Biochemical Characterization of Recently Identified Grain Mold Resistant Sorghum Lines

Rajan Sharma, M Peter Vijay, V P Rao and R P Thakur

International Crops Research Institute for the Semi-Arid Tropics, Patancheru - 502 324, Andhra Pradesh, India.
E mail: r.sharma@cgiar.org

Grain mold, the most important and widespread disease of sorghum worldwide, is a major constraint to sorghum productivity. Production losses due to this disease range from 30 to 100% depending on cultivars, growing season and prevailing weather conditions during flowering to harvesting (Singh and Bandyopadhyay, 2000). Grain mold can be broadly defined as preharvest grain deterioration caused by several fungal species interacting parasitically and/or saprophytically with developing grain. Fungi belonging to more than 40 genera are associated with molded grains. Among them *Fusarium* species, *Curvularia lunata* and *Alterneria alternata* are major pathogens of worldwide significance. Some species of *Fusarium* (*F. andiyagi, F. proliferatum, F. thapsinum, F. sacchari* and *F. verticillioides*) involved in grain mold complex produce mycotoxins, such as fumonisins, moniliformin, trichothecenes, and fusaproliferin (Leslie *et al.* 2005). These toxins are harmful to human- and animal-health and also reduce the quality and marketability of grains as food/feed sources (Thakur *et al.* 2006). The disease is particularly important on improved, short- and medium-duration sorghum cultivars that mature during the rainy season in humid, tropical and sub-tropical climates.

Management of grain mold in the rainy season sorghum cultivars, particularly hybrids, is a difficult proposition. Of the several approaches that have been tried, host-plant resistance appears to be the more viable and effective method of managing grain mold. Resistance to grain mold is a complex trait and several morphological/biochemical traits have been shown to be associated with resistance (Audilakshmi *et al.*, 1999). Total phenols and phenolics have long been considered as important defense related compounds whose levels are naturally high in the resistant plants. Phenolic compounds in sorghum caryopses are reported to improve resistance to insects, fungi and other pathogens (Butler, 1982), and cultivars with high tannin content have been used as a source of mold resistance (Esele *et al.*, 1993). To determine the association of phenolics with grain mold resistance in sorghum, we estimated Flavon-4-ols and peroxidase activity in six susceptible (SGMR 3-3-5-6, PVK 801-4, IS 36469C 1187-1-2-9-8-2, SP 72521-2-6-6-6, SPV 104, Bulk Y) and 11 resistant (IS 12932-2, IS 13969-1, SGMR 24-5-1-2, SGMR 11-3-5-1, ICSB 377, IS 8219-1, SGMR 40-1-2-3, IS 41397-3, ICSB 402-3, ICSB 402-1-2, SPV 462-3) lines of sorghum. The sorghum lines with diverse physio-morphological traits were selected for the study (Table 1).

Two sets of seed, one from crop raised in *Rabi* (healthy) and second from the *Kharif* crop raised under natural epiphytotic conditions (infected) were used for flavon-4-ols estimation. Grain samples were extracted with methanol and acidified methanol. To 0.5 mL aliquot of the extract, 7 mL water saturated butanol was added. Simultaneously a blank was prepared by mixing methanol, water saturated butanol and 0.1 N acetic acid in a 70:15:15 ratio v/v. The tubes along with the blank were rotated in the test tube rotator for 1 h. The absorbance was read at 550 nm in a spectrophotometer. Results were calculated as A_{550} g^{-1} dry weight sample (Butler 1982).

Isozyme analysis for peroxidase enzyme was carried out through native Polyacrylamide gel electrophoresis (Native-PAGE). The seed samples were surface sterilized with chlorax for 3 minutes and then washed 2-3 times with sterile distilled water. The sterilized seeds were then plated in humidity chambers (50 seeds/chamber) and incubated at 30°C for 5-6 days. The plumules of the seedlings were used as the source material for analysis. The proteins were extracted by grinding 500 mg of sample in 2 mL of Tris HCl buffer (0.05M, pH 7.4) with a pre-cooled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C in a cooling centrifuge (Beckmann). The protein content in the supernatant was determined by the method of Lowry *et al.* (1951). The samples were prepared for native-PAGE in a sample buffer (Tris buffer 0.5M, glycerol and 0.1% Bromophenol blue) and loaded into the wells such that each well contains 50 µg of protein. Electrophoresis was performed at 50V for 7-8 h in a vertical gel electrophoresis unit using a gradient gel of 10-15% concentration. Peroxidase isoforms were visualized by incubating the gel for 30 min in a solution containing 250 mg O-Dianisidine dissolved in 140 mL of 95% ethanol and 28 mL of 0.2M acetate buffer (pH 4.8) and the final volume made up to 200 mL with water. Hydrogen peroxide

(3%) was added to obtain a final concentration of 0.005%. The reaction was stopped by placing the gel into a large volume of acetic acid for 10 min. The relative front (R_f) values were then calculated by measuring the distance traveled by each band relative to the total distance traveled by the dye front (McDonald and Smith, 1972).

Table 1. Estimation of flavon-4-ols in infected (I) and healthy (H) grains of grain mold resistant (R) and susceptible (S) sorghum lines

Entry	Disease reaction	DTF	Plant ht (cm)	Panicle type	Glumes cov (%)	Glumes color	Grain color	Grain used	Mean Flavon-4-ols	
									Methanol	H+/ Methanol
IS 12932-2	R	77	295	SL	75	W	B	H	3.6	4.3
								I	3.4	1.1
IS 13969-1	R	78	250	C	50	B	B	H	9.6	5.2
								I	10.9	4.6
SGMR 24-5-1-2	R	80	165	SL	50	R	R	H	1.4	1.9
								I	3.9	3.6
SGMR 11-3-5-1	R	80	170	SL	50	W	W	H	1.2	1.5
								I	1.6	1.2
ICSB 377	R	71	175	SC	50	B	B	H	8.6	3.0
								I	8.7	4.2
IS 8219-1	R	80	210	SC	50	B	B	H	11.0	5.1
								I	4.3	3.8
IS 41397-3	R	66	165	SC	25	W	W	H	0.6	0.3
								I	0.2	0.8
SGMR 40-1-2-3	R	58	155	SC	25	B	B	H	3.2	3.1
								I	5.3	3.6
ICSB 402-3	R	62	160	SL	25	B	B	H	1.3	3.3
								I	7.6	4.4
ICSB 402-1-2	R	62	175	SC	25	B	B	H	4.4	3.9
								I	6.2	3.1
SPV 462-3	R	66	255	SC	25	W	W	H	0.3	0.6
								I	2.8	0.4
PVK 801-4	S	81	185	SC	25	R	W	H	0.8	0.3
								I	0.8	0.4
SGMR 3-3-5-6	S	77	180	SL	25	R	W	H	0.4	0.6
								I	0.8	0.4
IS 36469C1187-1-2-9-8-2	S	70	175	SC	50	R	W	H	0.5	0.2
								I	0.3	0.5
SP 72521-2-6-6-6	S	85	130	SC	25	W	W	H	0.3	0.2
								I	0.3	0.2
SPV 104	S	70	180	C	25	W	W	H	0.2	0.3
								I	0.3	0.4
Bulk Y	S	50	125	L	25	R	W	H	0.1	0.3
								I	0.4	0.3
Mean									3.2	2.0
LSD (P<0.05)									0.4	0.3

DTF=Days to 50% flowering; Panicle type: C=Compact; SC=Semi-compact; SL=Semi-loose; L=Loose
Glumes/grain color: W=White; B=Brown; R=Red

Mean Flavon-4-ols values of grain mold resistant and susceptible sorghum lines were distinctly different from each other. More Flavon-4-ols accumulated in the methanol and acidified methanol extracts of both infected as well as healthy grains of resistant lines than those in susceptible lines (Table 2). Mean flavon-4-ols of all 11 resistant lines in the methanol and acidified methanol extracts from infected grains were 4.97 and 2.77 and those for healthy grains were 4.10 and 2.90, respectively. Similarly, for all six susceptible lines, mean values of flavon-4-ols accumulated in infected grain were 0.46 (methanol) and 0.32 (acidified methanol), and 0.37 (methanol) and 0.29 (acidified methanol) in healthy grains. Range of Flavon-4-ols extracted both in methanol (0.15-10.85 in infected and 0.30-10.95 in healthy grains) as well as acidified methanol (0.35-4.6 in infected and 0.25-5.10 in healthy grains) was much greater in resistant lines than that in susceptible lines (0.1-0.75). Significantly higher levels of Flavon-4-ols were detected in the colored grains than in the white grains (Table 1). Majority of grain mold resistant germplasm lines have been reported to have a pigmented testa and high tannin levels (Menkir *et al.*, 1996). In general, infected grains recorded more Flavon-4-ols than healthy grains (Table 1). Considerable increase in the Flavon-4-ols content in the methanol extract was also observed in the infected grains of a white-grain resistant line SPV 462-3. This indicates that expression levels of Flavon-4-ols in the biosynthetic pathway are enhanced in response to the pathogen attack. Therefore, differential levels of Flavon-4-ols could be used as a biochemical marker for grain mold resistance.

Table 2. Comparison of Flavon-4-ols in the resistant and susceptible sorghum lines

			Flavon-4-ols content			
			Mean		Range	
Disease reaction	Number of lines	Infected/healthy	Methanol	H+/Methanol	Methanol	H+/Methanol
Resistant	11	Infected	4.97	2.77	0.15-10.85	0.35-4.6
		Healthy	4.10	2.90	0.30-10.95	0.25-5.1
Susceptible	6	Infected	0.46	0.32	0.25-0.75	0.15-0.45
		Healthy	0.37	0.29	0.10-0.75	0.15-0.55
Trial LSD ($P<0.05$)		0.40	0.34			

Native-PAGE profiles of peroxidase enzymes in resistant and susceptible sorghum lines are presented in Figure 1. Eight isoforms of peroxidase were observed across the resistant and susceptible lines. Of the eight isoforms, one was common in all the lines. Other isoforms had differential expressions. Two distinct isoforms (R_f 0.25 and 0.32) were present in the resistant lines IS 13969, ICSB 377 and IS 8219-1 indicating line/genotype-specific association of peroxidases with disease resistance. Peroxidase has been reported to be involved in cross-linking extension molecules to form lignin (Brisson *et al.*, 1994). Increased lignin

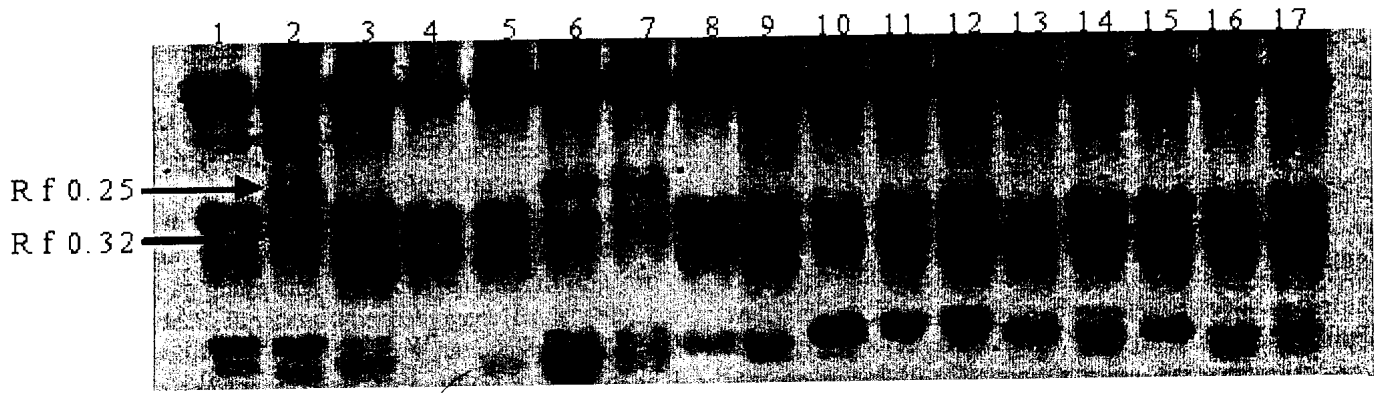

Figure 1. Native-page profiles of peroxidase enzymes in grain mold resistant (1, 2, 3, 4, 6, 7, 9, 10, 11, 12 and 13) and susceptible (5, 8, 14, 15, 16 and 17) sorghum lines
Lane 1-17 represent sorghum lines (sl no. 1-17) in Table 1.

deposition is believed to play a role in barricading the pathogen from invading the plant through physical exclusion (Milosevic and Slusarenko, 1996).

It is concluded from the results of this study that high levels of flavon-4-ols (about 4.0 in methanol and 3.0 in H+/ methanol extracts) and peroxidase (isoforms at R_f 0.25 and 0.32) in the sorghum lines could govern grain mold resistance. Therefore, measurement of Flavon-4-ols and peroxidase activity in the sorghum seed can give an initial indication about the grain mold reaction in sorghum cultivars.

References

Audilakshmi S, Stenhouse J W, Reddy T P and Parasad M V R 1999. Grain mold resistance and associated characters of sorghum genotypes. *Euphytica* **107 :** 91-103.

Brisson L F, Tenhaken R and Lamb C J 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* **6 :** 1703-1712.

Butler L G 1982. Relative degree of polymereization of sorghum tannin during seed development and maturation. *Journal of Agriculture and Food Chemistry* **30 :** 1090-1094.

Esele J P, Frederiksen R A and Miller F R. 1993. The association of genes controlling caryopsis traits with grain mold resistance in sorghum. *Phytopathology* **83 :** 490-495.

Leslie J F, Zeller K A, Lamperecht S C, Rheeder J P and Marasas W F O 2005. Toxicity, pathogenicity, and genetic differentiation of five species of Fusarium from sorghum and millet. *Phytopathology* **95 :** 275-283.

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951. Protein measurement with the Folin-Phenol reagents. *The Journal of Biological Chemistry* **193 :** 265-275.

McDonald T and Smith H H 1972. Variation associated with an Aegilops chromosome segment incorporated in wheat. II. Peroxidase and leucine aminopeptidase isoenzymes. *Genetics* **72 :** 77-86.

Menkir A, Ejeta G, Buttler L and Melakeberhan A 1996. Physical and chemical kernel properties associated with resistance to grain mold in sorghum. *Cereal Chemistry* **73 :** 613-617.

Milosevic N and Slusarenko A J 1996. Active oxygen metabolism and lignification in the hypersensitive reaction in bean. *Physiological and Molecular Plant Pathology* **49 :** 143-157.

Singh S D and Bandyopadhyay R 2000. Grain mold. Pages 38-40 *in* Compendium of Sorghum Diseases. Second Edition, The American Phytopathological Society, (Frederiksen RA and Odvody GN, eds). St. Paul, MN, USA. APS Press.

Thakur R P, Reddy B V S, Indira S, Rao VP, Navi S S, Yang X B and Ramesh S 2006. Sorghum grain mold. Information Bulletin no. 72, Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp.

Received : 20-05-09 Accepted : 22-12-09