Physiological Changes in Sorghum Antifungal Proteins

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Changes in sorghum caryopsis antifungal proteins (AFP) in different tissues and during development, imbibition, and germination, were determined. Sormatin, chitinase, and glucanase levels increased during caryopsis development and peaked at physiological maturity (30 days after anthesis (DAA)). Ribosome-inactivating protein levels were higher at 15 DAA and decreased subsequently. Sormatin and chitinase levels were significantly different between sorghum cultivars. Sormatin content at physiological maturity correlated with mold rating ($R^2 = 0.65$). Seed AFPs were present in the endosperm and migrated toward the exterior of caryopsis upon imbibition. AFPs leached out of immature seeds but were retained in the pericarp of mature seeds. Levels of these proteins also changed significantly during seed germination and were present in the shoots of germinating caryopsis.

Keywords: *Sorghum; chitinase; sormatin; glucanase; RIP; seed development; germination; grain molding*

INTRODUCTION

Deterioration of seeds by fungi in the field is called grain molding (Glueck and Rooney, 1980; Williams and Rao, 1981). Grain mold infections result in poor seed quality, ranging from cosmetic deterioration of the pericarp to substantial deterioration in quality attributes (Rooney and Serna-Saldivar, 1991) such as smaller seed size, lower test weight, endosperm density and decortication yield, increased amylase, protease, and lipase activities, and darker color of milled products. While several genera of fungi are associated with grain molds, *Fusarium moniliforme* Sheldon and *Curvularia lunata* (Wakker) Boedjin are the most predominant causal agents worldwide (Castor and Frederiksen, 1980).

Biochemical investigations have increased our understanding of the mechanisms of seed deterioration by fungi and breeding efforts have yielded modest improvements in seed resistance. Breeding for resistance has been largely unsuccessful because grain molding is sensitive to weather conditions making screening difficult and inconsistent. Our interest is to determine whether seed antifungal proteins (AFP) have a role in minimizing grain molding.

Plants respond to fungal invasion using an array of passive and active defense mechanisms (Dixon and Harrison, 1990). Factors that contribute to sorghum caryopsis defense are panicle architecture, endosperm hardness, presence of condensed tannins, phenolic compounds, etc. (Ellis, 1972; Glueck and Rooney, 1980; Rooney and Miller, 1982; Murty and House, 1984; Hahn and Rooney, 1985; Doherty et al., 1987). AFPs identified in cereal caryopses such as maize, barley, and sorghum are hypothesized to play a role in the defense of seeds against pathogen invasion (Vigers et al., 1991; Bass et al., 1992; Kumari and Chandrashekar, 1992; Darnetty et al., 1993). Hydrolytic enzymes, which are well-known pathogenesis-related proteins, degrade structural polysaccharides of the fungal cell wall such as chitin and glucan polymers (Legrand et al., 1987; Mauch et al., 1988b; Joosten and DeWit, 1989). Permatins (sormatin in sorghum; zeamatin in maize) act by permeabilizing the fungal cell membrane (Vigers et al., 1991), while ribosome-inactivating proteins (RIP) readily modify foreign ribosomes and inactivate them (Taylor et al., 1994). While AFPs have shown promise against certain diseases in transformed plants (Leah et al., 1991; Zhu et al., 1994; Jach et al., 1995), their role in caryopsis defense and the nature of their activity in caryopsis is unknown because most studies on changes in AFPs have been on leaves or other plant tissues.

A few studies have documented changes in seed AFPs during caryopsis development, germination or imbibition paving the way for hypotheses on modes of action of these proteins in seeds. Chitinase and glucanase levels increased during the maturation of pea pods in a greenhouse study (Mauch et al., 1988a). These proteins were further reported to be induced in immature pea pods following stress, such as wounding, exposure to ethylene or pathogen infection. Maize RIP levels increased 10 days after pollination and decreased subsequently from 16 to 20 days after pollination (Bass et al., 1992). Frederiksen (1995, personal communication) observed an increase in sorghum AFPs during seed development but did not obtain a good correlation between AFP levels in mature seeds and grain mold resistance.

Zeamatin levels increased during the first 3 days of maize germination and RIP activity also increased during germination of maize caryopses (Bass et al., 1992; Vigers, 1992). Further, one glucanase and three chitinase isoforms were induced in the embryo and vegetative tissues of maize seedlings in response to *F. moniliforme* infection (Cordero et al., 1994). Swegle et al. (1992) reported the selective release of different chitinases into the surrounding aqueous medium upon imbibition of mature barley caryopses. The release of these proteins was hypothesized to play a role in caryopses defense during germination.

AFPs have also been identified in sorghum caryopses, and attempts to correlate their levels with different pathogens have been unsuccessful. No significant cor-

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Table 1. Effect of Development of Sorghum Caryopses on Antifungal Proteins

cultivar/ hybrid	sormatin (μg/g) 15 DAA	sormatin (μg/g) 30 DAA	chitinase (μg/g) 15 DAA	chitinase (μg/g) 30 DAA	mold rating 30 DAA	pigmented testa
R 9025	52.2	190.4	0.3	11.0	1.5	no
Malisor 84-7	41.8	183.1	2.1	21.8	1.7	no
SC748-5	149.5	173.1	3.0	10.3	2	no
IS2319	41.4	153.9	6.6	9.2	2	yes
SC630-11E	3.2	7.6	1.3	1.5	2	no
BTx 635	123.8	113.9	0.3	2.1	2.5	no
Dorado	162.2	170.5	0.3	4.9	2.5	no
Pioneer 8305	0.1	88.0	4.4	4.1	3	no
Pioneer 8310	0.1	59.7	2.2	4.5	3	no
Pioneer 8313	0.1	685	1.8	5.6	3	no
BTx 3197	24.3	88.3	4.9	17.9	3	no
BTx 638	0.1	0.1	1.7	5.6	3.2	no
TX2536	6.5	11.7	9.8	3.5	5	no
Heg-Dobb	0.1	104.3	0.1	6.9	1.5	yes
Barnard Red	0.1	120.9	0.1	7.2	2.0	yes
Tam2566	0.1	84.1	0.1	4.9	2.5	yes
ARK 1097	0.1	84.2	0.1	5.5	3.0	yes

relations between permatin levels in caryopses and resistance to *Helminthosporium turcium*, *Helminthosporium maydis* or *F. moniliforme* in 10 lines of sorghum or maize were observed (Vigers, 1992). Considerable variability was observed in the levels of chitinases, glucanases, and permatin among different sorghum cultivars (Darnetty et al., 1993). Further, sorghum extracts were reported to be more inhibitory than maize or wheat extracts against the several fungi tested. The extent of inhibition correlated with the level of sorghum chitinases (Darnetty, 1990). Three, as yet uncharacterized, sorghum endosperm proteins have been identified that inhibited the growth of *F. moniliforme* (Kumari and Chandrashekar, 1992). Hard endosperm caryopses had higher levels of these proteins and lower incidences of mold infection compared to soft endosperm caryopses.

Our objectives, therefore, were to investigate the changes in seed AFPs during development under field conditions, determine the tissue distribution of these proteins in caryopses, and establish the relationship of AFPs during development to grain mold resistance.

In this paper, we document changes in sormatin and chitinase in developing sorghum caryopses and the ability of these proteins to migrate from the endosperm. The inability to link AFPs with extent of grain molding in the past can be explained by our observations. Sorghum AFPs are inhibitory against the two grain molding pathogens of sorghum and other fungi (our unpublished observations), and therefore, AFPs have the potential to limit grain molding. These observations enhance our understanding of the usefulness of AFPs and their potential to limit grain molding and other seed diseases.

MATERIALS AND METHODS

Plant Material. Sorghums (*Sorghum bicolor* (L.) Moench) were grown at the Texas A&M Agricultural Research Farm at College Station, TX (Table 1). Sorghum panicles were tagged at anthesis and caryopses sampled at three stages during development up to physiological maturity (15, 22, and 30 days after anthesis (DAA)) in 1994 and at eight stages of development up to combine harvest (7, 9, 11, 13, 15, 30, 40, and 50 DAA) in 1995. Composited caryopsis samples were frozen and lyophilized. Grain mold ratings were recorded at physiological maturity (30 DAA) and at final harvest (50 DAA) using conventional techniques (Forbes et al., 1989). Most sorghums evaluated did not contain tannins (type I) and will be discussed without further clarification. However, sorghums containing tannins (type II or III) were evaluated and will be identified accordingly.

Antibodies. Antibodies against zeamatin (maize permatin) and bean chitinase were provided by Dr. Claude Selitrennikoff (University of Colorado, Denver, CO) and Dr. Karen Broglie (DuPont Co., Wilmington, DE) respectively. Most of the study was conducted using these antibodies. Preliminary screenings were also conducted using antibodies against tobacco glucanase and maize RIP, which were provided by Dr. Michel Legrand (Universite Louis Pastuer, Paris, France) and Dr. Rebecca Boston (North Carolina State University, Raleigh, NC), respectively.

Sample Preparation and Immunoblotting. Samples were extracted and immunologically assayed (Western blots) for AFPs using the procedure described by Vigers (1992). Briefly, lyophilized seeds were ground using an UDY mill (1.0 mm mesh screen, Model 3010-030 cyclone mill, Udy Corp., Fort Collins, CO) and proteins extracted by shaking 0.10 g of flour for 1 h in 5.0 mL of cold extraction buffer (50 mM NaCl, 25 mM sodium phosphate, pH 7.2, and 5 mM EDTA). The slurry was centrifuged at 10 000*g* for 20 min and the supernatant collected and filtered through a 0.45 μm filter. Proteins were fractionated under reducing conditions using 12% SDS−PAGE gel (Bio-Rad minigel apparatus, Bio-Rad, Hercules, CA). Proteins from the gel were transferred to nitrocellulose membranes (Gelman Sciences, Ann Arbor, MI) using the manufacturer's directions (Owl Scientific Apparatus, Woburn, MA). Following incubation with antibodies, the antigen−antibody complex was detected using goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (Boehringer Manneheim, Indianapolis, IN).

Relative amounts of sormatin and chitinase were calculated by measuring the band intensities of known amounts of pure zeamatin and barley chitinase respectively (provided by Dr. Claude Selitrennikoff at University of Colorado, Denver, CO, and Dr. Muthukrishnan at Kansas State University, Manhattan, KS, respectively), in each immunoblot. Blots were scanned using a Hewlet-Packard ScanJet 3C. The images and band intensities were determined using NIH Image software (version 1.59).

Imbibition Study. Whole caryopses (50 DAA) were surface sterilized using 70% ethanol for 5 min. Caryopses were rinsed twice with water, for 5 min each time. Seeds (5.0 g) were then placed in a covered sterile petri dish containing 7.0 mL of water at room temperature. Caryopses and water were sampled after 24 h. The endosperm and bran (pericarp and embryo) tissues were manually separated. Whole unsoaked caryopses (50 DAA) were abrasively decorticated for 2 min, to remove the bran, using a tangential abrasive dehulling device (Reichert et al., 1982). Whole caryopses, decorticated endosperm, and bran fractions were collected. Immature unsoaked caryopses (9 DAA) were hand peeled and separated into endosperm and pericarp fractions. All the fractions were extracted as above and assayed for sormatin and chitinase. Proteins in the leachate were precipated with ice cold acetone, rinsed, and redissolved in sample buffer.

Germination Study. Sorghums used in a malting study (Beta et al., 1995) were sampled and screened for changes in sormatin and chitinase. Briefly, mature caryopses (50 DAA) were surface sterilized using 2% sodium hypochlorite, steeped in water overnight, germinated for 5 days in a warm (27 °C) moist (85% RH) chamber, and sampled everyday. Proteins were extracted from these samples as above and assayed for sormatin and chitinase. Shoots at 5 days of germination were also assayed.

RESULTS

Changes during Development. Zeamatin, bean chitinase, tobacco β-glucanase, or maize RIP antibodies each reacted to one major cross-reacting band when tested with sorghum extracts. Sormatin, chitinase, and

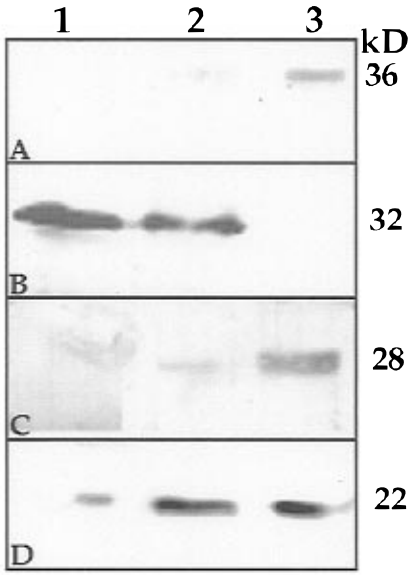

Figure 1. Changes in sorghum (Malisor 84-7) antifungal proteins during caryopses development: A, β-glucanase (36 kDa); B, RIP (32 kDa); C, chitinase (28 kDa); D, sormatin (22 kDa). Lanes: 1, 15 DAA; 2, 22 DAA; 3, 30 DAA.

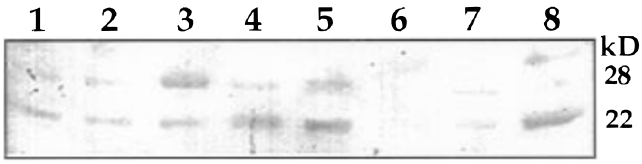

Figure 2. Changes in chitinase (28 kDa) and sormatin (22 kDa) of clean and moldy sorghum caryopses. Lanes: 1 and 2, Malisor 84-7 clean and moldy caryopses, respectively; 3 and 4, BTx378 clean and moldy caryopses, respectively; 5 and 6, TAM2566 clean and moldy caryopses, respectively; 7 and 8, SC103-12E clean and moldy caryopses, respectively.

glucanase levels increased during maturity while RIP levels decreased (Figure 1). Further investigations using tobacco β-glucanase or maize RIP were conducted with a few sorghum cultivars, due to limited availability of antibody, to confirm trends observed using sormatin or chitinase.

Further, clean and moldy caryopses from a few cultivars were separated from a panicle and assayed for changes in chitinase and sormatin. Western blots were developed using a mixture of bean chitinase and zeamatin antibodies. Levels of 28 kDa chitinase decreased in moldy caryopses of all four cultivars screened when compared to clean caryopses from the same panicle, but an additional cross-reacting band (33 kDa) was observed in SC103-12E (Figure 2). Changes in sormatin level in moldy caryopses were not consistent between cultivars; sormatin increased in BTx378 and SC103-12E, decreased in TAM2566, and remained the same in Malisor 84-7. These observations suggest that caryopsis AFP levels are affected by grain molds.

To further elucidate changes in AFPs during the entire span of caryopses development from anthesis to combine harvest, sorghums were sampled at seven stages from 7 to 50 DAA in 1995. Chitinase and sormatin levels peaked at physiological maturity (Figure 3, lane 6) and decreased subsequently.

Correlation of AFPs to Grain Mold Rating. Sormatin and chitinase levels increased up to physiological maturity in most sorghums screened (Table 1). Seed AFP (chitinase + sormatin) levels ranged from 5 to 200 μg/g. Chitinase and sormatin levels in sorghums increased 50−1000-fold between 15 and 30 DAA. Mold-tolerant sorghums (≤2.5 mold rating) usually contained more sormatin at 15 and 30 DAA (Figure 4). The correlation coefficient was higher at 30 DAA ($R^2 = 0.65$) than at 15 DAA ($R^2 = 0.17$). Chitinase levels did not correlate significantly with mold resistance in our study ($R^2 = 0.11$).

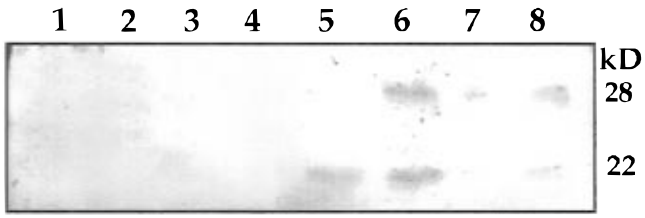

Figure 3. Changes in chitinase (28 kDa) and sormatin (22 kDa) of sorghum (Malisor 84-7) caryopses during development. Lanes: 1, 7 DAA; 2, 9 DAA; 3, 11 DAA; 4, 13 DAA; 5, 15 DAA; 6, 30 DAA; 7, 40 DAA; 8, 50 DAA.

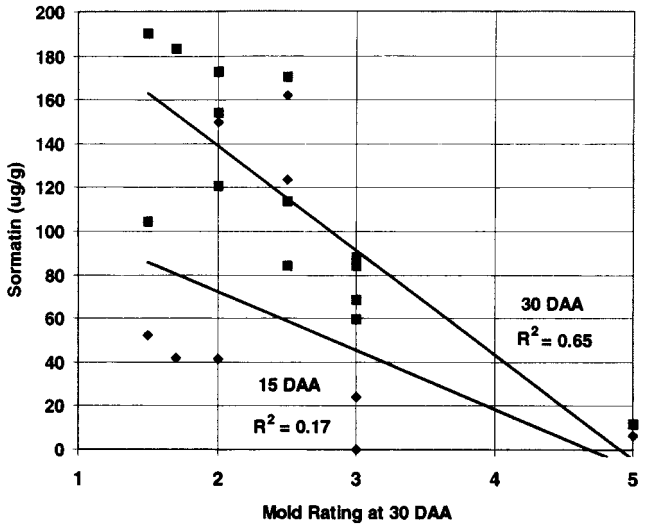

Figure 4. Relationship of mold rating and sormatin content of sorghums at 15 and 30 DAA.

SC630-11E is the notable exception with very low levels of sormatin and chitinases. Mold resistance of this cultivar is recessive and appears to be inherited differently from many sorghums (Rosenow, 1995, personal communication).

Two weeks after harvest (>50 DAA) several sorghum cultivars that exhibited good mold resistance were sampled. These sorghums were scored for mold rating and screened for sormatin and chitinase. Mold ratings of these sorghums ranged from 2.2 to 3.8; and their chitinase and sormatin levels ranged from 2 to 10 and 20 to 100 μg/g, respectively (Table 2). However, neither chitinase ($R^2 = 0.04$) nor sormatin ($R^2 = 0.01$) correlated with mold ratings.

Tissue Distribution and Mobility of Proteins. Sormatin was present predominantly in the endosperm tissue of mature (Figure 5E) and immature (Figure 6) caryopsis. Several isozymes of chitinase were detected in both the endosperm (Figure 5B) and pericarp (Figure 5C) tissues at physiological maturity, while they were present only in the endosperm of immature caryopsis (9 DAA) (Figure 6).

The mobility of these proteins from the endosperm was intriguing. We therefore investigated the mobility of these proteins within the caryopsis, similar to experiments conducted using mature barley caryopses (Swegle et al., 1992).

Table 2. Antifungal Protein in Sorghum Caryopses at 50 DAA

hybrid/cultivar	chitinase (μg/g)	sormatin (μg/g)	mold rating	pigmented testa
A868*SC120	5.1	52.9	3.8	no
ORO Edge (MPS)	5.2	90.1	3.8	yes
(A8618*CR4244*R6956)-c5-c3-c2-c1	7.0	73.4	3.6	no
Golden Acres Chico (Mycogen Plant Sci.)	9.0	74.8	3.5	no
A8618*(SC120*Tx7000)40-4-6-2-2-1*(R4244)CF2-c1-c9-c3-c1-c1	2.4	21.5	3.5	no
(SC719-11E*S6630*11E) 90B2662	10.7	55.6	3.3	no
ATx638*(R4244*R6956)-c13-c4-c2-c1	5.5	65.3	3	no
(A8618*CRT435*SC103-12)CF2-b5-b2-b2	7.8	95.3	2.8	no
(Tx433*(SC748*SC630))-87BH8606-6	4.8	37.6	2.8	no
AP9210 (Agripro Seeds)	6.8	71.7	2.8	no
DK64 (Dekalb)	4.7	33.0	2.8	no
A8618*SC103-12E	11.2	104.9	2.5	no
ATx626*R9105	7.8	66.9	2.5	no
ORO Baron (MPS)	2.7	36.8	2.5	no
A8618*(SC103-12E*SC237-14E)-1-1-b5-b1-b1-bbk-b4-b1-b3-b1-b1	3.0	36.7	2.2	no

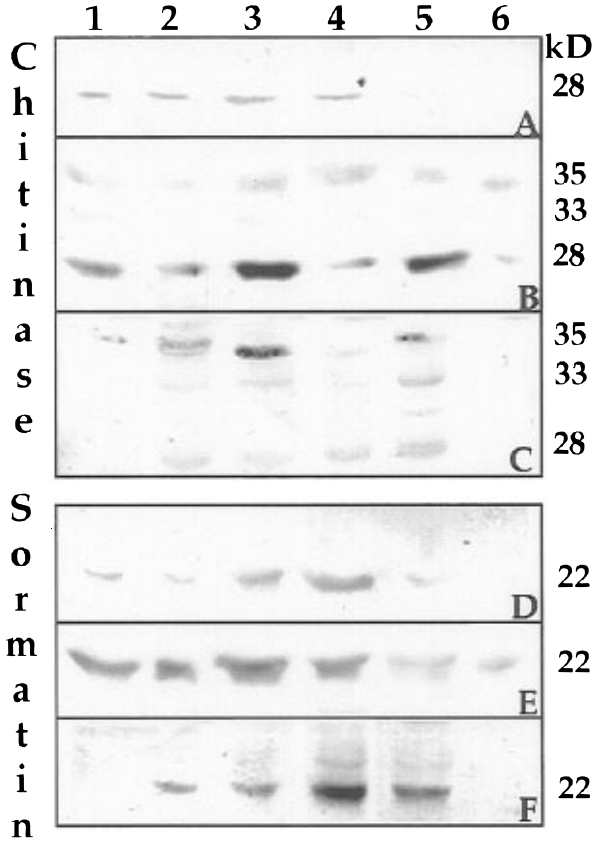

Figure 5. Tissue distribution and protein mobility of sormatin (22 kDa) and chitinases (28 and 33 kDa) in mature sorghums of types I (Malisor 84-7), II (IS2319) and III (SC10312-E). A and D, whole seed; B and E, endosperm; C and F, pericarp. Lanes: 1 and 2, Malisor 84-7 dry and soaked tissues, respectively; 3 and 4, IS2319 dry and soaked tissues, respectively; 5 and 6, SC10312-E dry and soaked tissues, respectively.

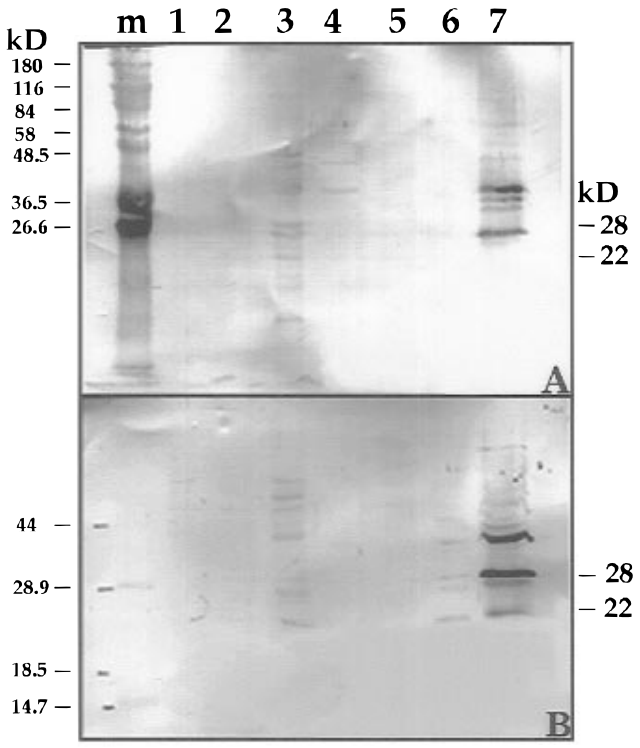

Figure 6. Tissue distribution and protein mobility of chitinase (28 kDa) and sormatin (22 kDa) in immature (9 DAA) sorghums (A, TAM2566; B, IS2319). Lanes: m, molecular weight marker; 1, whole caryopses; 2, soaked whole caryopses; 3, endosperm; 4, soaked endosperm; 5, pericarp; 6, soaked pericarp; 7, 24 h leachate.

Chitinase and sormatin levels in endosperm tissue decreased (Figure 5B,E) following soaking for 24 h of physiologically mature sorghum caryopses. Pericarp tissues of type I sorghums (Figure 5C,F), however, contained higher levels of sormatin and chitinase. Only trace levels of these proteins were observed in the leachate (figure not shown). AFPs appeared to be retained or bound by the pericarp.

To further test the hypothesis of proteins binding to pericarp tissue, immature caryopses (9 DAA) were soaked and screened for chitinase and sormatin. Substantial amounts of AFPs migrated from the endosperm (Figure 6, lane 3) into the leachate (Figure 6, lane 7). AFP levels in the pericarp increased from undetectable to detectable levels after soaking (Figure 6, lane 6).

Tissue distributions of sormatin and chitinase in type II (condensed tannins present in pigmented layer) and type III (condensed tannins present in pigmented layer and pericarp) sorghums were also studied. Detectable levels of chitinase and sormatin were present in the pericarp of unsoaked physiologically mature type II and III sorghums (Figure 5C,F; lanes 3 and 5), whereas, they were not detectable in type I (no tannin) sorghums (Figure 5C,F; lane 1). Upon soaking, chitinase levels remained unchanged and sormatin levels increased in the pericarp of type II sorghum, while chitinase and sormatin were undectable in the pericarp of type III sorghum. AFPs were not detected in the leachate from type II or III sorghums. Significant differences in the mobility and extractability are, therefore, evident between sorghums of types I, II, and III.

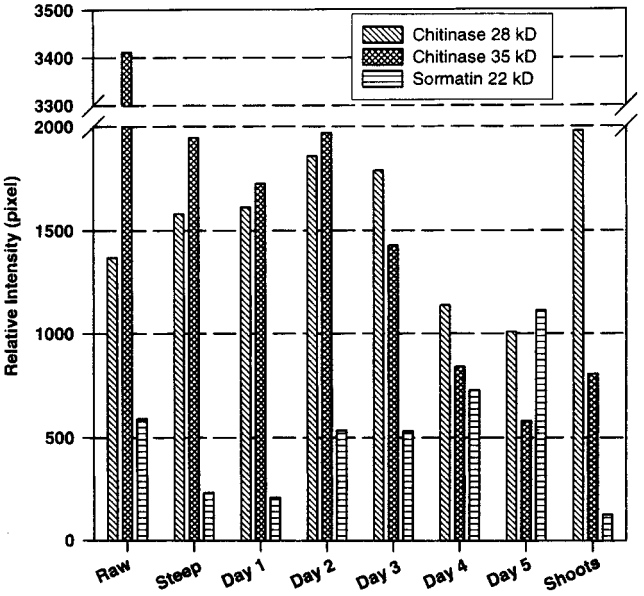

Figure 7. Effects of sorghum germination on sormatin (22 kDa) and chitinase (28 and 35 kDa) levels in caryopses.

Changes during Germination. Steeping and germination during the malting process significantly affected sormatin and chitinase levels in caryopses (Figure 7). Sormatin and a 34 kDa chitinase isozyme in the caryopses significantly increased during germination. The 26 and 18 kDa chitinase isozymes peaked between 2 and 3 days of germination. Shoots contained significant amounts of the higher molecular weight chitinase isozyme and lower levels of sormatin and the two lower molecular weight chitinase isozymes.

DISCUSSION

The identification, mobility, and bioactivity of AFPs in cereal caryopses offers an effective route to decrease grain molding. Efforts are underway to map disease resistance genes of cereals or create transformed plants with increased resistance. Concurrent investigations on the physiological aspects of AFPs provide useful insights to maximize their potential.

Significant changes in sorghum antifungal proteins during caryopsis development and postmaturation were observed. Sormatin, chitinase, and glucanase increased during caropysis development through physiological maturity (30 DAA) and decreased subsequently. These observations are similar to those reported in developing pea pods and maize caryopses, respectively (Mauch et al., 1988a; Bass et al., 1992). Darnetty et al. (1993) did not observe any cross-reacting bands using barley RIP antibody. Our results show that sorghums contain an isozyme of RIP that cross reacts with maize RIP, and the levels of RIP decrease after 15 DAA.

Measurement of AFPs in mature, combine harvest grain does not significantly correlate with resistance (Darnetty, 1990; Vigers, 1992). Four observations from this study indicate that mature caryopses may be unsuitable test materials to screen for sorghum grain mold resistance: (a) AFP levels decreased in most cultivars after physiological maturity; (b) the levels of AFPs were lower in moldy caryopses compared to clean caryopses from the same panicle; (c) sorghums that exhibited good mold resistance 1 week after final harvest (50 DAA) had moderate levels of sormatin (40−105 μg/g) that did not correlate with mold rating (R^2 = 0.01); and (d) sormatin levels at 30 DAA correlated significantly with mold ratings (R^2 = 0.65). The inability to draw clear conclusions, in prior studies, about the role of AFPs on grain molding could result from one or more of these observations. Therefore, we suggest that seeds sampled at or slightly before physiological maturity would contain a wider range of AFPs which can be correlated to grain molding and, therefore, be a better test material for screening for mold resistance.

The mobility of these proteins out of immature caryopses and within mature caryopses is an interesting phenomenon. This may enable the seed to synthesize and store large amounts of constitutive defense proteins in the endosperm. Mobility of AFPs from the endosperm to the periphery upon imbibition corresponds to environmental conditions conducive to fungal invasion and growth. The retention of AFPs in the pericarp permits their accumulation in a very narrow region of the caryopsis after protein synthesis ceases (postphysiological maturity, >30 DAA). The bioactivity of AFPs bound to the pericarp and/or tannins, however, is not known.

Further, AFPs need to be active during conditions of high risk, i.e., during the early stages of development and high humidity. Therefore, the synthesis and mobility of bioactive proteins from immature caryopses (9 DAA) into the surrounding environment (inside the glume) would be an additional strategy to limit *F. moniliforme* and *C. lunata* under such conditions (Castor, 1981).

Significant differences were also observed in the mobility and extractability of these proteins from sorghums of types I, II, and III. Sorghum caryopses are exposed, not covered like barley, rice, or wheat. Therefore, sorghums containing condensed tannins confer a degree of resistance against bird, insect, and mold damage (Hahn and Rooney, 1985; Jambunathan et al., 1990). Retention of AFPs by the pericarp of type I sorhugms under humid conditions becomes more important in the absence of condensed tannins. Swegle et al. (1992) reported the presence of chitinases in the leachate 2 h following imbibition of mature barley caryopses. Similar leaching patterns in exposed caryopses would deplete the antifungal potential of the caryopses under humid conditions. In our trials, AFP levels increased in the endosperm 2 h after imbibition (data not shown) but we were unable to detect AFPs in leachate that early. These observations probably indicate the mobilization and retention of defense proteins within the caryopsis. Differences of this nature between different caryopses need to be further investigated.

While we use the term mobility to explain the above observations in this study, it is possible that these changes could be due to immobilization (inextractibility) and/or synthesis/degradation. However, some reports suggest that enzymes (proteases or amylases) are preformed and stored in the endosperms of cereals such as sorghum or maize, while they are synthesized *de novo* in the aleurone layer in cereals such as barley or oats (Adam and Novellie, 1975). Further, degradation of AFPs could also result from proteases, but this does not adequately explain the concurrent accumulation of AFPs in other tissues or in the leachate. We are conducting further studies to confirm these observations.

The changes in AFPs during sorghum germination were similar to those reported in other studies (Bass et al., 1992; Vigers, 1992). We have additionally shown that chitinases and sormatin are also present in the

shoots. Chitinases that leach out of caryopses are hypothesized to protect the seed during germination (Swegle et al., 1992). Cordero et al. (1994) also reported the induction of chitinases and glucanases upon fungal infection in the vegetative tissues (radicle and coleoptile) of germinating maize. Additionally, by sampling germinating seeds from the malting process, we have highlighted a potential use for AFPs in malting of cereals for brewing since molding of malted grain significantly deteriorates malt quality (Flannigan et al., 1982).

Grain molding is a serious impediment in obtaining good yields and quality of sorghums, especially in the third world countries. Techniques to rapidly screen for resistant cultivars are valuable tools for breeders. Screening large numbers of samples using antibodies against AFPs will enhance the predictive capabilities of a breeding program. Our observations, in summary, allow us to target the timing, location, and/or levels of AFPs within the caryopsis to result in increased grain mold resistance. The *in vitro* inhibitory effects of sorghum caryopsis AFPs against the grain mold pathogens (Seetharaman et al., unpublished data) also suggest that AFPs have potential to enhance grain mold resistance.

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