to that of apigeninidin was observed at Rt of 19.3 min. Tan plants accumulated apigenin (Rt of 39.3 min) which extracted in ethyl acetate (Figs. 3 and 4). Two other peaks with spectra similar to apigenin were observed at Rt of 42.0 and 44.5 min. On hydrolysis with mineral acids, the two compounds were converted to apigenin.

DISCUSSION

Although plant pigments in sorghum have been implicated in the resistance/ susceptibility of

TABLE 5. Chemical analysis of some resistant sorghum lines

Designation (Plant colour)	Tissue	Flavan-4-ols ($A_{550/g}$)	Proanthocyanidins ($A_{550/g}$)	Pigments (mg/g)
CMS 180R (Purple)	Seeds	0	1	0.0
	Sheath	1	12	0.1
	Glumes	0	1	0.1
	Leaves	0	0	0.1
B-Var1 (Tan)	Seeds	0	0	0.7
	Sheath	0	1	0.7
	Glumes	0	0	0.1
	Leaves	0	0	0.6
TAM 428 (Red)	Seeds	0	0	0.0
	Sheath	0	2	0.2
	Glumes	0	0	0.4
	Leaves	0	0	0.1
BR-64[a] (High tannin)	Seeds	2	44	
	Leaves	8	0	
P 954035[a] (Red)	Seeds	7	1	
	Leaves	47	0	
P-954035 (Red)	Seeds	6	1	0.4
	Sheath	1	0	2.9
	Glumes	1	0	10.1
	Leaves	45	0	2.0
Fejerita Ajebsido (Purple)	Seeds	0	5	0.2
	Sheath	1	9	1.6
	Glumes	2	10	1.4
	Leaves	0	3	0.1
M-66341 (Tan)	Seeds	0	1	0.0
	Sheath	0	1	0.8
	Glumes	0	0	0.8
	Leaves	0	0	0.6
BR-64[a] (High tannin)	Seeds	2	44	
	Leaves	8	0	
P 954035[a] (Red)	Seeds	7	1	
	Leaves	47	0	

[a]Watterson and Butler (1983)

sorghum to leaf diseases (Nicholson *et al*, 1987; Tenkouano *et al.*, 1993) we did not find any correlation between plant colour and resistance / susceptibility as determined in the field. Near-isogenic pairs which differed markedly in their pigment content did not show any significant differences in their reaction to rust or anthracnose in the field. Tan and red plants in each near-isogenic sorghum pair had similar reactions to rust and anthracnose at Isabella, Puerto Rico, and at Tifton, Georgia. When resistant and susceptible non-isogenic sorghums were analysesd for pigments, no correlation was found between plant colour and field resistance or susceptibility. Therefore pigment production beyond the seedling stage may not be a good indicator of resistance or susceptibility of sorghum to leaf diseases. Other compounds, perhaps in combination with pigments, could be responsible for the observed resistance to leaf diseases.

The red plants, especially the sheath and the glumes, accumulated apigeninidin while the tan plants accumulated apigenin. In tan plants synthesis of pigments appears to be blocked just before formation of flavan-4-ols (Fig. 5). Red plants do not accumulate precursors of sorghum pigments (flavan-4-ols), which are instead rapidly converted into the pigments. Apigeninidin has

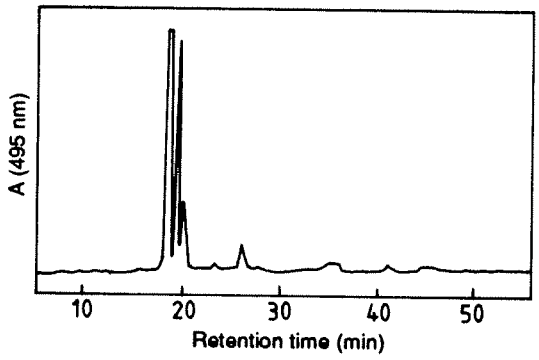

Figure 1. Methanol extract of a red sorghum plant was analysed by HPLC on a C_{18} column. Apigeninidin has a retention time of 18.8 min. The peak at 19.3 min had a spectra similar to apigeninidin. Luteolinidin had retention time of 20.0 min.

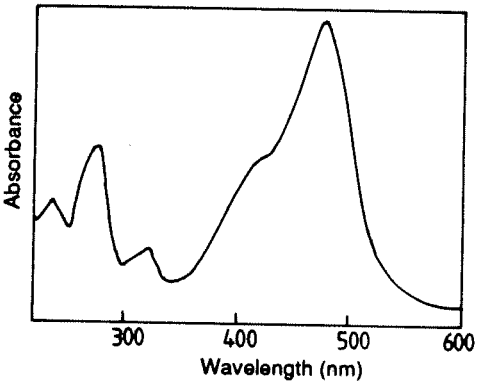

Figure 2. The UV-VIS spectrum of apigeninidin isolated from a red plant. The spectrum was identical to that of standard apigeninidin (λ_{max} = 475 nm)

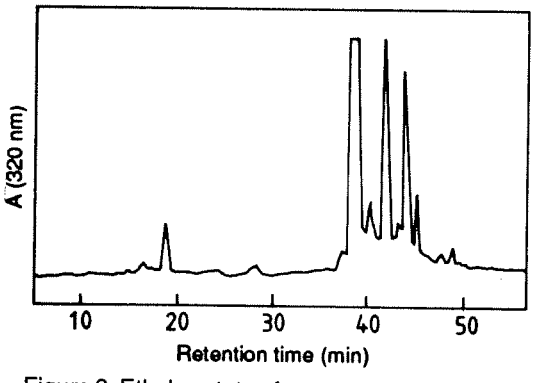

Figure 3. Ethyl acetate of a tan sorghum plant was analysed by HPLC on a C_{18} column. Apigenin had a retention time of 39.3 min. The peaks at 42.0 and 44.5 min had a spectra similar to apigenin.

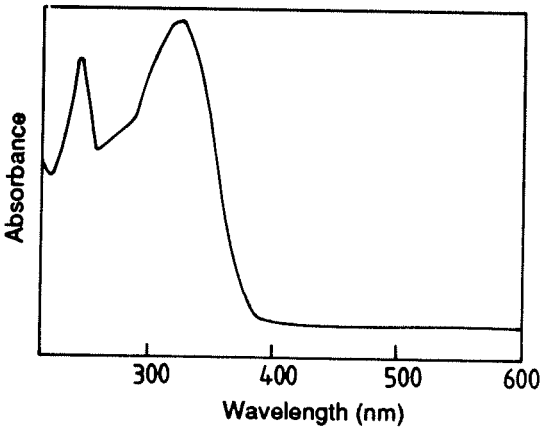

Figure 4. The UV spectrum of apigenin isolated from a tan plant. The spectrum was identical to that of standard apigenin (λ_{max} = 329 nm).

been shown to inhibit the growth of certain fungi (Schutt and Netzly, 1991) and bacteria (Stonecipher *et al.*, 1993). Therefore, red plants may fight off fungal and bacterial invasion better than the tan plants. We do not know how apigenin would affect fungal and bacterial growth.

ACKNOWLEDGMENTS

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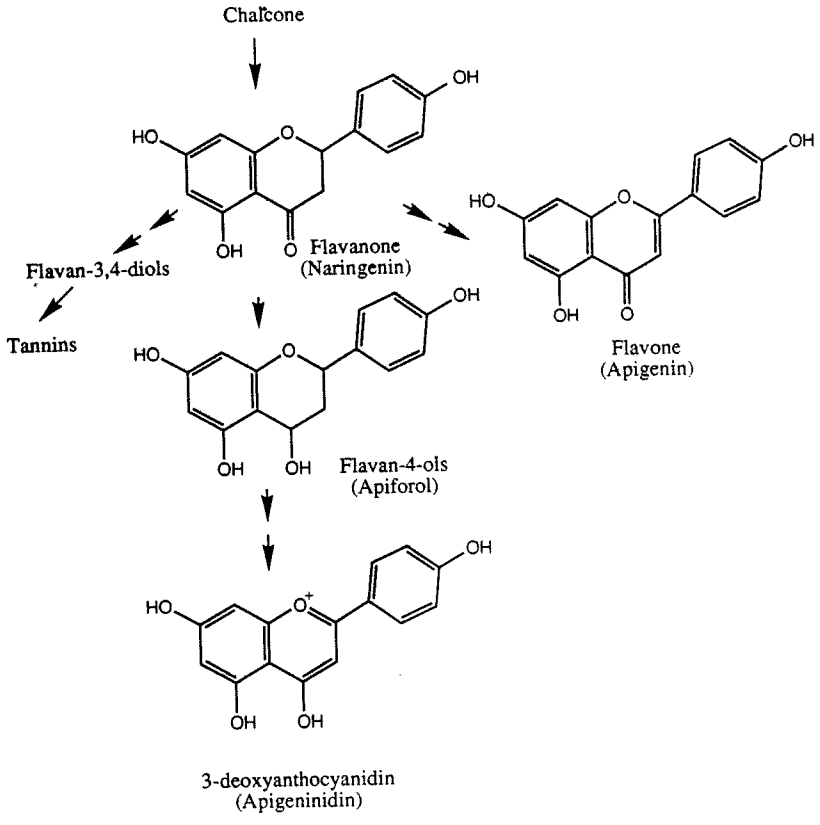

Figure 5. Outline of biosynthetic pathways toward the formation of pigments, tannins and flavones.

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