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IMPACT OF PHENOLIC COMPOUNDS AND RELATED ENZYMES IN SORGHUM VARIETIES FOR RESISTANCE AND SUSCEPTIBILITY TO BIOTIC AND ABIOTIC STRESSES

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Abstract—Contents of phenolic compounds and related enzymes before and after sorghum grain germination were compared between varieties either resistant or susceptible to biotic (sooty stripe, sorghum midge, leaf anthracnose, striga, and grain molds) and abiotic (lodging, drought resistance, and photoperiod sensitivity) stresses. Independent of grain germination, sorghum varieties resistant to biotic and abiotic stresses had on average higher contents of proanthocyanidins (PAs), 3-deoxyanthocyanidins (3-DAs), and flavan-4-ols than susceptible varieties. Results show that content of 3-DAs is a good marker for sorghum resistance to both biotic and abiotic stresses because it correlates with resistance to all stresses except for photoperiod sensitivity. The second good marker for stress resistance is content of PAs. Total phenolic compounds and the activities of related enzymes are not good markers for stress resistance in sorghum grains.

Key Words—Sorghum, proanthocyanidins, 3-deoxyanthocyanidins, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, stress, biotic, abiotic.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.)] Moench is a C4 plant grass of the hot, semiarid tropics and genetically diverse. On the basis of genetic diversity, some sorghum varieties are more resistant to stresses than others (Tao et al., 1993, 2003; Deu et al., 1994). The constraints of sorghum production are both biotic and abiotic stresses. Among biotic stresses, leaf anthracnose caused by the fungus *Colletotrichum graminicola*, sooty stripe incited by the fungus *Ramulispora sorghi*, and striga caused by the parasitic plant *Striga hermonthica* (Del.) Bent are the most damaging pests in West Africa in general, and particularly in Burkina Faso (Tenkouano, 1995; Neya and Le Normand, 1998; Trouche et al., 2001). In addition, sorghum midge caused by insects (*Contarinia sorghicola*, *Sitodiplosis moselana*, *Stenodiplosis sorghicola*, etc.) attack developing sorghum grains (Sharma and Hariprasad, 2002; Tao et al., 2003). Also, grain mold is a high constraint of sorghum production, and several molds are associated with sorghum caryopse damage (Melake-Berhan et al., 1996; Waniska et al., 2001). In Africa, the absence of grain mold resistance has been cited as a constraint to adoption of improved cultivars (Audilakshmi et al., 1999).

Important abiotic stresses limiting sorghum production in West Africa are drought, photoperiod sensitivity, and lodging (Tenkouano, 1995; Trouche et al., 2001). Lodging may be indirectly related to fungal infections, which weaken the plant (Waniska et al., 2001).

An increase in the activities of phenolic related enzymes and the accumulation of phenolic compounds have been correlated with resistance of cereals to biotic stresses (Mohammadi and Kazemi, 2002). Plant resistance to biotic and abiotic stresses is often regulated by the metabolism of phenolic compounds. Sorghum phenolic compounds, e.g., phytoalexins (3-deoxyanthocyanidins) or allelochemicals (*p*-hydroxybenzoates, *p*-coumarates, and flavanols), are involved in plant resistance to all kind of stresses (Lo et al., 1999; Weston et al., 1999, Weir et al., 2004).

Both biotic (fungi, insects, viruses, etc.) and abiotic (drought, temperature, photoperiod, nutrient deficiencies, etc.) stresses induce phenylalanine ammonia lyase (PAL; EC 4.3.1.5) synthesis (Chalker-Scott and Fuchigami, 1989; Tovar et al., 2002). PAL activity has been detected in the green shoots and leaves (Stafford, 1969; Mohan et al., 1988) of sorghum, and in sorghum, the infection of the plant with pathogens involves a rapid accumulation of PAL mRNA (Cui et al., 1996).

Also, peroxidases (POXs, EC 1.11.1.7; donor: H_2O_2 oxidoreductase) play an important role in stress-related resistance. One of the important physiological roles of POXs is the synthesis of cell-wall polymers (lignin and suberin), which constitute physical barriers for both biotic and abiotic stresses (Cosgrove, 1997).

In sorghum, POXs are involved in thermal tolerance (Choudhary et al., 1993) and resistance to fungal infection (Luthra et al., 1988).

Polyphenol oxidases (PPOs, EC1.14.18.1; monophenol, 3,4-*L*-dihydroxyphenylalanine: oxygen oxidoreductase) play an important role in plant defense via the oxidation of endogenous phenolic compounds into *o*-quinones, which are toxic to invading pathogens and pests (Mohammadi and Kazemi, 2002). PPO activity in plants increases under abiotic stress conditions (Mayer and Harel, 1991) and upon fungal infections (Luthra et al., 1988).

Several studies in other plant species have shown that the levels of phenolic compounds, and the activities of PAL, POX, and PPO are different between resistant plants and plants susceptible to stresses (Lo et al., 1999; Mohammadi and Kazemi, 2002). Comparing the effects of germination on the levels of phenolic compounds (Dicko et al., 2005a) and the activities of phenolic compounds related enzymes (Dicko et al., 2005b), the levels of these compounds were found to be highly variable in sorghum varieties. Whether the levels of phenolic compounds and related enzymes in sorghum grain could be linked to the grain or plant resistance or susceptibility to stress is unknown. The aim of the present study was to identify possible markers for the grain or plant resistance or susceptibility to these stresses. This was done by comparing the levels of endogenous phenolic compounds and related enzymes in ungerminated and germinated sorghum kernels of known grain or plant agronomic properties for resistance or susceptibility to biotic and abiotic stresses.

METHODS AND MATERIALS

Chemicals. 4-Hydroxyanisole (4-HA) and gallic acid (3,4,5-trihydroxybenzoic acid) were obtained from Aldrich (Steinheim, Germany). 3,4-Dihydroxyphenylpropionic acid (DHPPA) was from Acros Organics (Geel, Belgium). Folin-Ciocalteu's reagent, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), insoluble polyvinylpyrrolidone (PVP), bovine serum albumin (BSA), and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) were from Sigma Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide was from Merck (Darmstadt, Germany). Sorghum apigeninidin, isolated and characterized by Kouda-Bonafos et al., (1996), was a gift of Dr. Eloi Palé from the Laboratory of Natural Substances, University of Ouagadougou, Burkina Faso. Cyanidin chloride was from Extrasynthèse (Lyon, France). Apple ciders procyanidin oligomers (average degree of polymerization = 7.4) were kindly provided by Dr. Stephanie Prigent (Wageningen University, Wageningen, The Netherlands) and Dr. Catherine M. G. C. Renard (INRA, Rennes, France). These procyanidins were purified by RP-HPLC and characterized by thiolysis-HPLC as described by Guyot et al. (2001). All other chemicals were of analytical grade.

TABLE 1. SORGHUM VARIETIES AND THEIR AGRONOMIC PROPERTIES[a]

Code	Variety name	Gen.[b]	Country of origin	Race	Grain testa	Color of grain/ glume/plant	Known particular properties
V1	Ajabsido	LR	Sudan	C	+	W/R/R	Preflowering drought resistant
V2	BF 88-2/31-1	IL	Burkina Faso	C	−	W/P/tan	–
V3	BF 88-2/31-3	IL	Burkina Faso	C	−	W/R/tan	Preflowering drought susceptible, sooty stripe susceptible, grain mold susceptible, postflowering drought resistant, lodging resistant
V4	BF 89-18/139-1-1	IL	Burkina Faso	C	−	W/P/tan	Postflowering drought susceptible
V5	Cauga 108-15	IL	Burkina Faso	GC	−	W/P/R	Photoperiod insensitive
V6	Cauga 22-20	IL	Burkina Faso	GC	+	W/P/R	Photoperiod sensitive
V7	CE 180-33	IL	Senegal	C	+	W/R/tan	Leaf anthracnose susceptible, photoperiod insensitive, lodging resistant
V8	CEF 322/53-1-1	IL	Burkina Faso	C	−	W/P/R	Postflowering drought resistant
V9	CEF 395/9-2-3	IL	Burkina Faso	GC	−	W/P/tan	Hard grains (PSI < 10)
V10	CEF 396/12-3-1	IL	Burkina Faso	GC	−	W/P/R	Hard grains (PSI < 10)
V11	CEM 326/11-5-1.1	IL	Mali	GC	−	W/P/tan	Leaf anthracnose resistant, photoperiod insensitive, postflowering drought susceptible, lodging resistant, Hard grains (PSI < 10)
V12	CGM 1/19-1-1	IL	Mali	G	−	W/P/R	Sorghum midge susceptible
V13	CGM 19/9-1-1	IL	Mali	G	−	W/B/R	Striga susceptible
V14	CGM 19/9-1-2	IL	Mali	G	−	W/P/R	–
V15	CK 60	IL	USA	K	−	W/P/R	Striga susceptible
V16	F2-20	IL	Burkina Faso	C	−	W/R/tan	Leaf anthracnose resistant, sorghum midge resistant, photoperiod sensitive, lodging resistant
V17	Farkakofsi 781	LR	Burkina Faso	G	+	R/B/R	–
V18	Framida	IL	South Africa	KC	+	R/C/R	Striga resistant, photoperiod sensitive
V19	G 1296	LR	Burkina Faso	GC	−	R/R/R	Good for dyeing, grain mold resistant

V20	G 1414	LR	Burkina Faso	G	−	W/P/R	Photoperiod sensitive
V21	G 1636	LR	Burkina Faso	G	−	W/P/tan	Photoperiod sensitive, Soft grains (PSI > 16)
V22	ICSV 1002	IL	Burkina Faso	C	−	W/P/tan	Leaf anthracnose resistant
V23	ICSV 1049	IL	Burkina Faso	C	−	W/P/tan	Sooty stripe resistant, photoperiod insensitive, postflowering drought resistant, lodging resistant
V24	ICSV 745	IL	India	C	−	W/P/tan	Sooty stripe susceptible, sorghum midge resistant
V25	IRAT 10	IL	Niger	C	−	W/B/R	Grain mold susceptible, drought resistant, photoperiod insensitive, lodging resistant
V26	IRAT 174	IL	Burkina Faso	C	−	W/C/R	Photoperiod sensitive, lodging resistant
			Senegal	C	+	W/R/tan	Preflowering drought resistant, photoperiod insensitive, lodging susceptible
V27	IRAT 202	IL					
V28	IRAT 204	IL	Senegal	C	−	W/P/tan	Sooty stripe and anthracnose susceptible, postflowering drought susceptible, photoperiod insensitive, lodging susceptible
V29	IRAT 277	IL	Burkina Faso	C	−	W/R/tan	Leaf anthracnose resistant, photoperiod insensitive, Soft grains (PSI > 16)
V30	IRAT 9	IL	Cameroon	C	+	R/C/R	Grain mold resistant, photoperiod insensitive
V31	IS 15401	LR	Cameroon	GC	−	W/P/R	Striga resistant
V32	Kaapelga	LR	Burkina Faso	G	−	W/P/tan	Photoperiod insensitive, postflowering drought resistant, Hard grains (PSI < 10)
V33	Kapla-57	LR	Burkina Faso	G	+	R/P/R	Sorghum midge susceptible
V34	Kokologho	LR	Burkina Faso	C	+	W/B/R	Postflowering drought resistant
V35	90L1235	IL	USA	GC	−	W/B/R	Sorghum midge resistant
V36	Magadji 1-509	LR	Burkina Faso	GC	−	R/B/R	Photoperiod insensitive
V37	Nafo-Natogué 775	LR	Burkina Faso	G	−	R/B/R	–
V38	Nazongala tan	IL	Burkina Faso	G	−	W/B/tan	Soft grains (PSI > 16)
V39	Nongomsoba	LR	Burkina Faso	G	−	W/B/tan	Soft grains (PSI > 16)
V40	S 29	LR	Burkina Faso	G	−	W/R/R	Striga susceptible
V41	Sariaso 10	IL	Burkina Faso	C	−	W/R/R	Postflowering drought resistant, photoperiod insensitive

TABLE 1. CONTINUED

Code	Variety name	Gen.[b]	Country of origin	Race	Grain testa	Color of grain/ glume/plant	Known particular properties
V42	Sariaso 11	LR	Burkina Faso	G	+	W/P/tan	Sooty stripe resistant, leaf anthracnose sensitive, striga resistant, postflowering drought resistant, photoperiod sensitive
V43	Sariaso 12	LR	Burkina Faso	G	+	W/B/R	Photoperiod insensitive, postflowering drought resistant
V44	Sariaso 14	IL	Burkina Faso	C	−	W/P/tan	Grain mold resistant, sorghum midge resistant, postflowering drought resistant, photoperiod insensitive, thumbtacks sensitive, lodging resistant
V45	Sariaso 9	LR	Burkina Faso	G	−	W/B/R	Sooty stripe resistant, photoperiod sensitive
V46	Segaolane	IL	Botswana	C	−	W/P/R	Preflowering drought resistant
V47	SRN 39	IL	Sudan	C	−	Y/P/tan	Striga resistant
V48	Tiamassie 289	LR	Burkina Faso	G	+	W/B/R	–
V49	Tx 7000	IL	USA	C	−	W/P/R	Postflowering drought susceptible
V50	Zugilga	LR	Burkina Faso	G	+	R/B/R	–

[a] C = Cautadum; G = Guinea; GC = Guinea-Caudatum; D = Durra; K = Karif; KC = Karif-Caudatum, R = red; W = white; Y = yellow; IL = inbred line; LR = Landrace; PSI = particle size index. Grain with (+) or without (−) pigmented testa layer; (–) = not known.
[b] Gen. = genetic type.

Sorghum Grains. Fifty sorghum varieties were grown during the rainy season of 2002 at the experimental station of Saria, in Burkina Faso (West Africa). The environment was semiarid (temperature: 30–42°C; annual precipitation: 850 mm). Growth conditions have been described previously (Dicko et al., 2005a). For convenience, the sorghum varieties were classified in alphabetic order of their name followed by arabic numbers: V1 to V50 (Table 1). Mature grains (≥60 days after anthesis) were harvested, surface-sterilized, and germinated as described previously (Dicko et al., 2002, 2005a). Germinated and ungerminated sorghum grains were dried, ground, and stored (Dicko et al., 2005a). Flours of both germinated and ungerminated sorghum varieties were analyzed. The varieties were grouped according to their resistance or susceptibility to biotic stresses, e.g., sooty stripe, sorghum midge, leaf anthracnose, striga, and grain molds; and abiotic stresses, e.g., lodging, drought resistance, and photoperiod sensitivity (Table 2). Information on resistance and susceptibility to these specific stresses was obtained from sorghum breeders from the experimental station of Saria and Farakoba (Institut National pour l'Environnement et la Recherche Agronomique, Burkina Faso).

Extraction and Quantification of Phenolic Compounds. Sorghum phenolic compounds were extracted and quantified as previously described (Dicko et al., 2002, 2005a). Results were expressed as phenolic equivalent per gram of flour (w/w), on dry matter basis. Phenolic compounds were extracted from 50 mg of sorghum flour by continuous stirring with 1.5 ml of 1% (v/v) HCl in methanol at 25°C for 20 min, followed by centrifugation (5000 *g*, 10 min, 25°C), and supernatant collection. The residue was reextracted with 0.5 ml HCl/methanol as described above, the supernatants were pooled, and denoted total phenolic extract. The total phenolic extract was used directly for analysis or kept in the dark at −30°C. The same extract was used for quantification of total phenolics, proanthocyanidins (PAs), 3-deoxyanthocyanidins (3-DAs), and flavan-4-ols. The total phenol content was determined using the Folin-Ciocalteu's method adapted to a 96-well plate assay. To 10 µl of extract, 25 µl of Folin-Ciocalteu's reagent (50%, v/v) were added. After 5 min of incubation, 25 µl of 20% (w/v) sodium carbonate solution and 165 µl water were added. Blanks were prepared for each sorghum sample by replacing Folin-Ciocalteu's reagent with water. Gallic acid was used as a standard. The standard was always freshly prepared. The absorbances were measured after 30 min at 760 nm.

PAs and flavan-4-ols were assayed essentially as described by Melake-Berhan et al. (1996) with miniaturization to adapt the assay to a 96-well plate format as follows. To determine flavan-4-ols, 50 µl of the extract were added to 700 µl of reagent A (30%, v/v 12 M HCl in butan-1-ol) or to 700 µl of reagent B (15%, v/v 0.1 M acetic acid; 15%, v/v, methanol and 70%, v/v, butan-1-ol). The sample in reagent A was mixed by vortex and left at 25°C, for 1 hr, to allow formation of anthocyanidin pigments derived from flavan-4-ols (Melake-Berhan

TABLE 2. AGRONOMIC PROPERTIES OF SORGHUM VARIETIES

Type of stress	Plant/grain agronomic properties	Variety code
Biotic	Sooty stripe resistant ($N = 3$)	V23, V42, V45
	Sooty stripe susceptible ($N = 3$)	V3, V24, V28
	Anthracnose resistant ($N = 4$)	V11, V16, V22, V29
	Anthracnose susceptible ($N = 3$)	V7, V28, V42
	Sorghum midge resistant ($N = 4$)	V16, V24, V35, V44
	Sorghum midge susceptible ($N = 2$)	V12, V33
	Striga resistant ($N = 4$)	V18, V31, V42, V47
	Striga susceptible ($N = 3$)	V13, V15, V40
	Grain mold resistant ($N = 3$)	V19, V30, V44
	Grain mold susceptible ($N = 2$)	V3, V25
Abiotic	Lodging resistant ($N = 7$)	V3, V10, V16, V23, V25, V26, V44
	Lodging susceptible ($N = 2$)	V27, V28
	Preflowering drought resistant ($N = 3$)	V1, V27, V46
	Preflowering drought susceptible ($N = 1$)	V3
	Postflowering drought resistant ($N = 8$)	V3, V23, V32, V34, V41, V42, V43, V44
	Postflowering drought susceptible ($N = 4$)	V4, V11, V28, V49
	Photoperiod insensitive ($N = 14$)	V5, V7, V11, V23, V25, V27, V28, V29, V30, V32, V36, V41, V43, V44
	Photoperiod sensitive ($N = 7$)	V6, V18, V20, V21, V26, V42, V45

et al., 1996). Aliquots of the mixture (150 µl) were put in duplicate into a 96-multiwell plate, and the absorbance was read at 550 nm to quantify anthocyanidins formed from flavan-4-ols (Melake-Berhan et al., 1996). Cyanidin was used as standard to estimate the total amount of the anthocyanidins derived from flavan-4-ols. For quantification of PAs, the sample remaining in the tube with reagent A was further heated at 100°C, for 2 hr. Under these conditions, PAs are converted to anthocyanidins, and the unstable pigments formed from flavan-4-ols are destroyed. After cooling, 200 µl of the sample were put in duplicate in a 96 multiwell plate, and the absorbances of anthocyanidin compounds derived from PAs were read at 550 nm. Sample mixtures with reagent B, which were not heated, served as blanks for the quantification of both PAs and flavan-4-ols. Apple procyanidins with an average degree of polymerization of 7.4 and treated as indicated above were used as standards for sorghum PA quantification (Dicko et al., 2005a). For direct spectrophotometric quantification of 3-DAs, 50 µl of the total phenolic extract were mixed with 150 µl of methanol and the absorbances were read at 475 nm (Melake-Berhan et al., 1996). Sorghum apigeninidin was used as standard.

Enzyme Extraction and Determination of Protein Concentration. Enzyme extraction and total protein quantification were performed as described

previously (Dicko et al., 2002, 2005b). Enzyme extracts were prepared by mixing 250 mg of sorghum flour with 1.2 ml of 50 mM Tris–HCl buffer (pH 7.3) containing 0.5 M $CaCl_2$ and 2% (w/v) polyvinylpyrrolidone, at 4°C for 1 hr. The homogenate was centrifuged (14,000 *g*, 4°C, 45 min), and the resulting supernatant was denoted enzyme extract of PPO, POX, and PAL. Total protein was quantified by the linearized method of Bradford, using bovine serum albumin as standard.

Determination of Enzyme Activities. PAL activity was evaluated by measuring *trans*-cinnamic acid formation from L-phenylalanine as described previously (Tovar et al., 2002). Commercial sodium *trans*-cinnamate was used as standard. The spectrophotometric assay for PPO was performed as described previously (Dicko et al., 2002). 4-Hydroxyanisole (4-HA) and 3,4-dihydroxyphenylpropionic acid (DHPPA) were used as substrates to determine the monophenolase and *o*-diphenolase activities of PPO, respectively. The enzyme extract (10 μl) was incubated with 150 μl 50 mM sodium acetate, pH 5.5, 10 μl 40% (v/v) DMF and 10 μl 50 mM 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) at 25°C for 5 min. The reaction was started by addition of 20 μl 100 mM of the phenolic substrate (prepared in 0.15 mM phosphoric acid). The reaction was monitored at 500 nm. Blanks, in which the enzyme extracts or substrates were replaced by buffer, were performed. One unit of PPO activity (U) is defined as the amount of enzyme producing 1 μmol MBTH-*o*-quinone adducts/min resulting from the oxidation of 4-HA or DHPPA. Prior to determination of POX activity, extracts from ungerminated and germinated sorghums were diluted 400- and 1000-fold, respectively, in 50 mM Tris–HCl, pH 7.3, containing 0.5 M $CaCl_2$ and 1 mg/ml bovine serum albumin. POX activity was measured spectrophotometrically by monitoring the H_2O_2-dependent oxidation of ABTS, at 25°C (Dicko et al., 2002). The reaction mixture consisted of 10 μl of 200-fold diluted crude enzyme extract, 20 μl of 100 mM ABTS, 10 μl of 100 mM H_2O_2, and 160 μl of 50 mM sodium acetate, pH 4.0. Blanks, in which the enzyme extract or substrates were replaced by buffer, were performed. The reaction was monitored at 414 nm. One unit of POX activity (U) is defined as the amount of enzyme releasing 1 μmol ABTS radical/min under the assay conditions.

Statistical Analyses. Statistical analyses were carried out as previously described (Dicko et al., 2002, 2005a). All spectrophotometric assays were carried out in 96-well microtiter plates (Nunclon, Denmark) using a multiwell plate reader (μQuant Bio-Tek Instrument, Inc.) interfaced with a personal computer. Absorbances and slopes of absorbances per min (OD/min) were automatically recorded using KC junior software version 1.31.5 (Bio-Tek Instrument, Inc, USA). All assays were carried out at least in duplicate. Results were subjected to ANOVA performed with Statistica version 4.5 software for Windows. Significant differences in mean performance for each composition among sorghum varieties were determined by Student's *t*-test, where $P < 0.05$ implies significance.

RESULTS AND DISCUSSION

The contents of phenolic compounds and the activities of related enzymes in varieties grouped according to their agronomic properties are presented in Table 3. Difference in composition was determined using Student's *t*-test between varieties resistant and varieties susceptible to specific biotic or abiotic stresses (Table 4). Furthermore, the levels of significance were confirmed with ANOVA.

Correlations between Phenolic Compounds and Related Enzymes and Resistance or Susceptibility to Biotic Stress. In the present study, independent of grain germination, no significant difference in total phenolic compound content between sorghum varieties susceptible and those resistant to biotic stresses was found. However, leaves of sorghum resistant to fungi contained a higher content of total phenolics than leaves of susceptible ones upon pathogen challenge (Luthra et al., 1988). This suggests that total phenolic compounds content in sorghum grains, which are not challenged by pathogens, is not a good indicator for resistance to biotic stress.

When focusing on individual classes of phenolic compounds, it can be seen that PA levels were higher on average in varieties resistant to sooty stripe, sorghum midge, and grain mold than susceptible ones. In contrast, resistance to anthracnose and striga was not correlated with content of PAs. The high content of PAs in varieties resistant to mold is in agreement with previous observations showing that high PA containing sorghums are generally resistant to grain molding and weathering (Waniska et al., 2001). Sorghum midge resistance is also associated with PAs content in another study (Sharma and Hariprasad, 2002).

3-DA content is higher in all varieties resistant to the referred biotic stresses compared to that of susceptible ones. This indicates a function as phytoalexins (Weiergang et al., 1996; Lo et al., 1999). For example, the content of 3-DAs, notably apigeninidin, is an indicator of grain resistance to fungi such as *Colletrichum graminicola*, *Fusarium oxysporum*, *Gibberelle zeae*, and *Gliocladium roseum* (Schutt and Netzly, 1991) and of sorghum resistance to anthracnose (Lo et al., 1999). Colored phenolic compounds, probably 3-DAs, were suggested to be involved in sorghum resistance to striga (Arnaud et al., 1999). Recently, the importance of phenolic compounds as allelochemicals, involved in plant–parasite interactions, were indicated (Weir et al., 2004). Our results suggest that the content of 3-DAs in sorghum grain is not only an indicator of resistance to mold, but also to sooty stripe, anthracnose, sorghum midge, and striga.

The content of flavan-4-ols is on average higher in varieties resistant to mold than in susceptible ones. This agrees with previous findings showing that flavan-4-ol content is an indicator of grain resistance to molds (Schutt and Netzly, 1991; Melake-Berhan et al., 1996). Moreover, the results presented here show that

independent of germination, the flavan-4-ol content was on average higher in varieties resistant to sooty stripe, sorghum midge, leaf anthracnose, and striga than in susceptible ones. This suggests that the flavan-4-ol content in sorghum grain is a good indicator of plant resistance to sooty stripe, sorghum midge, and striga. For leaf anthracnose, no correlation could be found with the grain content of flavan-4-ols.

Before germination, PAL activity was higher in varieties resistant to sooty stripe and sorghum midge than in susceptible ones. However, after germination there was no difference. PAL activity in germinated varieties resistant to anthracnose was higher than in susceptible ones. This supports previous findings that high PAL activity in sorghum is associated with resistance to *Colletotrichum* species (Kamida et al., 2000).

POX and PPO activities before germination could not be linked to resistance or susceptibility to biotic stresses. Germination did not affect this trend for POX. After germination, the mono-PPO activity increased in the sooty stripe resistant varieties and decreased in anthracnose resistant varieties. These results are in agreement with findings that PPO may confer resistance to some fungal species such as *R. sorghicola* (sooty stripe agent) (Luthra et al., 1988).

Correlations between Levels of Phenolic Compounds and Related Enzymes, and Resistance Susceptibility to Abiotic Stress. Varieties resistant to lodging had significantly lower PAs than susceptible ones, both before and after germination. From these results, it may be inferred that the PA content of the grain is a marker for plant resistance to lodging. Independent of grain germination, varieties resistant to both preflowering and postflowering drought contained apparently more PAs, 3-DAs, and flavan-4-ols than susceptible ones. Results indicate the importance of polyphenols in drought resistance.

For other plants (*Mangifera indica*), the phenolic compound content is influenced by their response to light (Tovar et al., 2002). However, no significant difference in content of total phenolic compounds, PAs, 3-DA, or flavan-4-ols was found between the grain of sorghum varieties resistant or susceptible to photoperiod.

POX activity in ungerminated grains was not significantly different between varieties resistant or susceptible to abiotic stresses. After germination, POX activity appeared to be on average higher in varieties resistant to lodging than in susceptible ones. High activity of POX in varieties resistant to lodging could be related to the role of POX in the formation of physical polymeric barriers such as suberin and lignin (Cosgrove, 1997; Quiroga et al., 2000), which might confer the plant with high rigidity.

Specific POX isoenzymes in leaves, notably the cationic ones, are correlated with photoperiod sensitivity in sorghum, and play a role in plant adaptation (Pao and Morgan, 1988). However, in the present study POX activity of the grain could not be linked to the plant sensitivity to photoperiod.

TABLE 3. PHENOLIC COMPOUNDS AND PHENOLIC ENZYMES IN GROUPS OF SORGHUM VARIETIES RESISTANT AND SUSCEPTIBLE TO STRESSES

Type of stress		Total phenolics (%)[a]	PAs (%)	3-DAs (%)	Flavan-4-ols (%)	PAL (mU/mg)[b]	POX (U/mg)	Mono-PPO (mU/mg)	Dipheno-PPO (mU/mg)
					Ungerminated grains				
Biotic	Sooty stripe resistant ($N = 3$)	0.79	0.22	0.06	0.18	6.2	24.5	0.9	47.3
	Sooty stripe susceptible ($N = 3$)	0.78	nd	nd	nd	2.3	20.7	0.7	43.6
	Anthracnose resistant ($N = 4$)	0.73	nd	0.02	nd	6.0	34.9	0.9	42.9
	Anthracnose susceptible ($N = 3$)	0.76	0.09	nd	nd	4.7	36.2	1.0	47.7
	Sorghum midge resistant ($N = 4$)	0.74	0.45	0.05	0.16	6.3	30.8	0.8	45.5
	Sorghum midge susceptible ($N = 2$)	0.62	0.05	nd	nd	2.7	55.1	1.0	41.4
	Striga resistant ($N = 4$)	1.06	0.39	0.05	0.24	6.7	37.6	1.1	48.1
	Striga susceptible ($N = 3$)	0.70	0.13	0.04	nd	9.0	43.2	0.9	41.8
	Grain mold resistant ($N = 4$)	1.65	1.31	0.25	0.35	4.5	21.7	0.8	48.0
	Grain mold susceptible ($N = 2$)	0.77	0.05	nd	nd	4.0	19.2	0.9	41.0
Abiotic	Lodging resistant ($N = 7$)	0.68	0.06	0.03	nd	5.4	29.7	0.9	46.4
	Lodging susceptible ($N = 2$)	1.08	0.53	nd	nd	2.2	15.5	0.9	46.8
	Preflowering drought resistant ($N = 3$)	0.80	0.24	0.04	nd	nd	40.2	1.0	45.2
	Preflowering drought susceptible ($N = 1$)	0.72	nd	nd	nd	2.3	17.4	0.7	39.3
	Postflowering drought resistant ($N = 8$)	0.71	0.26	0.04	0.17	4.9	22.6	0.9	46.4
	Postflowering drought susceptible ($N = 4$)	0.69	0.07	nd	nd	3.2	38.1	0.7	46.0
	Photoperiod insensitive ($N = 14$)	0.82	0.23	0.05	0.23	4.5	38.6	0.9	45.2
	Photoperiod sensitive ($N = 7$)	1.03	0.44	0.04	0.22	4.6	28.6	0.9	47.6

					Germinated grains				
Biotic	Sooty stripe resistant ($N = 3$)	0.70	0.14	0.04	0.12	7.06	64.3	1.3	31.8
	Sooty stripe susceptible ($N = 3$)	0.59	nd	nd	nd	6.80	50.9	0.8	22.8
	Anthracnose resistant ($N = 4$)	0.72	nd	nd	nd	3.04	89.3	0.4	16.8
	Anthracnose susceptible ($N = 3$)	0.68	0.05	nd	nd	11.81	66.6	1.0	25.9
	Sorghum midge resistant ($N = 4$)	0.73	0.34	0.07	nd	8.54	85.0	1.1	34.7
	Sorghum midge susceptible ($N = 2$)	0.89	0.06	0.02	nd	8.37	96.3	0.9	29.0
	Striga resistant ($N = 4$)	1.00	0.29	0.04	0.17	7.49	87.9	1.6	28.3
	Striga susceptible ($N = 3$)	1.02	0.17	0.05	nd	12.38	97.6	1.0	16.7
	Grain mold resistant ($N = 4$)	1.59	1.02	0.14	0.32	8.86	82.0	1.6	27.0
	Grain mold susceptible ($N = 2$)	0.71	0.05	nd	nd	6.10	89.2	0.8	20.8
Abiotic	Lodging resistant ($N = 7$)	0.76	0.09	0.03	nd	5.33	92.3	0.8	22.1
	Lodging susceptible ($N = 2$)	0.92	0.28	nd	nd	6.47	16.0	0.7	16.1
	Preflowering drought resistant ($N = 3$)	0.96	0.16	0.05	nd	7.59	54.2	1.0	22.6
	Preflowering drought susceptible ($N = 1$)	0.69	nd	nd	nd	9.86	79.9	0.8	24.4
	Postflowering drought resistant ($N = 8$)	0.78	0.24	0.05	0.15	10.65	58.7	1.4	35.6
	Postflowering drought susceptible ($N = 4$)	0.64	0.06	nd	nd	7.71	71.8	0.8	20.1
	Photoperiod insensitive ($N = 14$)	0.84	0.17	0.03	0.20	9.39	74.0	1.1	24.2
	Photoperiod sensitive ($N = 7$)	0.96	0.32	0.04	0.16	8.04	79.4	1.1	22.5

PAs = proanthocyanidins (w/w, procyanidin equivalents); 3-DAs = 3-deoxyanthocyanidins (w/w apigeninidin equivalents); PAL = phenylalanine ammonia lyase; POX = peroxidase; mono-PPO = monophenolase activity of polyphenol oxidase (PPO); dipheno-PPO = diphenolase activity of PPO.

[a] Total phenolic compounds (gallic acid equivalents).

[b] All enzymes activities are expressed in terms of specific activities (U/mg of protein in the crude extracts).

TABLE 4. STUDENT *t*-TEST RESULTS INDICATING LEVEL OF SIGNIFICANCE IN COMPOSITION OF PHENOLIC COMPOUNDS AND RELATED ENZYMES FOR PAIRED COMPARISON BETWEEN SORGHUM VARIETIES RESISTANT AND SUSCEPTIBLE TO STRESSES

Type of stress	Group of varieties	Total phenolics	PAs	3-DAs	Flavan-4-ols	PAL	POX	Mono-PPO	Di-PPO
					Ungerminated grains				
Biotic	Sooty stripe resistant ($N = 3$)	NS	+	+	+	+	NS	NS	NS
	Sooty stripe susceptible ($N = 3$)	NS	−	−	−	−	NS	NS	NS
	Anthracnose resistant ($N = 4$)	NS	−	+	NS	NS	NS	NS	NS
	Anthracnose susceptible ($N = 3$)	NS	+	−	NS	NS	NS	NS	NS
	Sorghum midge resistant ($N = 4$)	NS	+	+	+	+	NS	NS	NS
	Sorghum midge susceptible ($N = 2$)	NS	−	−	−	−	NS	NS	NS
	Striga resistant ($N = 4$)	NS	NS	+	+	NS	NS	NS	NS
	Striga susceptible ($N = 3$)	NS	NS	−	−	NS	NS	NS	NS
	Grain mold resistant ($N = 4$)	NS	+	+	+	NS	NS	NS	NS
	Grain mold susceptible ($N = 2$)	NS	−	−	−	NS	NS	NS	NS
Abiotic	Lodging resistant ($N = 7$)	NS	−	+	NS	+	NS	NS	NS
	Lodging susceptible ($N = 2$)	NS	+	−	NS	−	NS	NS	NS
	Preflowering drought resistant ($N = 3$)	NS	+	+	NS	−	NS	NS	NS
	Preflowering drought susceptible ($N = 1$)	NS	−	−	NS	+	NS	NS	NS
	Postflowering drought resistant ($N = 8$)	NS	+	+	+	NS	NS	NS	NS
	Postflowering drought susceptible ($N = 4$)	NS	−	−	−	NS	NS	NS	NS
	Photoperiod insensitive ($N = 14$)	NS	NS	NS	NS	NS	NS	NS	NS
	Photoperiod sensitive ($N = 7$)	NS	NS	NS	NS	NS	NS	NS	NS

					Germinated grains				
Biotic	Sooty stripe resistant ($N = 3$)	NS	+	+	+	NS	NS	+	NS
	Sooty stripe susceptible ($N = 3$)	NS	−	−	−	NS	NS	−	NS
	Anthracnose resistant ($N = 4$)	NS	−	+	NS	+	NS	−	NS
	Anthracnose susceptible ($N = 3$)	NS	+	−	NS	−	NS	+	NS
	Sorghum midge resistant ($N = 4$)	NS	+	+	NS	NS	NS	NS	NS
	Sorghum midge susceptible ($N = 2$)	NS	−	−	NS	NS	NS	NS	NS
	Striga resistant ($N = 4$)	NS	NS	+	+	NS	NS	NS	NS
	Striga susceptible ($N = 3$)	NS	NS	−	−	NS	NS	NS	NS
	Grain mold resistant ($N = 4$)	NS	+	+	+	NS	NS	NS	NS
	Grain mold susceptible ($N = 2$)	NS	−	−	−	NS	NS	NS	NS
Abiotic	Lodging resistant ($N = 7$)	NS	−	+	NS	NS	+	NS	NS
	Lodging susceptible ($N = 2$)	NS	+	−	NS	NS	−	NS	NS
	Preflowering drought resistant ($N = 3$)	NS	+	+	NS	NS	NS	NS	NS
	Preflowering drought susceptible ($N = 1$)	NS	−	−	NS	NS	NS	NS	NS
	Postflowering drought resistant ($N = 8$)	NS	+	+	+	NS	NS	+	+
	Postflowering drought susceptible ($N = 4$)	NS	−	−	−	NS	NS	−	−
	Photoperiod insensitive ($N = 14$)	NS	NS	NS	NS	NS	NS	NS	NS
	Photoperiod sensitive ($N = 7$)	NS	NS	NS	NS	NS	NS	NS	NS

PAs = proanthocyanidins; 3-DAs = 3-deoxyanthocyanidins; PAL = phenylalanine ammonia lyase; POX = peroxidase; mono-PPO = monophenolase activity of polyphenol oxidase (PPO); dipheno-PPO = *o*-diphenolase activity of PPO.
NS, not significant ($P > 0.05$); +, significantly high ($P < 0.05$); −, significantly low ($P < 0.05$).

Before grain germination, PPO activities could not be linked to abiotic stresses. After germination, both the monophenolase and *o*-diphenolase activities of PPO were higher in postflowering drought resistant varieties than in susceptible ones. This suggests a role of PPO in postflowering drought resistance, in agreement with earlier findings (Mayer and Harel, 1991). Independent of grain germination, PPO activities could not be related to resistance to lodging or photoperiod. PAL activity in the grain cannot be used as a marker for resistance to lodging, drought, and photoperiod variation as well.

Overall Impact of Phenolic Compounds and Related Enzymes in Stress Resistance. Results in Table 4 show that 3-DA content is a good marker for sorghum resistance to both biotic and abiotic stresses because 3-DAs are positively correlated with resistance to all stresses except for photoperiod sensitivity. The second marker for stress resistance is PA content. Total phenolic compounds and the activities of related enzymes are not good markers for stress resistance in sorghum grains. For photoperiod sensitivity, none of the screened biochemical compounds could be used as a marker of resistance in sorghum grain.

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