Reaction of Sorghum to Grain Mould in Relation to Its Biochemical and Morphological Traits

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Abstract

Seeds of eight sorghum cultivars from Ethiopia and South Africa were evaluated for factors that might relate to grain mould resistance. Ergosterol content and extent of discoloration of seeds were used to measure seed invasion by the mould fungi. Ergosterol content of seeds was compared with their chemical and morphological characters to establish relationships between them. Cultivars Gambella1107, NK286, Buster and SNK3939 showed significantly low ergosterol concentration (< 24 µg/g seed) suggesting low colonization of the cultivars by grain mould fungi. NK283 and Seredo contained high ergosterol level (> 100 µg/g) indicating an increased development of grain mould on these cultivars. PAN8446 and IS9302 had ergosterol content of 48 and 63 µg/g seed, respectively, which indicated moderate level of grain mould development on the two cultivars. NK283 and Seredo also showed significantly higher seed discoloration (55 and 35%, respectively) than others (< 15%). Cultivars with relatively low levels of ergosterol contained significantly great amount of glume proanthocyanidin (tannins), seed flavan-4-ol, apigeninidin and/or luteolinidin than those cultivars with higher ergosterol. Among the cultivars with low ergosterol, most had colored seed pericarps and glumes, while some had white seeds. The cultivars varied in number of days to flowering (65–68). However, there was no significant correlation between ergosterol concentration and days to flowering.

Key words: Sorghum, grain mould, resistance, Ethiopia, South Africa

Introduction

Grain mould is a major disease of sorghum worldwide (Singh and Agarwal 1993) including Ethiopia (Girma et al. 1995). The disease is caused by a complex of fungi, primarily *Fusarium thapsinum, Curvularia spp., F. semitectum* and *Phoma sorghina* (Singh and Bandyopadhyay 2000). Grain mould reduces the yield and quality of sorghum seeds. Furthermore, some mould-causing pathogens produce mycotoxins that can poison animals which may feed on sorghum seeds that are contaminated with such toxins (Williams and Rao 1981, Singh and Agarwal 1993).

The most economical and effective control method of sorghum grain mould is the use of genetic resistance (Audilakshmi et al. 1999). An understanding of host plant resistance mechanisms is essential for the effective breeding of mould-resistant sorghum varieties. Resistance mechanisms involving both morphological and biochemical characters of seeds are associated with grain mould resistance (Abebe et al. 1996, Melake-Berhan et al. 1996). Phenolic compounds are one of the most important chemical factors that offer resistance to grain mould, and a variety of the compounds are known to involve in sorghum grain mould resistance (Melake-Berhan et al. 1996, Abebe et al. 1996).

The concentration and type of phenolic compounds in seeds, and hence the degree of their contribution to grain mould resistance, vary according to variety and environmental conditions (Abebe et al. 1996). Breeding for mould resistance needs to determine the kinds and quantities of phenolic compounds present in varieties that grow in different environments. The different resistance factors identified in such varieties may be combined together into a single genotype, thereby developing a variety with a range of grain mould resistance factors.

Some morphological characters of sorghum are reported to have some role in resistance to grain mould These include, for instance, seed pericarp color, glume color and cover, panicle shape and days to flowering or maturity. It is also to be noted that evidences supporting the involvement of some of the traits are not conclusive (Singh and Agarwal 1993, Garud et al. 1994, Abebe et al. 1996, Audilakshmi et al. 1999).

Despite the importance of grain mould in Ethiopia and South Africa, many sorghum cultivars adapted to the two countries have not been evaluated for their reactions to grain mould and for traits that may offer resistance to the disease. The present study was therefore conducted to determine the reactions of selected improved sorghum cultivars grown in Ethiopia and South Africa to grain mould resistance. The study also sought to assess the seed phenol content and morphological traits of the cultivars and thereby examine the relationship of grain mould development with morphological and biochemical characters.

Materials and Methods

Eight improved sorghum cultivars — three from Ethiopia (Gambella1107, Seredo, IS9302) and five from South Africa (NK286, PAN8446, SNK3939, Buster, NK283) — were used for the study. Seeds of each cultivar were sown in plastic pots with a capacity of 5 kg soil and two plants per pot were retained. The experiment was a completely randomised design with three replications. Two weeks after emergence, nitrogen in the form of limestone ammonium nitrate was applied at a rate of 1 g/kg soil.

At the soft dough growth stage (Zadoks et al. 1974), panicles were inoculated with suspensions prepared from mixtures of four known grain mould pathogens (*F. proliferatum, F. subglutinans, C. lunata* and *P. sorghina*). Each pathogen was grown on malt extract agar (MEA) at 25 °C for 10 days. Spore suspensions were prepared by homogenizing cultures in sterile distilled water and decanting the suspension through sterile cheesecloth. After adjusting concentration to 10^5 spores/ml using a haemocytometer (Prasada et al. 1995), the inoculum suspension was sprayed onto panicles until run-off using a hypodermic syringe. Panicles were then covered with moistened plastic sheets for seven days to enhance humidity and thereby facilitate infection. The average minimum air temperature in the greenhouse from inoculation of panicles to physiological maturity of seeds was 12.8 °C and the maximum was 29 °C.

Ergosterol analysis

Ergosterol was analysed based on the modified method of Jambunathan et al. (1991). Two panicles from each replication of each cultivar were harvested and threshed manually. Seed samples from the two panicles were uniformly mixed and a sufficient amount was ground using an Udy Cyclone mill. Flour of 20 g was extracted in 50 ml methanol by vigorously mixing in a 100 ml beaker for 30 minutes using a magnetic stirrer. When the suspension settled, 25 ml clear extract was added to test tubes containing 3 g KOH and agitated vigorously on a vortex mixer to dissolve the KOH. About 10 ml of n-hexane was added to the

mixture and then incubated in a water bath at 75 °C for 30 minutes.

After adding 5 ml of distilled water and mixing thoroughly, the mixture was cooled to room temperature. The upper hexane layer was transferred to clean beakers, while 10 ml hexane was added to the remaining suspension and then thoroughly mixed. The hexane layer was again removed and added to the previous aliquot, and this procedure was repeated once more. Finally the pooled hexane extracts were evaporated to dryness on a hot water bath. The residue was then re-dissolved in 5 ml high performance liquid chromatograph (HPLC) grade methanol and filtered through 0.45 μm filters (Millex, Millipore Corporation, Bedford, USA).

Ergosterol content was determined from the filtrate using HPLC (Waters 600E). The extract was loaded on a reverse-phase column (C18 125A 10 μm particle size, 3.9 mm x 300 mm). The mobile phase was methanol-water (96:4 v/v) at a flow rate of 1.2 ml/minute. The column temperature was maintained at 50 °C. To calibrate the equipment, standard ergosterol (Sigma) was injected several times at a concentration of 5 μg and the HPLC peak area of the standard was recorded. Subsequently, each sample was injected by mixing with the standard ergosterol (5:5 μg ratio). The standard ergosterol had a retention time of ±7 minutes. The peak area of the sample and standard mixture was then recorded. The differences between the peak area of the sample plus the standard and the peak area of the standard alone gave the peak area of the sample. Based on these data, the concentration of ergosterol in μg/g of seed was calculated.

Phenol analysis

Tannin and flavan-4-ol contents of seeds and glumes were analysed using the methods of Watterson and Butler (1983) as modified by Abebe et al. (1996) and Melake-Berhan et al. (1996). After harvest, seeds and glumes were threshed manually from the panicle. After grinding of both the glumes and the seeds separately, 250 mg flour from each cultivar was extracted in 15 ml of 0.5% HCl in methanol for 20 minutes. The suspension was centrifuged for 5 minutes at 5000 rpm and the supernatant was retained. From the supernatant 1 ml aliquot was taken and mixed with 14 ml of 30% HCl in 1-butanol.

Blanks for both flavan-4-ol and tannin analyses were prepared by adding 0.5 ml of the extract to 7 ml of a mixture of methanol, 0.1 N acetic acid and butanol (15:15:70 v/v). The sample and the blank were vortexed and left for one hour. The absorbance of the supernatant was then measured using a spectrophotometer set to 550 nm for flavan-4-ols after correcting for blanks. Then after, test tubes containing the supernatant were maintained in boiling water for 2 hours. After cooling at room temperature, the concentration of tannins (proanthocyanidins) was determined by reading the absorbance at 550 nm after correcting for blanks.

In order to determine the apigeninidin and luteolinidin content, 250 mg flour of seeds was extracted in 15 ml of 100% ethyl acetate for 30 minutes. The supernatant was retained and the residue was re-extracted with 15 ml of 0.5% HCl in methanol for 20 minutes. Acid-treated poly-vinylpyrrolidone (PVP) was prepared as described in Watterson and Butler (1983). The PVP was boiled for 10 minutes in 10% HCl and repeatedly washed with distilled water. The PVP was again washed with acetone and the residue was filtered and allowed to dry. The acid-treated PVP (0.4 g) was then added to 8 ml of the extract and mixed thoroughly using a vortex mixer. The mixture was incubated at room temperature for 10 minutes and centrifuged at 5000 rpm for 5 minutes. Absorbance of the supernatant was determined at 475 nm for apigeninidin and at 495 nm for luteolinidin.

The results of phenol concentrations were expressed as A550/g dry sample for seed and glume proanthocyanidins and flavan-4-ols. Apigeninidin and luteolinidin concentrations

were presented as A475 and A495/g dry sample (Melake-Berhan et al. 1996 and Abebe et al. 1996). Analyses were done twice for each replication. Correlation analysis was made between the phenol and ergosterol contents of the seeds.

Morphological traits and mould severity

Data on morphological traits including panicle compactness, seed and glume color and number of days to flowering were recorded using standard methods (IBPGR and ICRISAT 1993, Abebe et al. 1996). The color of seed pericarp was white for Gambella1107, red for IS9302, brown for Buster and NK286, and light-brown for all other cultivars. Glume color was orange for Gambella1107, red for SNK3939, purple for Buster and PAN8446, and greyed-orange for the rest of the cultivars. The panicle shape was compact and elliptic for Gambella1107, IS9302 and Serodo; while for the other cultivars the shape was semi-compact and elliptic.

The number of days to 50% flowering was about 65 for Buster, 67 for NK286 and PAN8446, 86 for Seredo and 78 for the other cultivars. The degree of discoloration of threshed seeds was measured after harvest at physiological maturity, i.e., ± 13% moisture content. Seeds of 30 g collected from each replication were spread over a glass Petri dish (Audilakshmi et al. 1999). Using a stereo microscope (30X magnification), grain mould severity was assessed as a visual estimate of the percentage of seed surface discolored.

Analysis of variance and Pearson correlations were made using the Minitab Statistical Package for Windows (Minitab 1998).

Results

Ergosterol and phenol analysis

The results of the ergostrol and phenolic compounds of the different cultivars are presented in Table 1. Cultivars NK283 and Seredo contained higher ergosterol (135.6 and 117.8 µg/g, respectively) and showed higher seed discoloration (55 and 35%, respectively). Conversely, these cultivars had significantly lower glume proanthocyanidin concentration than the other cultivars. Furthermore, NK283 and Seredo showed lower seed flavan-4-ol than PAN8446, SNK3939 and Buster.

On the other hand, Gambella1107, NK286, Buster and SNK3939 had significantly lower concentration of ergosterol (3.56–23.58 µg/g seed) and lower seed discoloration (5–10%) than the other cultivars. Among these four cultivars, NK286 and SNK3939 had a relatively higher concentration of seed proanthocyanidins. Gambella1107 and NK286 did not have seed flavan-4-ol, while SNK3939 had the highest concentration followed by PAN8446 and Buster. Significantly higher levels of apigeninidin and luteolinidin were observed in NK286, SNK3939 and Buster than in other cultivars.

Cultivar SNK3939 had significantly higher concentration of glume flavan-4-ols than the other cultivars, while Gambella1107 had the lowest concentration. Gambella1107, NK286, PAN8446 and SNK3939 contained significantly higher level of glume proanthocyanidin than the other cultivars. PAN8446 and IS9302 had intermediate levels of ergosterol concentration (48.24 and 63.20 µg/g, respectively).

Morphological traits and mould severity

The flowering dates of most of the cultivars ranged 65–86 days after planting, and three of the cultivars flowered in less than 70 days. NK283 flowered at the same time with Gambella1107 or SNK3939; however, it had significantly higher level of ergosterol content and seed discoloration. In addition, cultivars like Seredo that flowered relatively late (> 80 days) showed significantly greater ergosterol content and seed discoloration than early-flowering cultivars such as Buster and NK286.

Table 1. Mean per cent seed discoloration, concentrations of ergosterol and phenolic compounds in seeds and glumes of different sorghum cultivars

Cultivar	Seed discolo-ration (%)[1]	Ergosterol (µg/g)	Seed proantho-cyanidins (A550/g)[1]	Glume proantho-cyanidin (A550/g)	Seed flavan-4-ol (A550/g)	Seed Apigenini din (A475/g)	Seed luteolinidin (A495/g)	Glume flavan-4-ol (A550/g)
NK286	10.0cd	12.37f[2]	1.85a	2.07a	0.00d	1.85a	1.86b	0.02d
PAN8446	14.7c	48.24d	1.61b	2.02ab	0.06b	0.68c	0.66c	0.02d
SNK3939	5.0e	23.58e	1.83ab	2.01ab	0.09a	1.85a	2.03a	0.08a
Buster	8.0de	12.77f	1.76ab	1.79c	0.06b	1.04b	0.73c	0.03c
NK283	55.0a	135.60a	1.72ab	1.58d	0.01cd	0.28e	0.03e	0.06b
Gambella-1107	5.0e	3.56g	1.69ab	2.00ab	0.00d	0.03f	0.00e	0.01e
Seredo	35.0b	117.80b	1.67ab	1.70cd	0.01cd	0.48d	0.44d	0.03e
IS9302	7.0de	63.20c	1.79ab	1.84bc	0.01cd	0.19e	0.09e	0.06b
SE	1.98	2.27	0.07	0.06	0.01	0.04	0.05	0.002

[1]Arc sine transformation used for analysis but means presented here are not transformed.
[2]Means within a column followed by the same letter are not significantly different at 5% level based on Duncan's Multiple Range Test.

Except Gambella1107 that had white seed pericarp, all the cultivars had colored seed pericarps (red, light-brown or brown) and glumes (greyed orange to purple). Some cultivars that differed in their degree of seed invasion by mould and the consequent ergosterol level were observed sharing similar pericarp colors. For instance, NK283 and Seredo which had higher ergosterol level (> 100 µg/g) had light brown pericarp color as SNK3939 that contained significantly lower ergosterol (23.58 µg/g). Moreover, Gambella1107 which had white pericarp color had significantly lower ergosterol level than cultivars with brown, red or purple pericarp colors.

Similarly, cultivars like NK283 that had higher ergosterol levels (> 135 µg/g) had the same glume color as NK286 (12.37 µg/g ergosterol and 10% seed discoloration). There were also cultivars that varied in their glume color while their ergosterol content and degree of seed discoloration were equal. All the cultivars used in the present study had either semi-compact or compact panicle shape. Some cultivars that had the same panicle shape, for instance NK283 and NK286 (both semi-compact) and Gambella1107 and Seredo (both compact), differed significantly in their ergosterol content. Moreover, Gambella1107 and NK286 had different panicle shape, but they showed equal level of ergosterol.

There was highly significant negative correlation ($r = -0.83$, $n = 8$, $p < 0.01$) between seed ergosterol level and glume proanthocyanidin level. Different degrees of negative correlations were observed between ergosterol content and the various phenols contained in the seeds and/or glumes. There was no significant correlation between seed ergosterol level and days to 50% flowering. The degree of seed discoloration also did not significantly correlate with days to 50% flowering ($r = 0.40$, $p > 0.05$).

Table 2. Correlation between ergosterol concentration, grain discoloration and different biochemical and morphological traits of seeds and glumes of sorghum cultivars

Character	Correlation coefficient (r)
Seed proanthocyanidin	-0.11[ns]
Seed flavan-4-ols	-0.27[ns]
Seed apigeninidin	-0.42[ns]
Seed luteolinidin	-0.46[ns]
Glume flavan-4-ols	0.35[ns]
Glume proanthocyanidin	-0.83**
Seed discoloration	0.91**
Days to 50% flowering	0.58[ns]

** Highly significant ($p = 0.01$); $n = 8$ with and each figure is an average of 3 observations
ns Not significant ($p > 0.05$)

Discussion

The quantification of fungal biomass in seeds using biochemical characteristic specific to fungi (ergosterol method) is one of the sensitive methods used in detecting the degree of seed colonization by fungi (Singh and Agarwal 1993). According to Audilakshmi et al. (1999), the ergosterol content of most sorghum cultivars resistant to grain mould was not more than 20 µg/g although some resistant cultivars had 25 µg/g. In the present study, cultivars Gambella1107, NK286, SNK3939 and Buster had lower concentrations of ergosterol (< 24 µg/g) than other cultivars. The result, therefore, indicated a lower colonization of these cultivars by grain mould fungi.

In contrast, Audilakshmi et al. (1999) reported that majority of the cultivars known to be susceptible to grain mould had ergosterol level of > 100 µg/g. But moderately resistant cultivars showed ergosterol levels of 30–56 µg/g. In the present study, the high ergosterol concentration in Seredo and NK283 (> 100 µg/g) showed an increased development of grain mould on these cultivars which therefore might also be an indication of their susceptibility to grain mould. Moreover, the results for the other cultivars whose levels of ergosterol were between the highest (> 100 µg/g) and lowest (< 24 µg/g) levels indicated that they were moderately colonized by the grain mould fungi.

In the present study, ergosterol level strongly correlated with seed discoloration ($r = 0.91$, $p \leq 0.01$) (Table 2). The result showed the usefulness of the ergosterol method used as a measure of mould severity. Hence, cultivars that contained > 100 µg/g ergosterol significantly differed in their seed discoloration from those with low ergosterol level (< 25 µg/g). In some cultivars, however, degree of seed discoloration did not differentiate between cultivars that significantly differed in ergosterol content. The result indicated the insensitivity of the seed discoloration method to measure grain mould severity compared with the ergosterol method. A similar result was also reported by other studies (Singh and Agarwal 1993). Seitz et al. (1983) found a significant and positive correlation between ergosterol content and seed discoloration. However, the authors reported that seed discoloration did not give sufficient indication of the extent of colonization by grain mould fungi.

NK286 had higher seed apigeninidin, luteolinidin and glume proanthocyanidin, suggesting a possible role of these compounds in limiting seed invasion by mould fungi. Levels of glume proanthocyanidin are known to affect resistance of sorghum to grain mould (Abebe et al. 1996). Likewise, the low ergosterol level in SNK3939 might be due to its seed and glume proanthocyanidin, although the cultivar also had higher level of seed flavan-4-ol that might had contributed to the low seed colonization. Flavan-4-ols were reported to impart resistance to grain moulds in sorghum (Melake-Berhan et al. 1996).

In addition to increased amount of seed flavan-4-ol, the cultivar Buster which had low ergosterol (12.77 µg/g), had greater amount of seed apigeninidin and luteolinidin. Thus, the low ergosterol observed in Buster might be attributed to these factors. Furthermore, PAN8446 and IS9302 with ergosterol levels of 48.24 and 63.20 µg/g, respectively, had relatively higher levels of apigeninidin and/or luteolinidin compared to cultivars with high ergosterol (> 100 µg/g). Apparently, apigeninidin and luteolinidin contributed to the relatively limited development of grain mould on these cultivars. Abebe et al. (1996) reported the involvement of apigeninidin, which is highly associated with luteolinidin), in sorghum resistance to grain mould.

Gambella1107, which contained the lowest ergosterol level (3.56 µg/g), had either comparatively lower or none of some of the phenols mentioned above. But the cultivar

contained almost as high level of glume proanthocyanidin as NK286, PAN8446 and SNK3939. Hence, Gambella1107 might had resisted colonisation by grain mould partly through its glume proanthocyanidin. Mansuetus (1990) reported that compared to susceptible varieties, resistant ones contained higher levels of phenolic compounds in their glumes and that such glumes were less colonized by *F. thapsinum*.

In contrast, Audilakshmi et al. (1999) found a weak relationship between measures of invasion by grain mould and levels of glume phenol. Therefore, further studies are required about the influences of glume phenol on grain mould using a large number of cultivars. Other traits not considered in the current study, such as seed texture/hardness, may give resistance to grain mould in sorghum. Such traits might also have played important role in the low development of grain mould on Gambella1107 that had no as high phenolic compounds as cultivars like NK286 and Buster.

In the present study, cultivars with similar number of days to flowering significantly differed in their ergosterol content and seed discoloration. Furthermore, the correlation between seed ergosterol level and days to 50% flowering was not significant, suggesting the absence of relationship between the two parameters. In other studies (Garud et al. 1994), flowering date appeared to influence grain mould severity with late flowering types showing lower mould levels. In contrast, Abebe et al. (1996) found that late flowering might not always be related to resistance to grain mould.

Cultivars used in this study were closely related in their panicle shape, i.e., some had semi-compact shape and others compact shape. Some cultivars with different panicle shape exhibited comparable ergosterol level and there were also cultivars of the same panicle shape that had significantly different ergosterol level. Thus, a relationship between panicle shape and degree of colonisation by grain mould fungi was not evident from the present results.

Colored pericarp seeds may better resist grain mould than white seeds (Hiremath et al. 1993). However, the susceptibility of seeds with colored pericarp has been reported (Williams and Rao 1981, Audilakshmi et al. 1999). In the present study, colored pericarp was not consistently associated with low level of grain mould (ergosterol). The present finding that cultivars with low ergosterol level possessed colored glumes is in agreement with that of Audilakshmi et al. (1999) who found a strong relationship between darker glume colors (dark purple to black) and grain mould resistance. In the present study, a cultivar with lighter glume color (greyed orange) was also observed having significantly lower ergosterol level and seed discoloration.

Generally, the results of the current study revealed that cultivars with diverse and increased levels of phenolic compounds had relatively reduced level of ergosterol and seed discoloration. The results provide information on biochemical and morphological seed characters of some cultivars adapted to Ethiopia and South Africa in relation to their reaction to grain mould under greenhouse condition. Once these results are confirmed under field conditions, they may offer a possibility of using some seed characters to predict cultivar resistance to grain mould.

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