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Review

Disease and pest resistance in grains of sorghum and millets

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Abstract

In this review available information on the mechanisms of resistance to insect pests and fungal pathogens in sorghum and millets is discussed. The primary source of resistance lies in the chemical and physical make up of the grain. Phenolic compounds such as ferulic acid and tannins present in some sorghums are potent inhibitors of pests and pathogens. Grain hardness is a major deterrent to infection and infestation in low tannin grains. The prolamins, the grain storage proteins of sorghum, are organized into protein bodies and provide a physical and a nutritional barrier since they are resistant to digestion by insect and fungal proteases. A plethora of proteins that belong to the 'pathogenesis related protein' group are distributed in various parts of the grain. Some of them are located in protein bodies. Notwithstanding, sorghum is still susceptible to insect pests and fungal pathogens. An understanding of the natural mechanisms of resistance in the grain is paramount for the development of durable resistance against pests and pathogens. The pyramiding of resistance genes and the development of transgenic lines based on this understanding are two sources of hope for the future protection of sorghum and millets.
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Keywords: Sorghum; Millets; PR proteins; Antifungal proteins; Insecticidal proteins; Phtyoalexins; Polyphenols; Tannins; Development of resistance; Grain hardness

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Abbreviations: AFP, antifungal proteins; AV, avirulence; *bmr*, brown midrib gene; daa, days after anthesis; HR, hypersensitive response; MAS, marker assisted selection; nsLTP, non specific lipid transfer protein; PRP, pathogenesis related proteins; QTL, quantitative trait loci; R, resistance gene products; RIP, maize ribosome-inactivating protein; SAR, systemic acquired resistance; TLP, thaumatin-like protein

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1. Introduction

Sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum typhoideum*), finger millet (*Eleusine coracana*), and minor millets (Table 1) are cultivated, small-seeded tropical grasses grown for food, feed or forage, providing the major source of dietary energy and protein for some one billion people in the semi-arid tropics (Belton and Taylor, 2004; Naylor et al., 2004; Rooney, 2004). The productivities of sorghum and millets are low. Even though total production has been increasing in recent years, this has only been achieved by cultivating more and more land (Belton and Taylor, 2004). The projected food demand for 2025 will require the yield of millets to rise from 2.5 to $4.5\,t\,ha^{-1}$ (Kothari et al., 2005). Apart from lack of irrigation and fertilizer for millet cultivation, insect pests and fungal and bacterial diseases cause huge losses in many parts of the world. Pest and/or pathogen attack causes 10–40% reduction in crop yield, depending on the geographical location (Repellin et al., 2001). The estimated loss of sorghum crop due to pests and pathogen attack is 30% (Reddy and Zehr, 2004). One way to increase the quantity and quality of food grain is to reduce crop damage by insects and by fungal and bacterial diseases. Such damage is commonly responsible for significant economic losses in the least developed countries, where tropical conditions allow insects and pathogenic agents to reproduce rapidly and to colonize unprotected grain.

Like all crops, sorghum and millets are subject to infectious diseases that can limit production. Infestation of grain is of primary concern here since the grains of sorghum and millets are consumed for food and feed, although diseases of other plant parts are also of great importance. In this review, mechanisms of resistance to pathogens and pests in the grains of sorghum and millets are discussed with emphasis on those pests and pathogens that effect grain quality.

Table 1
Commonly grown sorghum and millet species (Adapted from Kothari et al., 2005)

Species	Common name
Sorghum bicolor	Sorghum
Pennisetum glaucum	Pearl millet
Eleusine coracana	Finger millet (ragi)
Setaria italica	Foxtail millet; Italian millet
Paspalum scrobiculatum	Kodo millet; Barnyard millet
Panicum sumatrense	Little millet
Panicum miliaceum	Proso millet
Paspalum notatum	Bahia grass
Coix lachryma jobs	Job's tears

1.1. Pests and pathogens of sorghum and their point of entry

During the last few years there has been great interest in understanding the mechanisms by which plants fight infection. Fungal infection of grain is very much dependent on the ambient humidity and is always higher after rain (Bandyopadhyay et al., 2000). The data accumulated over many years indicate innate differences among sorghum grains in their ability to resist infestation. The mechanisms of resistance to pests and pathogens involve both the physical and chemical composition of the grain. The physical structure of the grain, for example, pericarp thickness and composition, endosperm texture, and various chemical constituents such as the hydroxycinnamic acid, ferulic acid, and various endosperm proteins that are directly antagonistic to pest and pathogens are involved in defense. The pericarp is the first site of infection as shown in maize using *Fusarium verticillioides* tagged with the green fluorescent protein (Oren et al., 2003). After penetration, fungi attack the endosperm. Infection by fungi often takes place at the flowering stage (Castor, 1981; Forbes, 1986). This is certainly true of the ergot fungus, *Claviceps sorghi* (Bandyopadhyay et al., 1998). Some fungi such as *Phoma* are restricted mainly to the perciarp. The pericarp of sorghum consists of an outer cellular layer, the epicarp, and a single or multiple mesocarp layer which overlies the aleurone (Earp and Rooney, 1982). Infection by *Phoma* appears to depend on the thickness of the mesocarp (Kumari et al., 1992). On the other hand, *Curvularia* and *Fusarium* spread from the hylar region on the pericarp surface, eventually penetrating the endosperm. Premature germination and digestion of endosperm starch, aids in this process (Bandyopadhyay et al., 2000). *Curvularia* mainly attacks the grain while *Fusarium* often attacks the stem and leaf (Little, 2000). Penetration of the fungus into the developing grain is often abetted by insect infestation for example by the sorghum head bug (*Eurystylus oldi*), shoot fly (*Atherigona soccata*), the midge (*Stenodiplosis sorghicola*) and the boll worm (*Helicoverpa armigera*). Marley and Malgwi (1999) correlate the incidence of fungi on the sorghum grain with attack by *Eurystylus* sp. Another fungus that occasionally attacks the sorghum head is *Colletotrichum sublineolum*, a fungus that causes the sorghum anthracnose disease mainly of the leaf.

After harvest wet grains are attacked by aspergilli some of which produce aflatoxins. However, when compared to maize and groundnut (*Arachis hypogea*) sorghum grain is less susceptible to infection by *Aspergillus parasiticus*, and hence aflatoxin contamination, due to its physical characteristics and biochemical composition. The lowest amounts of aflatoxin and ergosterol were observed in

sorghum genotypes with red pericarp, whereas higher amounts of both were found in white genotypes followed by maize and groundnut. Red genotypes of sorghum are relatively resistant to mold damage, their different polyphenol contents relate to the amount of aflatoxin produced (Ratnavathi and Sashidhar, 2003). The more mold resistant red pericarp sorghums especially contain more flavon-4-ols than the white pericarp sorghums (Dykes and Rooney, 2006; Menkir et al., 1996; Waniska et al., 2001). Dykes et al. (2005) reported that purple/red-pigmented sorghums with a thick pericarp have higher levels of flavon-4-ols (4.3–9.3 abs/ml/g) than purple/red-pigmented sorghums with a thin pericarp (3.0–3.6 abs/ml/g) and tan-pigmented sorghums (2.3–2.7 abs/ml/g). The flavon-4-ols are components of the phytoalexin defense system and are discussed in Section 1.2.1.

Stored grains of sorghum and pearl millet are attacked by the maize weevil (*Sitophilus zeamais*). These insects deposit their eggs near the germ end of the grain and the emerging caterpillar grows on the floury or soft endosperm (Landsberg et al., 1995). The seed coat of sorghum grains resistant to attack by the maize weevil is twice as thick as that of the seed coat of susceptible grains (Pendleton et al., 2005).

Ergot (*Claviceps fusiformis*), downy mildew (*Sclerospora graminicola*) and blast (*Pyricularia oryzae*: *Magnaporthe grisea*) are the major fungi affecting pearl millet (Ahmed and Reddy, 1993). Italian millet/foxtail millet (*Setaria italica*) is susceptible to blast (*Pyricularia setariae*), rust (*Uromyces setariae-italicae*), smut (*Ustilago crameri*) and downy mildew (*Sclerospora graminicola*). Finger millet, whose grain rich in polyphenols is resistant to grain mold, but not to leaf blast (*Pyricularia setariae*) or leaf blight (*Cochliobolus nodulosus*). The grains of finger millet are very resistant to insect attack and this may be attributed to the presence of a pigmented testa rich in tannins (McDonough et al., 1986).

1.2. Tannins, phenolic compounds and phytoalexins

Phenolic compounds belonging to three major categories: phenolic acids, flavanoids and tannins are found in sorghum grain (Chung et al., 1998; Dicko et al., 2006; Dykes and Rooney, 2006). In sorghum and millets the phenolic acids are present primarily in the pericarp, seed coat (testa) and aleurone layer (Hahn et al., 1984; McDonough et al., 1986). The testa is present only in some sorghums and is absent in sorghums used for food in India and for feed in the USA.

High levels of *p*-coumaric acid have been observed in some white pericarp, non-tannin sorghums that are susceptible to molding (Waniska et al., 1989). Phenolic acids increase during caryopsis development reaching a maximum at physiological maturity and decreasing thereafter (Doherty et al., 1987). *p*-Coumaric acid is the progenitor of ferulic acid and its conversion might be deficient in susceptible cultivars. Sorghum cultivars resistant to fungal attack contained both a greater variety and higher amounts of free phenolic acids, especially in the case of tannin containing sorghums (Hahn et al., 1983; Waniska et al., 1989). The presence of a pigmented testa (Esele et al., 1993) as well as seed phenols and glume color caused by phenolic pigments (Audilakshmi et al., 1999) also contribute to grain mold resistance. Funnell and Pedersen (2006) showed that both leaves and grain of sorghum bearing the gene for the brown midrib (*bmr*) trait are resistant to attack by various species of *Fusarium*. The *bmr* trait is a defect in the pathway of lignin biosynthesis and it has been proposed by Funnell and Pedersen (2006) that lignin biosynthetic intermediates accumulating in the *bmr* lines contribute to the lowered growth of *Fusarium*.

1.2.1. The phytoalexin response

Plants respond to infection by producing a variety of compounds that are toxic to the invading micro-organism. These compounds are known as phytoalexins. The phytoalexins of sorghum (Lo et al., 1999a; Snyder and Nicholson, 1990) are unique anthocyanins that in contrast common anthocyanins do not contain the hydroxyl group in the 3-position of the C-ring and thus are called 3-deoxyanthocyanins (Chung et al., 1998; Dicko et al., 2006; Dykes and Rooney, 2006). These compounds accumulate in intracellular inclusion bodies, towards the site of fungal penetration and then release their contents, killing both the fungus and the cells that synthesize them (Snyder and Nicholson, 1990; Snyder et al., 1991). The phytoalexin levels in infected host cells reached 150 μM (Snyder et al., 1991), which exceeds the amount required to kill the fungus et al., In an attempt to understand the genetic regulation of the biosynthesis of sorghum phytoalexins, Boddu et al., (2004) isolated a differentially expressed partial cDNA corresponding to a putative flavonoid 3′-hydroxylase. Transcription of the flavonoid 3′-hydroxylase was coordinately regulated with that of chalcone synthase and dihydroflavonol reductase, and expression of these genes was induced within the first 24 h of fungal challenge. Evidence for elicitation of secondary phenylpropanoid metabolism in sorghum-pathogen interactions has been provided by Castor (1981). Studies on inoculated sorghum seedling mesocotyls revealed that both chalcone synthase and phenylalanine ammonia-lyase, two enzymes necessary for the production of sorghum phytoalexins are induced in response to fungal inoculation (Cui, 1995; Cui et al., 1996; Nicholson and Wood, 2001). Inoculation of sorghum with pathogens such as the causative agent of downy mildew, *Peronosclerospora sorghi*, induced higher levels of both phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) in a resistant than in a susceptible control (Cui, 1995). A resistant cultivar accumulated phytoalexins and CHS mRNA more rapidly and to a higher level than did a susceptible cultivar when challenged with the causative agent of anthracnose, *Colletotrichum sublineolum* (Little, 2002; Little and Magill, 2003). Although phytoalexins of

sorghum grain have not been studied, the presence of flavon 4-ols have been correlated with resistance to fungal attack (Menkir et al., 1996; Waniska et al., 2001) Manipulation of the phytoalexin production may be one way of extending the defense capability of grains.

1.3. Grain texture

The endosperm lies beneath the pericarp and in sorghum, the endosperm like that of maize shows two forms. Usually the area just below the pericarp is translucent and encloses an inner opaque (corneous) area. The relative proportion of the two areas determines grain hardness. Grain hardness has been implicated in reducing mold infestation (Audilakshmi et al., 1999; Esele et al., 1993; Jambunathan et al., 1992; Kumari et al., 1992, 1994b). The corneous endosperm of sorghum is enriched in kafirins, especially γ-kafirins (Duodu et al., 2003; Mazhar and Chandrashekar, 1995; Kumari and Chandrashekar, 1994a; Kumari et al., 1992). The γ-kafirins (Belton et al., 2006) have the highest cysteine content among the storage proteins of the endosperm and form extensive intra-chain disulfide bonds, which may contribute both to texture and in resistance to fungal infection (Mazhar and Chandrashekar, 1993; Mazhar et al., 1993). The composition of the cell walls also varies between the corneous and floury endosperm and between hard and soft grains (Kavitha and Chandrashekar, 1992, 1993). Bueso et al. (2000) have shown that a combination of factors contribute to grain mold resistance. Sorghums with red pericarp and/or tannins are devoid of a hard, corneous endosperm. Sorghums that have no testa and hence are devoid of tannins derive resistance to pathogen attack either through the pigments in the pericarp or through the presence of a vitreous, hard endosperm. Harder grains have lower mold ratings after rain, or when stressed by sprinkling (simulated rain) or after inoculation with fungal pathogens.

Many of the same factors that contribute to varietal differences in resistance to fungl infection are responsible for varietal differences in resistance to pest attack. Ramputh et al. (1999) reported a strong negative correlation between emergence of the insect, *Sitophilus* and soluble phenolic content in sorghum, while Leuschner et al. (2000) found that the number of hatching *Sitophilus* was higher in soft and large grains and lower in grains that were hard and small.

1.4. Pathogenesis-related proteins in the sorghum caryopsis

There are a number of proteins present in the grain which have been shown to attenuate the growth of fungi. During the last few years there has been extensive work on such proteins from many plants. These proteins known as the pathogenesis-related proteins (PR-proteins) are by definition those increased during fungal infection or insect infestation (Jwa et al., 2006; Kitajima and Sato, 1999; van Loon and van Strien, 1999). Many of these proteins are antifungal in nature but not necessarily active against the pathogen involved in grain mold damage. The term "antifungal proteins" (AFP) in this review and in papers cited, refers to constitutive proteins whose action is directed against the pathogens of the grain (Selitrennikoff, 2001). "Defense-related proteins" often refer to proteins that are involved in the signal transduction events following infection and perhaps induce all proteins involved in defense. The genes encoding these proteins are also known as the "R" genes (Bergelson et al., 2001; Krupa, et al., 2006). The role of these proteins in the synthesis of PR proteins (antifungal proteins) in grains is yet to be determined.

Plant responses to attack by pathogenic microorganisms are complex, and involve the induction of expression of a large number of genes encoding diverse proteins, many of which are believed to have a role in defense. These provide a chemical barrier made up of antifungal PR-proteins, phenolics and phytoalexins and structural barriers involving for example, deposition of lignin-like polymers in the wall (Linthorst, 1991). The PR-proteins, defined as proteins encoded by the host plant, but induced specifically in pathological or related situations, not only accumulate locally in the infected area but are also induced systemically, associated with the development of systemic acquired resistance (SAR) against further infection by fungi, bacteria and viruses (van Loon and van Strien, 1999). The expression of genes encoding PR-proteins is generally used as an index of disease response in plants (Kitajima and Sato, 1999; Sarosh et al., 2005).

Initially, five main classes of PR-proteins (PR-1-5) were characterized in tobacco using both biochemical and molecular biological techniques and subsequently, PR proteins have been classified into 14 families (van Loon and van Strien, 1999) (Table 2). The antifungal proteins have been characterized according to their enzymatic properties ($(1\rightarrow3)$-β-glucan hydrolases, chitinases), their structure (e.g. cysteine rich) or their similarity in sequence to an already classified proteins (e.g. thaumatin-like proteins). The PR-proteins differ in molecular weight, isoelectric point, and immunological cross-reactivity (Muthukrishnan et al., 2001). Theis and Stahl (2004) have extensively reviewed the different cellular target sites of antifungal proteins. The wide range of hydrolase and inhibitory activities of PR-proteins are consistent with the notion that they have a role in defending the plant against pathogen infection, either by active hydrolysis of specific fungal cellular components or by a general toxic effect (Muthukrishnan et al., 2001). Most of the existing 16 families include members that are exported to the extracellular space. Proteins with oxalate oxidase or oxalate oxidase-like activity have been classified as 'PR-15'- and 'PR-16', respectively. Christensen et al. (2002) reported proteins belonging to a new family of plant PR-proteins, designated as 'PR-17' and these possess protease activity. Many PR-proteins have been purified to homogeneity and used for the preparation of specific antibodies.

Table 2
Families of pathogenesis related proteins (van Loon and van Strien, 1999)

Family	Type member	Properties	Mechanism of action
PR -1	Tobacco PR1a	Unknown	Unknown
PR -2	Tobacco PR-2	(1→3)-β-glucan hydrolase	Fungal cell wall digestion
PR -3	Tobacco P, Q	Chitinase type I, II, IV, V, VI, VII	Fungal cell wall digestion
PR -4	Tobacco 'R'	Chitinase type I, II	Fungal cell wall digestion
PR –5	Tobacco 'S'	Thaumatin like	Alteration of membrane permeability
PR -6	Tomato inhibitor I	Protease inhibitor	Inhibition of proteases
PR -7	Tomato P_{69}	Endo-protease	Protein digestion
PR -8	Cucumber chitinase	Chitinase type III	Fungal cell wall digestion
PR -9	Tobacco 'lignin forming peroxidase'	Peroxidase	Detoxification of H_2O_2, lignin synthesis
PR -10	Parsley 'PR-1'	'Ribonuclease like'	Activates ribonuclease, transport of sterols and cytokines
PR -11	Tobacco class V chitinase	Chitinase type I	Fungal cell wall digestion
PR -12	Radish Rs-AFP3	Defensin	Antimicrobial, membrane permeabilization
PR –13	Arabidopsis TH12.1	Thionin	Affects membrane properties
PR –14	Barley LTP4	Lipid transfer protein	Lipid transport, Membrane properties affected
Pr 15,16	Barley OXOLP	Protease oxidase	Produces hydrogen peroxide
PR –17	Barley HvPR-17		Protein digestion

The availability of antibodies also has led to the cloning of corresponding cDNAs and genes for PR-proteins (reviewed by Muthukrishnan et al., 2001). It is interesting that homologs of these proteins have also been found in animal systems (Kitajima and Sato, 1999).

Several proteins are constitutively expressed in developing sorghum and millet seeds and have antifungal properties. Generally, these proteins are not race- or species-specific and have a broad spectrum of activity. During the past few years, several types of bioactive and antifungal proteins have been identified and characterized in sorghum and millets. Some of these proteins are induced in response to pathogen attack.

Sorghum grains possess PR proteins with antifungal activity such as chitinases, (1→3)-β-glucan hydrolases, sormatins, proteinase inhibitors, ribosome inactivating proteins and thionins. Leslie et al. (1993) working with crude extracts of kernels (mostly endosperm) reported one chitinase band of approximately 29 kDa, and three additional bands ranging in size from 21–24 kDa. One (1→3)-β-glucan hydrolase band was identified, with an estimated molecular weight of 30 kDa, and so were sormatin bands at 22 and 29 kDa. Qualitative differences in isozyme profiles for different classes of AFPs from three different cereal grains have been found. Kumari and co-workers (Kumari and Chandrashekar, 1994a, b; Kumari et al., 1994) identified three proteins of 18, 26, and 30 kDa, which affected hyphal growth of *Fusarium moniliforme*. They concluded that the 18 kDa protein could be an enzyme acting on cell walls and that the 26 and 30 kDa components could be related to permeatins. More AFPs were observed in the hard endosperms from grain mold resistant sorghums and were related to prolamin content (Kumari and Chandrashekar, 1994a). Vigers et al. (1991) reported the presence of a protein with a molecular weight of 22 kDa in extracts of sorghum seeds, which cross-reacted with an antibody to zeamatin, a thaumatin-like protein (TLP) from maize. N-terminal sequencing of this sorghum protein revealed high sequence similarity to zeamatin (20 out of 22 residues were identical) and to other TLPs. Seetharaman et al. (1996) reported hyphal tip rupture and spore germination inhibition of *F. moniliforme* and *Curvularia lunata* by mixtures of chitinases, RIPs (ribosome-inactivating proteins), sormatin, and (1→3)-β-glucan hydrolases at 70–360 ppm. Higher levels of serine proteinase inhibitors were observed in developing hard versus soft endosperm sorghums (Kumari et al., 1992). Mincoff et al. (2006) report the isolation of a 30 kDa protein that inhibits the growth of *Candida parapsilosis, C. tropicalis* (MIC: 36 μg/ml), and *C. albicans*. Adherence of *Candida* to endosperm cells is a prerequisite for infection. These workers also noted that addition of the sorghum protein deformed the *Candida* cells and reduced their ability to adhere to glass slides and it was presumed that the pathogenicity of the cells had been attenuated.

Lo et al. (1999b) cloned the gene for the PR10 (ribonuclease-like) protein from sorghum. The PR10 transcript in sorghum accumulated rapidly (6 h post-inoculation), followed by an increase and then a gradual decrease in the mesocotyls of plants infected with the non-pathogenic *Cochliobolus heterostrophus*. Levels of PR10 mRNA (at 36 h post-inoculation) increased and remained at an almost constant level until the end of the experiment in the mesocotyls of plants infected with *Collectotrichum sublineolum* (the causal agent of anthracnose) (Lo et al., 1999b). PR-1 and PR-6 proteins are induced in sorghum in response to fungal infection (Lo and Nicholson, 1998; Lo et al., 1999b).

Levels of mRNA for four known defense-response genes, phenylalanine ammonia lyase (PAL1-1), chalcone synthase (CHS2G), (1→3)-β-glucan hydrolase (GLUC2-1) and chitinase (CHIT25-1) increased rapidly following inoculation of flowers with *Fusarium thapsinum* and *C. lunata* (Little and Magill, 2003). In a study conducted to gain an overview of gene expression patterns in *Colletotrichum*-infected plants, expressed sequence tags (EST) were compared from

susceptible and resistant interactions of 2- and 4-week old plants of sorghum with *C. sublineolum* (Goodwin et al., 2004). *Colletotrichum* species are causal agents of anthracnose, fruit rot and blight of a large number of crops. Excluding ESTs related to photosynthesis and other aspects of central plant metabolism, some of the most abundant ESTs related to biotic stress, such as $(1\rightarrow3)$-β-glucan hydrolase and osmotin, were highly represented in the susceptible Cs-4-week sorghum interaction, but not in healthy sorghum. ESTs representing cysteine proteinase were found in greater abundance in resistant sorghum interactions (two weeks after infection) compared to that found in the susceptible sorghum interaction (four weeks after infection). The authors concluded that the abundance of the cysteine proteinase may be related to plant cell death since cysteine proteinases have been implicated in programmed cell death (apoptosis) in both resistant and susceptible plant–pathogen interactions (Kruger et al., 2002). In contrast, the abundance of ESTs for heat shock proteins appeared to be inversely related to plant cell death. A very high redundancy of heat shock protein ESTs was found in the susceptible Cs-4-week sorghum interaction compared to that from resistant Cs-2-week sorghum interactions. Heat shock proteins belonging to the hsp70 and hsp90 families have been found to be related to host resistance and are believed to be involved in signal transduction of plant defense responses (Kanzaki et al., 2003). A detailed study of ESTs derived from RNA expressed in sorghum germ and seedling has been made (Pratt et al., 2005). Some libraries were made from seedlings exposed to *Colletotrichum graminicola* and leaves of plants exposed to darkness. A number of transcripts specific to susceptibility (46) or compatibility (40) were obtained. In addition to chitinases, thaumatin-like proteins, $(1\rightarrow3)$-β-glucan hydrolases, chalcone synthases and peroxidases that appear in the list of annotated sequences, many transcripts have been uniquely identified to be present in the diseased tissue. Their function needs to be elucidated. Bowman-Birk inhibitors (double-headed protease inhibitors) and LTPs occur among the list of RNAs expressed during drought (Pratt et al., 2005).

In the case of insect infestation, Zhu-Salzman et al. (2004) showed that genes elicited by green aphids attacking 2-week old seedlings of sorghum for at least 48 h were similar in sequence to those elicited by salicylic acid, but less similar to those elicited by methyl jasmonate. It would appear that the genes elicited by methyl jasmonate, which were more effective against aphids, were actually down regulated and it has been speculated that this may have been due to the release of ethylene. Some of the genes possibly involved in defense against fungi such as $(1\rightarrow3)$-β-glucan hydrolase, chitinase, thaumatin-like protein, were up-regulated, whereas the level of Bowman-Birk inhibitors, cysteine protease inhibitor, lipoxygenase, dhurrinase and other proteins of unknown function were actually down-regulated. Such differential regulation of defense related genes is also seen in the work of Pratt et al. (2005).

1.4.1. Chitinase and (1-3)-β-glucan hydrolases

Three of the chitinases isolated from sorghum, CH1, CH2, and CH3, inhibit the growth of *F. moniliforme* at 500 ng/ml (Krishnaveni et al., 1999a, b). Significant differences were observed in the induction of chitinase activity in red and white sorghum. Ratnavathi and Sashidhar (2004) observed that chitinase activity was significantly higher in red and white genotypes compared with that in yellow genotypes. They suggested that induction of chitinase during seed germination and during fungal infection is an important defense mechanism in sorghum grains. Similar observations had also been made by Ratnavathi and Sashidhar (1998) and Seetharaman et al. (1996). The enhanced chitinase activity in the low-phenolic genotypes could be a compensation for the loss of phenolics. Chitinase and sormatin contents in sorghum kernels increased between anthesis and physiological maturity and thereafter decreased in 17 sorghum varieties and hybrids naturally infected with grain mold (Seetharaman et al., 1996). Rodriguez-Herrera et al. (1999) detected higher contents of sormatin, $(1\rightarrow3)$-β-glucan hydrolase and chitinase in grain mold resistant cultivars than in susceptible cultivars in naturally infected fields. Bueso et al. (2000) observed a decrease in the amount of sormatin and chitinase in susceptible cultivars upon fungal inoculation, but resistant cultivars maintained or increased the levels of these proteins in the caryopsis. Grain mold resistance corresponded to induction of AFP synthesis in response to sprinkling, fungal stress and/or adverse field conditions. No strong association between resistance to grain mold and the accumulation of sormatin and chitinase was demonstrated in sorghum lines inoculated with *F. thapsinum* and *C. lunata* (Prom et al., 2005). This was attributed to factors such as environment, sorghum line, fungal species used, and the fact that sormatin and chitinase may not act alone but synergistically to impart resistance to grain mold. It was also suggested that certain moderately resistant to resistant sorghum cultivars, might employ other strategies to restrict fungal invasion either before or after physiological maturity.

Tiffin (2004) compared the sequences of chitinases of the Poaceae family including those from *Sorghum*, *Zea* and *Tripsacum* (a relative of maize found in the USA). Most sequences fell into one of two distinct groups and the sequences were more similar to one another than to any non-Poaceae sequences in GenBank. This suggests that sequences within each group may have diverged after the establishment of the grass family; however, it is possible that these sequences are products of duplication events predating the Poaceae. The work of Tiffin (2004) also suggested differential rates of evolution for two chitinase groups. Fig. 1 shows that the sequence of sorghum seed chitinase is more related to that of maize than those from wheat or from rye. The importance of these differences in their antifungal activity is not clear. Ary et al. (1989) reported the presence of an endochitinase from Job's tears which was inhibitory to *alpha*-amylase and a similar

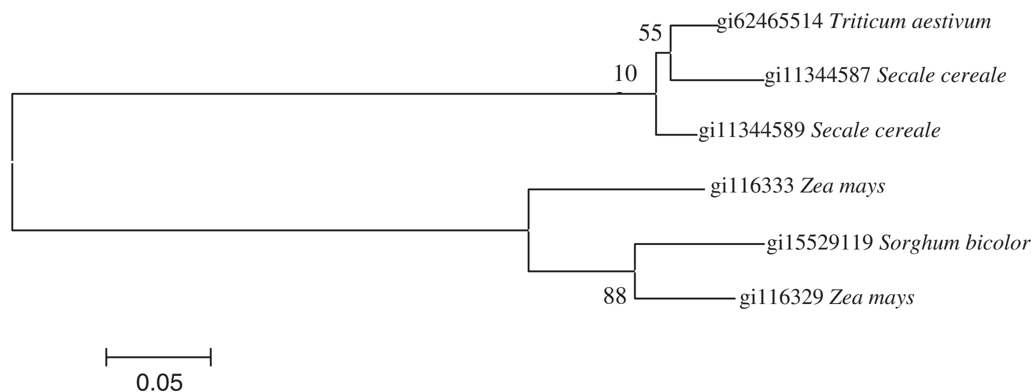

Fig. 1. Neighbor Joining analysis for chitinase sequences from various grains using the Mega-2 program (Kumar et al., 2001). The sorghum sequence is closer to that of maize than that of rye or wheat. Genbank accession numbers are appended.

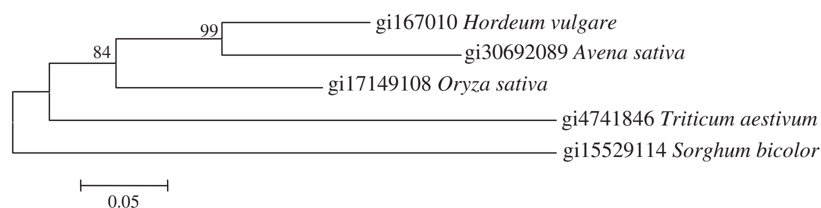

Fig. 2. Neighbor Joining analysis of sequences of (1→3)-β-glucan hydrolases from various grains using the Mega-2 program (Kumar et al., 2001). The sequence from rice is for the enzyme expressed in flowers. Clear divergence of sequences from each other is apparent. Genbank accession numbers are appended.

protein from *Phaseolus vulgaris* (Dayler et al., 2005) has a partial sequence matching that of some endochitinases. Radjacommare et al. (2004) described the purification of a 57 kDa chitinase from finger millet plants challenged with *Pyricularia grisea* after pretreatment with *Pseudomonas fluorescens*. The purified chitinase had anti-fungal activity against the blast fungus, *Pyricularia grisea*, *in vitro*.

The (1-3)-β-glucan hydrolase from sorghum endosperm is closer in sequence to that of wheat. (Fig. 2).

1.4.2. Proteins active against Aspergillus flavus

Mature seeds of sorghum showing toxicity to aflatoxigenic isolates of *Aspergillus flavus* showed four AFPs of molecular weight 20.5, 16.3, 13.9 and 12.2 kDa (Ghosh and Ulaganathan, 1996). The 20.5 kDa protein was found to be more potent than other proteins. The 13.9 kDa protein was also an active inhibitor of fungal growth *in vitro*. The 20.5 kDa protein was located in embryonic tissues of sorghum seeds (Ghosh and Ulaganathan, 2004). The localized expression of proteins involved in defense in embryonic tissues might constitute an effective barrier to microbial entry into the seed. Moore et al. (2004) reported the purification of a protein from a cultivated maize variety that at a concentration of 20 μg/ml inhibited the growth of *Aspergillus flavus* by 50%. There was no activity against *Fusarium moniliforme* at concentrations up to 1275 μg/ml. Maize ribosome-inactivating protein (RIP), was inhibitory to the growth of *A. flavus* and lethal to *A. nidulans* (0.5 mg/ml). Hyphal branching was profuse in *A. flavus* exposed to RIP. No effect was seen when the RIP was mutated. RIP isolated from maize was active only after proteolytic cleavage (Nielsen et al., 2001). The presence of an RIP in sorghum has been reported by Hey et al. (1995). Dowd et al. (1998) showed in maize that RIP after activation by protease is lethal to the caterpillars of the cabbage looper (*Trichoplusia ni*) and was an antifeedant to maize weevils (*Sitophilus zeamais*). Recently Chen et al. (2006) identified a protein over-expressed in maize kernels resistant to *A. flavus* infection. The protein was cloned and was active when expressed in both *E. coli* and in tobacco. The protein has ribonuclease activity and is closely related to the sorghum PR10 protein (Lo and Nicholson, 1998; Lo et al., 1999b) discussed in Section 1.3.

1.5. PR proteins of pearl millet and other millet grains

Downy mildew (*Sclerospora graminicola*) resistance in pearl millet involves lytic factors (Umesha et al., 2000). Lytic activity could be observed within 30 min, whereas the pathogen requires 6–24 h to establish itself in the host. Induced systemic resistance has been observed in the pearl millet-downy mildew system (Kumar et al., 1993), which is associated with increases in the activity of chitinases, (1→3)-*β*-glucan hydrolases and peroxidases. Although the exact role of (1→3)-*β*-glucan hydrolase in pearl millet–downy mildew interactions is still unknown, preliminary studies indicate the involvement of this enzyme in resistance of pearl millet against downy mildew (Kini,

Table 3
Inhibitors of insect pests and enzymes from sorghum and millets

Inhibitor	Source	Inhibitory activity against	Reference
SIα1, SIα2 and SIα3 (γ-Purothionin type)	Sorghum endosperm	*Locusta migratoria* and *Periplaneta americana*	Bloch and Richardson (1991)
Ragi Bowman Birk Inhibitor	Finger millet endosperm	Yellow meal worm (*Tenebrio molitor*) *alpha*-amylase	Strobl et al. (1998)
alpha-Amylase inhibitor	Finger millet, Little millet endosperm	*Callosobruchus chinensis, Locusta migratoria alpha*-amylase	Sivakumar et al. (2006)
alpha-amylase inhibitor; Chitinase Inhibitor	Job's tears seeds		Ary et al. (1989)
RIP	maize and sorghum endosperm	*Trichoplusia ni*	Dowd et al. (1998)

1998). The enzyme is highly basic (p*I* 9.6) with a molecular weight of 20.5 kDa on SDS–PAGE. It is thermostable with a broad temperature-activity profile between 37 °C and 70 °C and an optimum pH of 5.2. Identification of the basic isoform of (1→3)-β-glucan hydrolase in pearl millet suggested an important role for the enzyme in active defense of pearl millet against the downy mildew pathogen. The purified basic (1→3)-β-glucan hydrolase showed limited antifungal activity against *Trichoderma harzianum*, but was inactive against other test fungi such as *Fusarium oxysporum* and *A. flavus*. This may have been due to the inability of (1→3)-β-glucan hydrolase to act alone as an antifungal agent. Investigation of the involvement of lipoxygenase, phenylalanine ammonia-lyase, (1→3)-β-glucan hydrolase, ribonuclease and peroxidases in pearl millet-downy mildew interaction revealed increased activity for these enzymes in highly resistant seedlings and decreased enzyme activity in highly susceptible seedlings (Kini et al., 2000a, b; Nagarathna et al., 1992, 1993; Shivakumar et al., 2000, 2003). (Table 3).

A cysteine protease inhibitor from pearl millet, with a molecular mass of 24 kDa has strong antifungal activity against *Trichoderma reesei*, and species of *Claviceps*, *Helminthosporium*, *Curvularia*, *Alternaria*, and *Fusarium* (Joshi et al., 1998). Modification of a number of amino acid residues in this protein was shown to differentially affect its inhibitory and antifungal activities. Therefore, the antifungal activity was suggested to be an effect separate from its ability to inhibit fungal proteases (Joshi et al., 1999). A lipid transfer protein (LTP) from pearl millet, that has been purified to homogeneity by Velazhahan et al. (2001) has a molecular mass of 25 kDa. Its N-terminal sequence (25 residues) has homology to non-specific LTPs of cotton, wheat and barley. The purified LTP inhibited mycelial growth of *T. viride* and *Rhizoctonia solani*. A non-specific lipid transfer protein has been reported from finger millet (Campos and Richardson, 1983). Non-specific lipid-transfer proteins (nsLTPs) in plants are very basic and contain eight disulphide-linked cysteines (Kader, 1996) and show antifungal activity (García-Olmedo et al., 1995). A class of small polypeptides, isolated from seeds of barley and millet, has striking amino acid sequence identity with phospholipid transfer proteins (Bernhard and Somerville, 1989). As mentioned, hormones have an effect on the defense responses in sorghum (Zhu-Salzman et al., 2004). Ethylene appeared to increase the level of proteins that are antagonistic to the growth of fungi. Sarosh et al. (2005) assessed the effect of L-methionine, the precursor of ethylene (Bleecker and Kende, 2000), on disease development. A close association was found between L-methionine-induced resistance and the accumulation of defense related proteins indicating the changes in a number of signaling events in pearl millet seedlings after infection with *S. graminicola*. The mRNA levels of genes for PR-1a, (1→3)-β-glucan hydrolase, chitinase, peroxidase, lipoxygenases and chalcone synthase increased after inoculation with *S. graminicola*.

1.6. Thionins, protease and alpha-amylase inhibitors

Insects cause significant losses in grain sorghum and other millets, under field conditions as well as under storage. Among plants, proteinaceous enzyme inhibitors are found in cereals and legumes. These inhibitors act on key digestive hydrolases of the insect gut, the *alpha*-amylases and proteinases. Several kinds of *alpha*-amylase and proteinase inhibitors, present in seeds and vegetative organs, act to regulate numbers of phytophagous insects (Gatehouse and Gatehouse, 1998). These insects are cosmopolitan pests feeding on seed products. Plants have evolved defense strategies to counteract these effects through enzyme inhibitors impeding the action of insect gut digestive *alpha*-amylases and proteases. The properties and structures of insect *alpha*-amylases and their inhibitors and the different mechanisms of inhibition has been reviewed (Franco et al., 2002; Payan, 2004; Svensson et al., 2004). Proteinaceous *alpha*-amylase inhibitors can be grouped on the basis of their tertiary structures into six classes: lectin-like, knottin-like, cereal type, Kunitz-like, γ-purothionin-like and thaumatin-like (Payan, 2004).

γ-Hordothionin (SIα1, 5 kDa monomeric form), isolated from sorghum, was the first example of a γ-thionin capable of inhibiting insect *alpha*-amylases. This peptide inhibited *alpha*-amylases from the cockroach, *Periplaneta americana,* and the grasshopper, *Schistocerca americana*, but did not inhibit mammalian *alpha*-amylases (Bloch and Richardson,

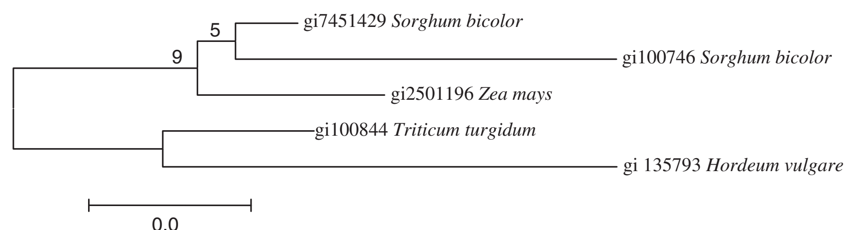

Fig. 3. Neighbor Joining analysis of the sequence of thionins from various grains using the Mega-2 program (Kumar et al., 2001). The sorghum and maize thionins form one clade while wheat and barley thionins belong to another. Genbank accession numbers are appended.

1991). Later, two inhibitors (SIα4 and SIα5) of *alpha*-amylases from insect and mammalian sources were purified from sorghum grain. Comparisons of their sequences with one another and with those of other enzyme inhibitor proteins indicated that the two sorghum proteins had significant similarities (21–42% identity) with members of the cereal superfamily of enzyme inhibitors (Bloch and Richardson, 1992). A form of hordothionin from barley, mutated to bear more lysine (Rao et al., 1994) but retaining antifungal activity, has been used to transform sorghum (Zhao et al., 2003). This protein reduced fungal attack while increasing nutritional value, but needs further evaluation.

Nitti et al. (1995) determined the primary structure of an *alpha*-amylase inhibitor (SIα1) from sorghum which exhibited effectiveness toward both bacteria and fungi. The amino acid sequences of the *alpha*-amylase inhibitors share a high degree of similarity with the related plant gamma-thionins (Nitti et al., 1995). γ-Thionins consist of small basic peptides of approximately 45–47 residues with 4–8 of the cysteine residues that are disulfide linked, and two clusters of basic amino acids located at either end of the polypeptide chain. They appear to play diverse roles in nature, showing anti-bacterial and/or anti-fungal activity (Bloch et al., 1998), inhibition of mammalian cell growth by membrane permeabilization and inhibition of insect *alpha*-amylases and proteases (Broekaert et al., 1995; Castro and Fontes, 2005). Sorghum thionin sequences are closer to those of maize than from other cereal grains (Fig. 3). On this basis a proposal has been made that transgenic plants expressing higher levels of γ-thionins from other plants sources could increase the resistance to pathogens, reducing crop losses (Pelegrini and Franco, 2005). It would be interesting to determine the relative efficiencies of thoinins from the Andropogoneae compared with those from grass seeds.

Serna et al. (2001) showed that transcripts of an antifungal protein could be located by hybridization in the basal placentochalazal region (cells involved in solute transfer) of maize seeds. These transcripts were found only in the endosperm from 7 daa (days after anthesis) to 18 daa. The protein is produced as an 8 kDa protein without the signal prepeptide, and as a 4.5 kDa protein after cleavage of a 31 kDa prodomain. The mature peptide showed strong antifungal activity against *A. flavus, Fusarium culmorum, Alternaria brassicicola, Fusarium moniliforme* with an IC_{50} (concentration of the antifungal protein required to inhibit 50% of fungal growth) ranging from 2–3 $\mu g\,ml^{-1}$. There are strong indications that the gene is present in sorghum, maize and teosinte (*Zea luxurians*) but absent from wheat and barley.

A separate class of inhibitors found in cereal seeds has been designated the *alpha*-amylase/trypsin inhibitor family or cereal inhibitor family (García-Olmedo et al., 1995; Strobl et al., 1995). These proteins are composed of about 120 amino acids and contain a large number of conserved cysteines forming four or five intramolecular disulfide bridges. All functional members of the family are either inhibitors of *alpha*-amylases or trypsin. The only member possessing both functions is the bifunctional *alpha*-amylase/trypsin inhibitor (RBI) from finger millet (Shivaraj and Pattabiraman, 1981). RBI shares 25–66% sequence identity with the other functional members of the inhibitor family (Strobl et al., 1995). It is a monomer of 122 amino acids (Campos and Richardson, 1983) with five disulfide bonds. The protein, purified from the seeds of ragi, has a molecular mass of 13,300 Da and a pI of 10.3. The structure of a bifunctional inhibitor from ragi seeds has been solved by X-ray diffraction analysis (Gourinath et al., 1999, 2000). The RBI is one of the most studied inhibitors from this family and is reported to be active against insect *alpha*-amylase (Alam et al., 2001; Strobl et al., 1998). The *alpha*-amylases from the yellow mealworm (*Tenebrio molitor* L.) and porcine pancreatic *alpha*-amylase (PPA) appear to be inhibited by slightly different mechanisms (reviewed by Payan, 2004) involving the N terminal residues, serine and alanine, and also the proline and cysteine residues at 52 and 55.

Pattabiraman (1986) compared the properties of purified proteinase inhibitors from the small-seeded grains like finger millet, sorghum, pearl millet, Italian millet and barnyard millet. Changes in the levels of inhibitory activities during germination and plant growth of finger and barnyard millets were observed. One of the major inhibitors of foxtail millet (FMTI-III) was shown to be specific for trypsin alone. The sequence of another major trypsin inhibitor (FMTI-II) purified from foxtail millet grain indicated that the protein contains 67 amino acid residues and is similar to FMTI-II, except for the replacement of the C-terminal glutamine by glutamic acid. This single amino acid substitution had no effect on inhibitor-enzyme association (Tashiro et al., 1991).

Sivakumar et al. (2006) tested ammonium sulfate fractions derived from the extracts of little and finger millet grains against the *alpha*-amylases from the storage insects: rice weevil, *Sitophilus oryzae* (Curculionidae); red flour beetle, *Tribolium castaneum,* (Tenebrionidae); pulse beetle, *Callosobruchus chinensis* (Bruchidae); rice moth, *Carcyra cephalonica* (Pyralidae) and from other insect pests such as the tobacco caterpillar, *Spodoptera litura* (Noctuidae); the gram pod borer,. *Helicoverpa armigera* (Noctuidae); castor semilooper, *Acaea janata* (Noctuidae) and the diamond-back moth, *Plutela xylostella* (Plutellidae). The extent of inhibition by the different insects varied from 8.0% to 69.9%. The highest inhibition was recorded for ragi (69.9%) and little millet protease inhibitors (50.0%) against the *Callosobruchus chinensis alpha*-amylase, whereas these inhibitors had the least effect on the *alpha*-amylase from *H. armigera* and *S. litura.*

2. Genetics of disease resistance in sorghum and millets

In sorghum, resistance to downy mildew (*P. sorghi*) is reported to be conferred by a dominant, major gene (Rana et al., 1982). Resistance to downy mildew has been identified from several sorghum sources (Sifuentes and Frederiksen, 1988). Inheritance of resistance to three pathotypes of *P. sorghi* indicated two dominant genes for resistance in QL 3 and one in SC 414-12. However, these sources are race specific and changes in race often lead to lines losing their resistance (Craig and Odvody, 1992). The inheritance of sorghum downy mildew resistance is oligogenic and the number of genes involved varies from 1 to 6 (Rana et al., 1982; Sifuentes and Frederiksen, 1988). Of the four smuts affecting sorghum, head smut caused by *Sporisorium relianum* is the most economically significant (Rooney, 2004). Magill et al. (1997) reported that two genes controlled head smut resistance.

2.1. Genetic markers associated with fungal disease resistance in sorghum and millets

Molecular markers have been linked to sorghum downy mildew resistance (Gowda et al., 1995; Oh et al., 1994, 1996). Klein et al. (2001) identified five quantitative trait loci (QTLs) for mold resistance from a cross between Sureno (resistant) and RTx430 (susceptible) on a sorghum chromosome map using molecular markers. Franks (2003) used the QTLs detected by Klein et al. (2001) in five different sorghum-breeding populations. The marker-assisted selection was efficient in the same population in which the trait was originally mapped and was ineffective in any of the other four populations. This suggests that additional research is required to identify markers that are applicable across populations. Grain mold disease may be best addressed using transgenes that would increase mold resistance in the grain (Rooney, 2004). Parh et al. (2004) have identified a QTL for resistance to ergot, a disease that attacks the flowers of sorghum. A number of individual loci implicated in tolerance in sorghum to the aphid greenbug (*Schizaphis graminum*) have been identified using QTLs (Nagaraj et al., 2005). Progress has been made towards the development of molecular tools for pearl millet (Allouis et al., 2001). QTLs for downy mildew have been mapped (Jones et al., 1995) in pearl millet and the downy mildew resistance genes identified to date exhibited race-specific resistance (Jones et al., 1995; Witcombe and Hash, 2000). This work may indicate that the resistance conditioned by these loci will be rapidly overcome through pathogen evolution. It is unclear whether the pearl millet gene pool contains additional forms of quantitative resistance that are more likely to contribute to durable resistance (Naylor et al., 2004). Markers have been identified for at least 22 different putative downy mildew resistance QTLs in pearl millet (Thakur and Mathur, 2002). Recently QTLs for the resistance against the shoot fly (*Atherigona soccata*) was reported by Dhillon et al. (2006). Table 4 lists some of the QTLs identified for sorghum.

Despite moderate to high heritability for grain mold resistance, improvement in grain mold resistance has been difficult. This is primarily due to each environment favoring a specific mechanism of grain mold resistance, with a consequence that genotypes react differently across a range of environments (Rodriguez-Herrera et al., 2000).

2.2. Transgenics for resistance to fungal disease

Much work has been performed to develop transformation techniques for sorghum and the millets (Able et al., 2001; Datta et al., 1999; Jeoung et al., 2002; Kothari et al., 2005; O'Kennedy et al., 2004, 2006; Zhao et al., 2000). The transformation of sorghum for increased resistance to disease has been reviewed by Muthukrishnan et al. (2001).

Table 4
Qualitative and quantitative resistance trait loci identified in sorghum and millets (Based on Rooney, 2004)

Resistance trait	Reference
Head smut (*Ustilago crameri*)	Oh et al. (1994)
Downy mildew (*Sclerospora graminicola*)	Gowda et al. (1995); Oh et al. (1996); Thakur and Mathur (2002)
Grain quality and mold resistance	Franks (2003); Klein et al. (2001)
Green bug (*Schizaphis graminarum*)	Nagaraj et al. (2005)
Midge (*Stenodiplosis sorghicola*)	Tao et al. (2003)
Sorghum shoot fly (*Atherigona soccata*)	Dhillon et al. (2006)

The approaches that have been taken by researchers using genetic engineering for fungal disease resistance in various crops can be grouped into five general categories (reviewed by Punja, 2001): (1) The expression of pathogenesis-related proteins (PR proteins) that are directly toxic to pathogens or that reduce their growth (Mauch et al., 1988). (2) The expression of gene products such as polygalacturonase, oxalic acid, lipase, polyphenols, and phytoalexins that destroy or neutralize a component of the pathogen. (3) The expression of gene products that can potentially enhance the structural defenses in the plant e.g. elevated levels of peroxidase and lignin. (4) The expression of gene products releasing signals that can regulate plant defenses. These include production of specific elicitors, hydrogen peroxide, salicylic acid, and ethylene. Hyper-expression of resistance gene (R) products which are transcriptional factors affecting expression of PR proteins and are involved in the hypersensitive response (killing or necrosis of area infected by the pathogen) has been shown to provide protection against fungal attack. Although the concepts of durable resistance and resistance gene deployment have been current for several decades, durable resistance has remained an elusive goal for most crop improvement programs (Michelmore, 2003).

The production of transgenic sorghum plants for increased disease resistance without using antibiotics or herbicides as selection agents has been reported (Gao et al., 2005). The reporter gene, *gfp*, encoding the green fluorescent protein (GFP), was used as a visual screening marker, and the target gene encoding thaumatin-like protein (TLP), was chosen for enhancing resistance to fungal diseases and drought. A complete correlation was observed between the GFP expression and the presence of the target gene, *tlp*, in these plants. The transgene segregated in various ratios among progeny, which was confirmed by examining seedlings showing GFP fluorescence. The progeny also showed different copy numbers of the transgenes.

Zhu et al. (1998) reported the introduction of a rice chitinase gene, *chi11* into sorghum under the control of a CaMV 35S promoter. A small proportion (about 20%) of the progeny showed evidence of silencing (reviewed by Muthukrishnan et al., 2001). Transgenic plants expressing the rice chitinase were significantly more resistant to *Fusarium thapsinum* than the non-expressing transgenic plants and control groups. The rice lipid transfer protein, TLP (PR-5 group) has also been introduced into sorghum plants by *Agrobacterium* mediated transformation (Muthukrishnan et al., 2001). The plants contained the selectable marker, *bar,* and the rice *tlp* genes. Several plants were resistant to spraying with phosphinothricin and expressed the 23 kDa rice TLP at quite high levels. Latha et al. (2005) produced transgenic finger millet plants for increased resistance to leaf blast disease using a gene encoding an antifungal protein (PIN) which is substantially homologous to the cationic, antimicrobial, lytic peptide cecropin A from prawns. The fungicidal PIN protein exerts antimicrobial activity, analogous to that of cecropin A, either by targetting anionic, microbial cell membranes and/or by altering transcriptional profiles, leading to cell death. The transgenic plants showed considerable resistance against leaf blast fungus (*Magnoporthae* spp.) at the seedling stage.

One of the main limitations to increased resistance to pathogens has been the relatively low level of resistance obtained with a single plant antifungal gene, a combination of genes is needed. The obvious strategy is to identify combinations of PR-protein genes that are maximally effective against specific pathogens (Muthukrishnan et al., 2001). Evidence for synergistically enhanced protection *in planta* was shown by the co-expression of a rice basic chitinase and an acidic $(1\rightarrow3)$-β-glucan hydrolase (Zhu et al., 1994). The combined expression in transgenic tobacco of a barley class II chitinase, a $(1\rightarrow3)$-β-glucan hydrolase, and a type-I RIP, significantly enhanced protection against fungi, indicating synergistic protective interactions of the antifungal proteins in vivo (Jach et al., 1995). A similar strategy has been applied in other crops with varying success (Anand et al., 2003; Bieri et al., 2003; Datta et al., 2002; Kim et al., 2003). The combined expression of various PR-protein encoding genes to enhance disease resistance in sorghum and millets is an area yet to be explored. However, caution has to be observed when choosing the genes to be used. For example, it has been reported that only specific chitinases exhibit anti-fungal activity and not all chitinases have anti-fungal action against pathogens (Roberts and Selitrennikoff, 1988). Furthermore, pathogens may adapt to the host chitinases (Vidhyasekaran, 1997). Hence, proper selection of chitinase genes and other PR-protein encoding genes are important for the development of transgenic plants with enhanced disease resistance. There may also be silencing of the innate plant chitinases, a possibility not explored so far.

Genetic engineering can be used to change metabolic pathways to increase the amounts of various flavonoids e.g. the deoxyanthocyanidin flavonoids (luteolinidin, apigenindin etc), which play an important role in host-plant resistance to insect pests and fungal diseases) in sorghum. The expression of phytoalexins in transgenic plants may be difficult due to complexities of their biosynthesis. However, stilbenes have been expressed in transgenic tobacco plants, exhibiting various degrees of inhibition of fungal growth (Heller and Forkman, 1993). Over-expression of genes encoding certain phytoalexins, such as *trans*-resveratrol and medicarpin, in transgenic plants resulted in delayed development of disease and symptom production by a number of pathogens on several plant species (Punja, 2001).

Gurr and Rushton (2005b) reviewed the proposed use of pathogen-inducible promoters, which may reduce the cost of resistance by restricting expression to infection sites, and the use of 'designer' synthetic promoters. Emani et al. (2002) suggested that methylation-based silencing is frequent in sorghum and probably responsible for several

cases of transgene inactivation reported earlier for this crop. To reduce gene instability and gene silencing problems, integration of transgenes by homologous recombination would favor the establishment of a simple integration pattern and allow insertion of a transgene into a known and stable region of the genome.

2.3. Transgenics for insect resistance

The development of transgenic plants with insect resistance has become very important during the last few years and has been reviewed (Christou et al., 2006; Pelegrini and Franco, 2005; Sharma et al., 2000, 2004). Researchers addressing host plant resistance against pests and diseases in food crops, both in the field and in storage, face the imposing challenge of enhancing resistance while maintaining the desired nutritional and processing qualities of the grain (Bergvinson and Garcia-Lara, 2004). Work on transformation of sorghum and the development of transgenic plants resistant to insect pests and fungal pathogens has been reviewed by O'Kennedy et al. (2006). Using various transformation techniques, several genes for insect resistance, including the *cry* gene from *Bacillus thuringensis* (Bt) and protease inhibitor genes, have been transferred to various crops. The use of plant derived genes for expression in transgenic plants for insect resistance has been reviewed by Babu et al. (2003). Protease inhibitors from plants are of particular interest because they are part of the natural plant defense system against insect attack. Protease inhibitors are also reportedly active against nematode, viral, bacterial, and fungal pathogens; thus, they may have a cumulative protective effect in plants (Haq et al., 2004). Genes encoding for inhibitors of *alpha*- and *beta*-amylases have been used in a variety of cases to produce insect resistant plants (Ishimoto et al., 1996). Various protease inhibitors have been shown to be capable of partially controlling certain weevil species (see e.g. Girard et al., 1998), and may be suitable for conferring resistance in stored grains. Of the protease inhibitors expressed in transgenic plants to date, those involving cysteine proteinase inhibitors have shown the most promising results, probably because most phytophagous insects employ these proteases in their digestive tracts (Haq et al., 2004). The use of bifunctional *alpha*-amylase/trypsin inhibitors in transgenic plants would help minimize the likelihood of the appearance of resistant insect strains.

Girijashankar et al. (2005) introduced the cry1Ac BT gene driven by a maize wound inducible promoter into sorghum by particle bombardment of shoot apices. The somatic embryos and plants subsequently generated from them produced 1–8 ng delta-endotoxin per gram of fresh leaf tissues and showed partial resistance to attack by the stem borer larvae. The development of transgenic pest resistant varieties with strong insecticidal activities has raised concerns on the development of resistance by insect pests with possible environmental consequences (Babu et al., 2003). To overcome development of resistance, the expression of multiple genes in plants for long-term durable resistance to insects has been emphasized (Datta et al., 2002). The strategy of using more than one foreign inhibitor in transgenic plants that affect different digestive proteases in the insect seems appropriate (Babu et al., 2003). Pyramiding of different genes would reduce the probability of resistance development, since multiple concurrent mutations would be needed in individual insects (Sharma et al., 2000, 2004). However, reports on the genetic engineering of pest resistance in sorghum and millets are lacking and this is an area that should be researched, to reduce losses due to insect pests in field and more importantly during grain storage.

2.4. Breeding options for resistance to grain mold

Breeding crop varieties with durable resistance to diseases is made difficult by the variability in the pathogen populations (Christ et al., 1987). Attempts in conventional breeding are often centered around pericarp color. As pointed out earlier (Section 1.2) pericarp color and resistance to fungal infection can be related. Red or brown glume and grain color and a grain coverage by the glume of more than 75% was important in resistance to grain mold in a breeding program (Reddy et al., 2006). In contrast, Showemimo (2003) reported that grain color was not important in resistance to the mealy bug, *Eurystylus oldi*, wherein open panicle and glume cover provided a greater degree of resistance. Efforts of the International Crop Research Institute for the Semi Arid Tropics to breed for resistance are summarized in Bantilan et al. (2004).

Conventional breeding can only go so far. Stacking or pyramiding of genes is a viable option to overcome pest and disease resistance. For example, multiple pest and disease resistance in rice against bacterial blight species, yellow stem borer species and sheath blight species was obtained by pyramiding transgenes encoding different resistance traits (Datta et al., 2002). The feasibility of delivering a large amount of genetic information in one experiment has been demonstrated by Chen et al. (1998) who co-introduced 13 genes into rice.

Resistance genes (R genes) are important components of the plant surveillance system. R genes directly or indirectly recognize the pathogen and this triggers a diverse array of defense mechanisms. Introduction of an R gene can confer on the plant the ability to recognize the pathogen and mount an effective defense (Gurr and Rushton, 2005a). To engineer durable resistance, more than one R gene needs to be introduced because resistance could be lost by a single loss-of-function mutation in the corresponding pathogen avirulence (Avr) gene. As already noted, one way of overcoming this problem is by pyramiding, in which multiple R genes, each recognizing a unique range of isolates of a pathogen, is incorporated into a single cultivar (Gurr and Rushton, 2005b). Transgenic rice plants resistant to the bacterium *Xanthomonas oryzae pv. oryzae*

have been developed by the introduction of four different R genes (Li et al., 2001).

With completion of genome sequencing projects in rice and *Arabidopsis*, and recent progress in genomics of other crops like maize and sorghum, newer candidate genes for enhancing disease resistance may become available in the near future. With this vast array of genetic information, use of ''genomics-guided transgenes'' (GGT) may be feasible (Naylor et al., 2004). Dominant alleles that confer important agronomic traits of interest (e.g. pest and disease control, drought tolerance) and those of low abundance in breeding populations can be identified and inserted into breeding lines. An example of this approach is the use of the Xa21 gene, which provides resistance to the bacterial blight disease species of rice. Xa21 was originally transferred from a wild relative to cultivated rice by conventional breeding. The gene was first moved from wild rice to cultivated lines through breeding and marker assisted selection (MAS). Transgenic plants bearing the gene have then been made (reviewed by Naylor et al., 2004).

3. Conclusions

Sorghum grain possesses a number of defense mechanisms against pathogens and pests. Some have been well characterized while others remain to be explored. Some sorghum proteins characterized so far are similar to those of maize while there are many PR proteins reported from maize whose presence and function in sorghum grain remains unstudied. There are clear differences in sequences of antifungal proteins from sorghum compared with those in wheat and rye, indicating that although the genes have a common origin there may be differences in their mode of action and efficacy. These differences also provide an opportunity for the use of proteins from other grains to protect sorghum grain. There is high transcriptional control of PR proteins but there is still no systematic understanding of regulatory factors in grains that contribute to disease resistance and or how their distribution within the kernel is regulated. Current information clearly indicates that many proteins have to be simultaneously expressed in different parts of the grain for effective resistance. It is obvious that this is normally achieved through the operation of transcription factors: very little information is available on the transcription factors that control the expression of proteins conferring resistance to pests and pathogens. The effect of hyper-expression of resistance genes, which are often transcription factors, on grain mold in sorghum needs investigation. The possibility of hyper-expressing proteins involved in the synthesis of polyphenols and phytoalexin also exists. There are no reports on the effect of transgenes on similar proteins already present in the plant or seed. For example, the effect of the expression of transgenic heterologous chitinases on the level of normally produced chitinase needs exploration.

A more complete understanding of the defense mechanisms employed by sorghum grains will help in the future development of disease resistant grains, but it must be remembered that insect pests and fungal pathogens will also acquire new offensive capabilities. For example, *Fusarium* produces two toxins: the trichothecenes and dioxynivalenol, that inhibit host protein production and possibly act in defense against other fungi (Nishiuchi et al., 2006). Indeed transgenic plants bearing the trichothecene pump from of the *Fusarium* protects barley against *Fusarium* (Manoharan et al., 2006). There is a link between resistance to insects and to fungi (Dowd and White, 2002; Wiatrak et al., 2005) and this is an area that needs exploration.

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