Role of Stalk-Anatomy and Yield Parameters in Development of Charcoal Rot Caused by *Macrophomina phaseolina* in Winter Sorghum

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Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. damages stalk tissue and weakens stalk strength, leading to lodging of crop and loss in grain yield in sorghum. In an attempt to understand the pathogenicity by *M. phaseolina* in relation to stalk characters and plant outputs, the effects of morphological (stalk-thickness), anatomical (bundle-number, bundle-density, and vascular tissue) and physiological characters (water and soluble sugar content) of the stalks of winter sorghum genotypes on charcoal rot development were analyzed. Bundle-number and stalk-thickness had a significant influence on internal spread of charcoal rot. A thicker stalk facilitated the spread of lesions more than did a thinner one. A stalk with densely packed vascular bundles inhibited lesion advancement. Field studies with 24 winter sorghum genotypes demonstrated that most of the parameters that could improve grain and stover yield also increased the length of charcoal rot lesions. Lesion development in stalks showed a high degree of association with grain yield (R^2=0.51) and 100-grain weight (R^2=0.42). The rate of symptom development in stalks differed during dough and maturity stages. It was concluded that anatomical characters of sorghum stalk, especially bundle-number, along with yield parameters played an important role in determining the extent of stalk damage by charcoal rot in winter sorghum.

KEY WORDS: Grain yield; stover yield; vascular bundle density.

INTRODUCTION

Charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. is an economically important disease worldwide. In India the disease assumes significance in a large area (around 5.0 million ha) because of damage to stover (dry fodder) and grain during the post-rainy (winter) season. The disease not only reduces grain yield (up to 64% in hybrids) but also intoxicates stover (1,4,12). Loss of grain yield is due chiefly to lodging of the crop and loss of stover quality is due to rotting of the stalk. Charcoal rot is systemic in nature and the pathogen moves through the xylem, causing extensive rotting of the stalk – resulting in lodging. Soil moisture stress predisposes to charcoal rot and increases the incidence (16). The disease becomes severe when plants are under physiological and environmental stress. Other physiological conditions in plants, such as levels of sugar (11), water in the stalk (14), sink/source balance (7) and non-senescence (15) are known to influence stalk rotting and level of host resistance. The strength of the basal part of the stalk plays a very important role in lodging resistance. Development of lodging-resistant

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cultivars has focused on improving the morphological and anatomical characters of the stalk (2,9,10). Studies on understanding the contribution of morphological and anatomical characters in stalk rotting are limited, and information on how stalk characters influence stalk rotting and host resistance, and how the disease progresses during different growth stages is lacking. It is known that the disease is more severe in high-yielding cultivars (12) as compared with the low-yielding local land races, but correlations of yield losses to severity of charcoal rot are missing.

The objectives of the present study were to (i) understand the influence of stalk characters on stalk rotting during different plant growth phases and (ii) correlate sorghum yields and charcoal rot.

MATERIALS AND METHODS

The sorghum genotypes used in the experiments were obtained from the Centre on Rabi Sorghum, Solapur, and the All India Coordinated Sorghum Improvement Programme.

Stalk characters in relation to symptom progression Seven sorghum genotypes – E36-1, CSV14R, CSH15R, M35-1, Sel-3, CSV8R and local land race LG146 – were selected for the study. Seeds were surface-sterilized with 0.01% $HgCl_2$ for 3 min, washed with sterile distilled water and sown in cement pots (45 cm diam × 45 cm height) previously filled with potting mixture (black soil, sand and farmyard manure, 3:2:2). Each genotype was grown in three replications (four pots of five plants constituted one replication). At 30 days, five plants with uniform growth and spacing were selected and the rest of the plants were removed. Equal amounts of water were given to each pot as required. Plant height and diameter of the basal part of the stalk (at the middle of the1^{st} internode) were recorded on the day of 50% flowering of the genotype. On the same day, eight plants (per replication) were selected randomly and collected by cutting at the collar region. These plants were used to study the stalk anatomy, stalk-sugar and stalk-water. Further watering was not provided for the remaining plants, which were used for artificial inoculation for recording colonization of *M. phaseolina* inside the stalk.

Anatomical and physiological characters Anatomical characters such as vascular bundle number, density and distribution patterns were recorded. A thin transverse section was taken from the 2^{nd} internode of every selected plant and observed under a microscope (Fig. 1). Number of vascular bundles was recorded separately in the outer peripheral region (1 mm thick) and central region through a compound light microscope (Magnus MRX, Olympus). The percentage area covered by bundle tissues (amount of mechanical tissue) was calculated by measuring the mean diameter and cross-sectional area of bundle tube in a transverse section of the stalk.

Basal stalks (50 cm long) were collected from four plants (per replication) at the flowering stage. Total soluble sugars (TSS) were analyzed by the colorimetric method of Dubois *et al.* (8). Absolute water content (AWC) was measured in the freshly collected stalks. The fresh stalk sample was weighed and dried at 70°C until constant weight was obtained in consecutive measurements of dry weight. AWC was calculated from the formula:

$$AWC(\%) = \frac{Fresh\ weight - Dry\ weight}{Dry\ weight} \times 100$$

Artificial inoculation of sorghum stalk Potted plants (12 per replication) were artificially inoculated with a virulent culture of *M. phaseolina* by inserting a pathogen-infested toothpick at the 2^{nd} internode on the 10^{th} day after flowering to facilitate *in situ* development of charcoal rot (17). Internal spread of symptoms in the inoculated plants was recorded in two phases: six plants at 20 days after inoculation (hard dough) and the remaining six plants at 40 days after inoculation (physiological maturity). Every diseased stalk was split open along with its roots and was observed for length of charcoal rot lesion caused by *M. phaseolina*. The pathogen was re-isolated from rotted stalks to confirm that it was responsible for the rot. The precise length of spread of rotting was measured from the point of inoculation up to the height of the stalk as described by Das *et al.* (5). Replicated data were analyzed.

Charcoal rot *vs* yield In another field experiment 24 sorghum genotypes including released varieties (Maulee, Sel-3, M35-1, CSV8R and CSV14R), hybrids (CSH15R and CSH19R) and improved sorghum lines (SPV1585, SPV1590, SPV1597, RSP3, AKR150, RS29, RS585, RS653, RS673, R354, RR9817, RR9818, IS2312, SLV3, SLV5, SLR5 and C43) with different maturity groups and yield potential were selected for the study.

Plants were raised in a charcoal-rot-sick plot (110 microsclerotia of *M. phaseolina* per g soil) at the farm of the Centre on Rabi Sorghum, during the winter (Rabi) season. Every genotype was grown in two 5-m-long rows in a randomized block design with three replications. Plot size was 5 m $\times$ 0.90 m, with 12 cm between plants. Irrigation to the crop was withheld at 65 days after emergence (DAE) (flowering stage) in order to induce moisture stress and facilitate development of charcoal rot. Time to 50% flowering and physiological maturity was noted for every genotype. Incidence of charcoal rot was recorded at 120 DAE. Discoloration at the base and soft-stalk (an indicator of charcoal rot development by pressing at the first internode) were noted. Plants showing discoloration or soft stalk were carefully split open (to minimize stalk injury) with a knife at the first internode to confirm the presence of charcoal rot symptoms. *M. phaseolina* was re-isolated from rotted stalks to confirm that it was responsible for the rot. Disease incidence was calculated in every genotype. Length of internal colonization of charcoal rot symptoms was recorded on eight randomly affected plants from each plot at 15 days after physiological maturity. Grain yield, stover yield (dry fodder) and 100-grain weight were recorded from 20 randomly selected plants in each plot after harvest. Data were analyzed with the SPSS (version 10.0) software package.

Data analysis Data were analyzed using statistical software package 'Statistix' (version 8.1), unless mentioned otherwise.

RESULTS

Anatomical and physiological characters Most of the morphological, anatomical and physiological characters of stalks under study, except bundle-density, showed variations among the genotypes. Thickness of the stalk was significantly greater in CSH15R and CSV8R than in the local land race LG146 (Table 1). In other genotypes (CSV14R, M35-1, E36-1 and Sel 3) there was not much difference in stalk thickness. Among the test genotypes, CSV8R had a significantly higher number of vascular bundles than Sel 3 and LG146 and was at a par with other genotypes (CSH15R, CSV14R, M35-1 and E36-1). Although variation in bundle-density was almost absent, its distribution at the peripheral region varied significantly among the genotypes. Bundle-density was greater at the peripheral region than in the central cortex. M35-1 had significantly higher peripheral bundle-density than Sel 3 and CSV14R and was at a par with other genotypes. Vascular tissue content in E36-1 was significantly higher than CSV8R, CSV14R, Sel3 and LG146. AWC differed significantly in most of the genotypes, being the highest in LG146 and E36-1. TSS was similar in most of the genotypes, except in E36-1 and CSH15R in which TSS was significantly higher. Mean lesion length was significantly higher in CSV8R than in all other genotypes; among the others, mean lesion length in CSH15R, CSV14R and M35-1 was significantly higher than in E36-1 and LG146.

Stalk rotting during different growth stages Progression of charcoal rot symptoms inside the stalk differed between the dough and physiological maturity stages. Rate of lesion spread was faster during the grain maturity stage than at the dough stage (Fig. 2). In most of the genotypes, except E36-1, stalk rotting occurred mainly during grain maturity. In E36-1 the rate was slow, with only a marginal increase during grain maturity.

Correlation matrix revealed that among the morphological and anatomical characters, stalk thickness and bundle-number had a positive influence, whereas bundle-density and vascular tissue had a negative influence on internal spread of charcoal rot lesions (Tables 1, 2). The influence of stalk thickness and bundle-number on mean lesion length was stronger than that of bundle-density and vascular tissue. AWC and TSS had strong negative effects on lesion development (γ = -0.64, $P<0.01$; and γ = -0.47, $P<0.05$, respectively). The thicker the stalk, the lower was the bundle-density and AWC (Table 2). Stalk with more vascular tissue contained more TSS (r= 0.66, $P<0.01$).

Influence of yield components on symptom progression Based on grain yield (sink size), 24 test genotypes could be categorized into four tentative sink groups (low: $\leq$20; low-medium: 21–30; medium: 31–40; and high sink: $\geq$40 g grain yield per plant) for studying the influence of each group in charcoal rot lesion development (Table 3). There were significant differences in the length of charcoal rot lesions among the different sink groups. Maximum and minimum lesion length was recorded in the high-sink (43 cm) and low-sink (11 cm) group, respectively. No significant differences were recorded in lesion length between low and low-medium, or between low-medium and medium sink groups. Stover yield (dry fodder) and biological yield (grain yield + stover yield) were significantly higher in the high-sink group than in the three other sink-groups, which were at a par. Seed weight was significantly high in the high-sink and low in the low-sink group.

All of the yield parameters under study (*viz.*, grain yield, stover yield, biological yield and seed size) showed a strong association with internal advance of charcoal rot lesions (Fig. 3). The higher the biological yield and seed weight, the greater was the internal spread of rotting. Regression analysis showed that biological and stover yield had the highest degree of association (R^2= 0.61) with lesion length, followed by grain yield (R^2= 0.51) and 100-grain weight (R^2= 0.42).

DISCUSSION

Sorghum is a top-heavy grass and is therefore prone to lodging, which occurs when the bending movement of a plant exceeds the strength of the stem base (3). The strength of the basal part of the stalk plays a very important role in reducing lodging – and thereby yields – in sorghum. Charcoal rot damages stalk tissue and weakens the strength of the

TABLE 1. Morphological, anatomical and physiological characters of stalks of selected winter sorghum genotypes

Genotype	Stalk thickness (diam, mm)	Bundle-number	Mean bundle density (no. mm^{-2})	Bundle density at periphery (no. mm^{-2})	Amount of vascular tissue (%)	Absolute water content (%)	Total soluble sugar (mg g^{-1})	Mean lesion length (mm)
CSH15R	12.4±0.5 a[z]	357±18.5 ab	3.0±0.2 a	6.6±0.1 ab	46.7±5.4 ab	406±5.3 de	26.7±2.1 b	99.0±9.1 b
CSV8R	11.9±0.6 ab	378±14.0 a	3.4±0.4 a	7.0±0.7 ab	41.1±4.4 b	395±4.6 e	17.1±1.9 c	120±18.1 a
CSV14R	11.5±0.8 abc	315±27.2 abc	3.1±0.6 a	6.1±0.4 b	41.4±9.1 b	381±2.6 f	18.4±1.0 c	93.9±12.4 b
M35-1	11.4±0.9 abc	355±18.6 ab	3.5±0.4 a	7.4±0.9 a	51.8±4.3 ab	437±6.0 c	20.8±1.6 c	87.8 ±7.5 b
E36-1	11.2±0.3 abc	341±7.0 ab	3.4±0.2 a	7.0±0.2 ab	65.4±7.3 a	517±3.0b	34.3±2.1 a	62.9±4.0 c
Sel-3	10.4±0.8 bc	269±40.2 c	3.2±0.5 a	6.0±0.7 b	40.0±8.8 b	413±5.0 d	20.6±0.5 c	80.8±6.1 bc
LG146	10.0±0.5 c	293±17.0 bc	3.8±0.6 a	7.1±0.8 ab	40.4±9.8 b	666±2.6 a	19.9±0.2 c	68.1±10.9 c
Correlation coefficient with mean lesion length	0.59**	0.49*	-0.33	-0.25	-0.41	-0.64**	–0.64**	1.00

[z] Within columns, means (± S.D.) with a common letter do not differ significantly by Duncan's Multiple Range Test (P=0.05).

*, ** = significant at 5% and 1% level, respectively.

TABLE 2. Correlations among winter sorghum stalk characters and mean charcoal rot lesion development

	Stalk thickness	Bundle-number	Bundle-density	Bundle-density (P)	Vascular tissue	AWC	TSS
Bundle-number	**						
Bundle-density	_**	ns					
Bundle-density (P)	ns	*	**				
Vascular tissue	ns	ns	ns	*			
AWC	_**	ns	*	ns	ns		
TSS	ns	ns	ns	ns	**	ns	
MLS	**	*	ns	ns	ns	_**	_*

*, ** = significant at 5% and 1% level, respectively.
ns= non-significant

TABLE 3. Spread of charcoal rot lesions in winter sorghum genotypes of different sink groups

Grain yield (g plant^{-1})	Sink group	Biological yield (g plant^{-1})	100-seed weight (g)	Lesion length (cm)
18–20 (3)^z	Low	49 b^y	2.0 c	11 c
21–30 (12)	Low-medium	58 b	2.3 bc	18 bc
31–40 (7)	Medium	70 ab	2.7 ab	24 b
41–50 (2)	High	98 a	3.4 a	43 a

^{z}Figures in parentheses indicate number of genotypes in the group.
^{y}Within columns, means with a common letter do not differ significantly by Duncan's Multiple Range Test (*P*=0.05).

stalk, leading to lodging. The present study indicated that stalk anatomy, especially the thickness of the stalk at the basal part and the total number of vascular bundles in the stalk, determines to some degree the role in development of charcoal rot in sorghum. A strong correlation between bundle-number and mean lesion length can be related to the fact that the vascular bundles direct the upward movement of the pathogen. Increase in bundle-number might have increased the chances of fungal movement through the stalk. Bundle numbers differed significantly between E36-1 and Sel 3, but mean lesion length did not. This indicated that factor(s) other than bundle-number might be involved. The amount of TSS, AWC and vascular tissue were significantly high in E36-1 and might have provided resistance to lesion development in this genotype. E36-1 is a stay-green genotype and is known for its resistance to stalk rotting and lodging (5). Similarly, in CSV8R mean lesion length was significantly greater than in CSH15R, CSV14R and M35-1, but there were no significant differences in bundle-number, bundle-density (mean) or stalk-thickness. A high level of TSS in CSH15R, bundle density in M35-1 (bundle density was negatively related with mean lesion) and grain mass in CSV14R (data not shown) might have led to resistance to lesion development in these genotypes. Earlier studies have demonstrated that stalk-sugar (11) and stalk-water (14) are indicators of physiological stresses in plants and when present in higher quantities, they lead to resistance to charcoal rot development in sorghum.

Variations in AWC and TSS observed among the test genotypes were due purely to response of the genotypes, since growth conditions (moisture stress, temperature) were similar for all. Such genotypic variations are common in sorghum (11,14). A strong correlation between TSS and vascular tissue suggested that most of the soluble sugars were

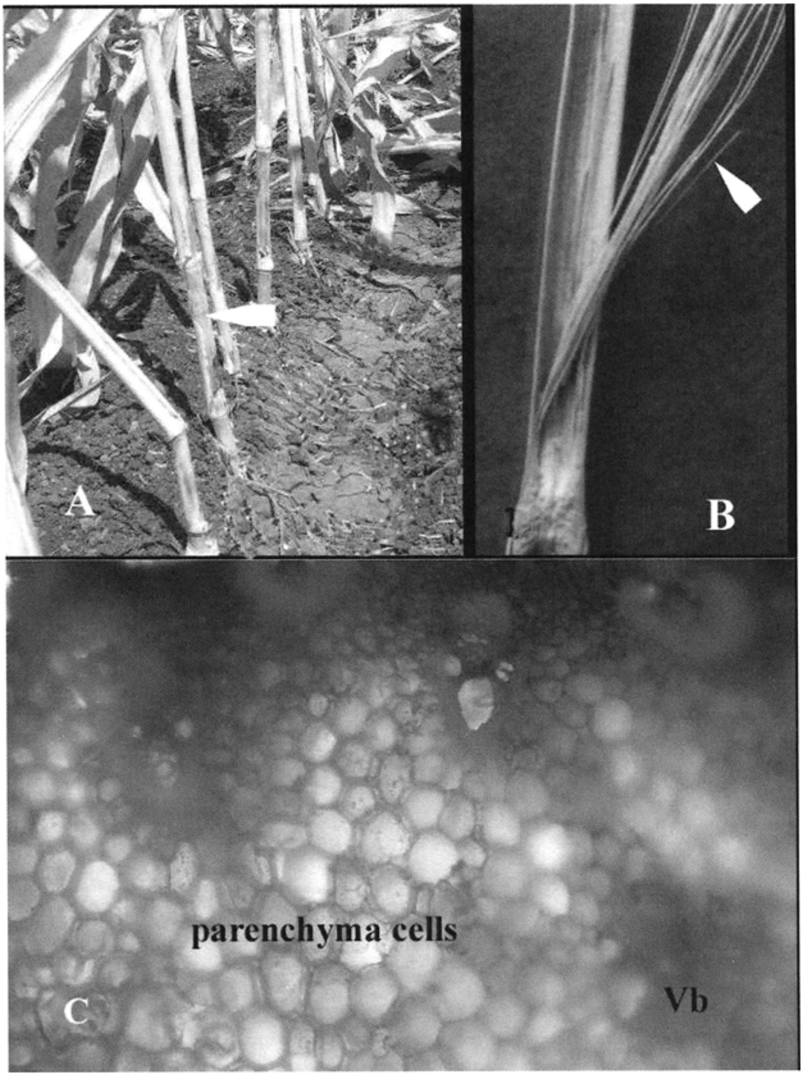

Fig. 1. (A) Charcoal rot-infected winter sorghum plants (arrow) in field. (B) Disintegration of parenchyma tissues and separation of vascular bundle tubes (arrow) in charcoal rot-infected sorghum stalk. (C) Transverse section of sorghum stalk at 2^{nd} internode showing distribution of vascular bundles (Vb) and parenchyma cells in the cortex.

present in the vascular tissue (phloem and xylem tissues) – because vascular tissues act as channels for movement of photosynthates. Lack of variations in bundle-density among the test genotypes (except in M35-1, in which peripheral bundle-density was quite high) is supported by our earlier observations that bundle-density is a relatively stable character in a sorghum genotype (6). A negative effect of bundle-density and vascular tissue on mean lesion length might be due to the inability of *M. phaseolina* to decompose the sclerotic mechanical tissues of the vascular bundles. The vascular tissues might have acted as a barrier to the advancement of the rotting lesion. Moreover, an increase in vascular tissue

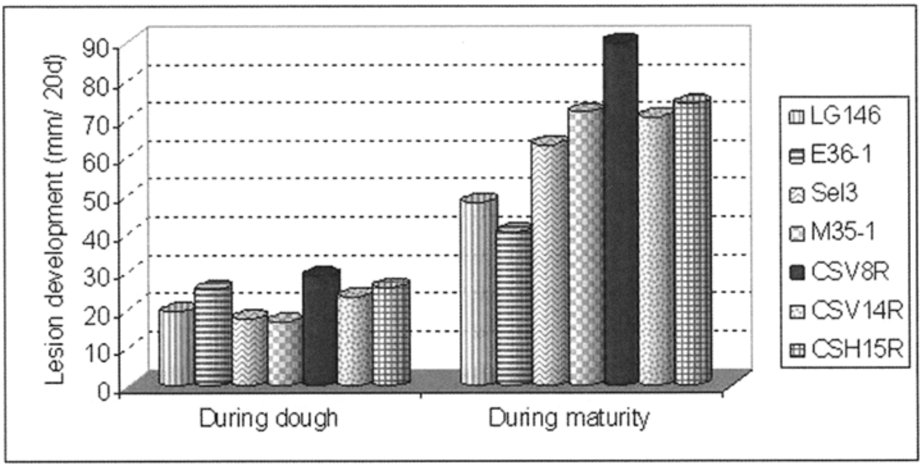

Fig. 2. Rate of charcoal rot development during two different winter sorghum growth stages.

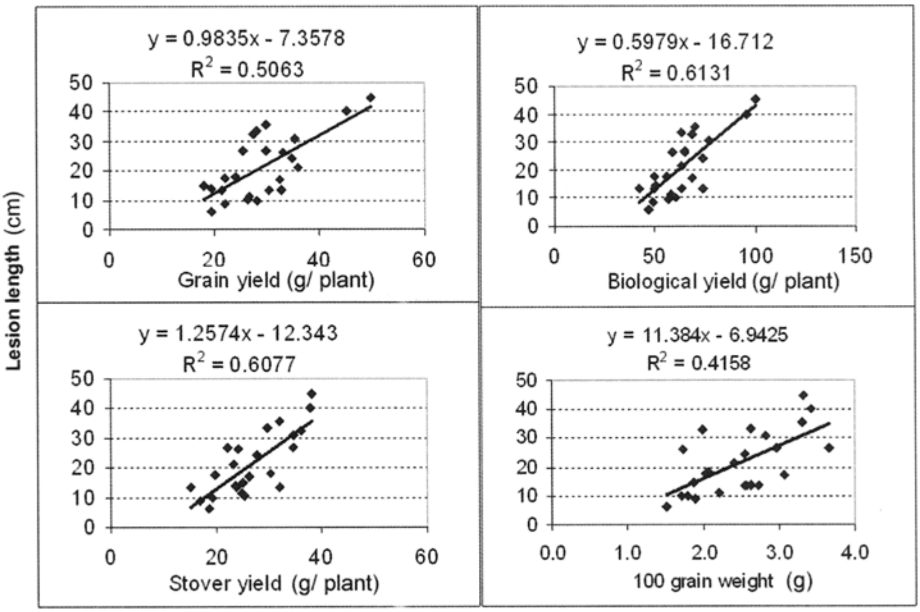

Fig. 3. Scatter diagram showing relation of lesion length to yield parameters in winter sorghum

in the stalk might have left less space for parenchyma tissue, which can be infected and colonized by the pathogen. Bundle-density being a less variable and more stable character (6), an increase in stalk-thickness causes an increase in bundle-number (strong positive relation with thickness; r= 0.66, $P<0.01$) and a concomitant decrease in AWC (strong negative relation with thickness; r= -0.57, $P<0.01$). This might be the reason why a thick-stalked genotype tended to produce a longer lesion than a thin one. As the growth advances from the milk stage, the level of physiological stresses (stalk-water, stalk-sugar) also increases (7) and parenchyma tissues placed around the vascular bundles lose turgidity and become increasingly vulnerable to infection and colonization by charcoal rot fungus. *M. phaseolina* infects cells which are in conditions of stress (13). This was the reason why rate of lesion development was higher during the mature than the dough stage. Present studies suggest that, in addition to physiological factors, anatomical factors – in particular bundle-number and stalk-thickness – play an important role in charcoal rot development in sorghum. A genotype with a thick stalk would be more prone to rapid rotting than one with a thin stalk. Most of the local land races grown in India are thin-stalked and possess tolerance to charcoal rot, which lends further support to these findings.

Most of the parameters that contributed to an increase in yield (grain or stover) also increased charcoal rot (lesions). A strong association of grain yield and 100-grain weight with charcoal rot lesions might be related to the post-flowering carbohydrate translocation pattern in sorghum. The higher the grain yield, the greater is the carbohydrate requirement of developing grains and translocation of the same from stalk to grain, leading to a condition of physiological stress that results in greater susceptibility of stalk tissue to invasion by *M. phaseolina*. This is also in consonance with the photosynthetic stress translocation balance concept of sorghum stalk rots (7). An increase in stover yield is achieved by increasing plant height and stalk-thickness, both of which facilitate lesion spread and disease development. Combining high yield with charcoal rot resistance requires a highly energy-efficient genotype (7) that can produce both the high yield and maintain turgid cells in the stalk until maturity (physiologically and metabolically active stalk).

It is concluded that the anatomical characters of sorghum stalks, especially the number of vascular bundles and stalk-thickness, play an important role in determining the extent of stalk damage by the charcoal rot fungus. The pace of charcoal rot development is different at different stages of plant growth and lesions spread faster during plant maturity than at the dough stage. Under a given growing environment, a grain sorghum genotype producing less biomass (total biomass of grain and stover) is expected to have less chance of damage from charcoal rot compared with a genotype producing a greater biomass. The findings will be useful for refining the criteria required for efficient screening for charcoal rot resistance in sorghum and developing resistant genotypes.

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