Evaluation of sweet sorghum hybrid parents for resistance to grain mold, anthracnose, leaf blight and downy mildew

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Abstract

Sweet sorghum has emerged as an important feedstock for bioethanol production in recent times. Towards an effort to develop hybrid cultivars with improved biomass and total fermentable sugar at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, we evaluated advanced hybrid parental lines for resistance to some important diseases (grain mold, downy mildew, anthracnose and leaf blight) that would likely affect the biomass and juice content of the stalk. With this objective 29 B-lines and 19 R-lines/varieties were field evaluated for their responses to anthracnose, leaf blight and grain mold in the respective disease nurseries during two rainy seasons and to downy mildew in the greenhouse by artificial inoculation. Of the 29 B-lines evaluated, 21 were resistant to anthracnose, 6 to leaf blight, 2 to grain mold and 4 to downy mildew. Similarly, of the 19 R-lines/varieties evaluated 7 were resistant to anthracnose, 4 to leaf blight, 3 to grain mold and one to downy mildew. Several B-lines and R-lines showed multiple resistance to 2 or 3 diseases. From these, agronomically superior parental lines having high Brix and sugar content will be utilized for developing hybrids for high ethanol yield coupled with multiple disease resistance.

Introduction

Sweet sorghum (*Sorghum bicolor*) is a promising feedstock for bioethanol production worldwide. It is a food-fuel-energy-industrial crop, which requires low water/fertilizer input, has moderate grain yield and high yield of biomass for biorefining and industrial utilization. It is grown in many countries of Asia, Africa and Americas. Sweet sorghum requires one-fourth the amount of water that sugarcane needs and has fermentable sugars in the juice extracted from the stalks (Almodares et al. 1994). Sweet sorghum is similar to grain sorghum but grows rapidly and produces higher biomass and has a wider adaptation (Reddy et al. 2005). Sweet sorghums are distinct due to higher sugar content in the stalks (Brix 10–18%) from flowering to maturity than that of grain sorghum (Brix 9–11%) during the same period (Srinivasa Rao et al. 2009). Thus, sweet sorghum being a multipurpose crop offers a solution to the food-versus-fuel issue particularly with reference to the requirements of developing nations like India.

Most of the constraints for the growth and production of sweet sorghum are similar to that of grain sorghum. Among biotic stresses, grain mold is common to both sweet and grain sorghum, whereas leaf diseases such as anthracnose, leaf blight and downy mildew are especially important for sweet sorghum. A best management strategy for these diseases in sweet sorghum is through host plant resistance. ICRISAT has a major research focus on development of hybrid parental lines, especially diversifying the genetic base of male-sterile lines, which are disseminated to public organizations and private seed companies for use in developing F_1 hybrid cultivars. Our objective in this study was to evaluate selected sweet sorghum hybrid parental lines to identify lines with resistance to single and multiple diseases.

Materials and methods

Seed material. Twenty-nine B-lines and 19 R-lines/varieties were evaluated for anthracnose (*Colletotrichum graminicola*), grain mold (complex of different species of *Fusarium*, *Curvularia*, *Alternaria*, *Phoma* and others) and leaf blight (*Exserohilum turcicum*) in the respective disease nurseries during the rainy season in 2008 and 2009 along with appropriate susceptible and resistant checks. These lines were also evaluated in greenhouse for downy mildew (*Peronosclerospora sorghi*) resistance.

Screening for grain mold resistance. The sweet sorghum hybrid parental lines along with susceptible check H 112 were evaluated in the sorghum grain mold nursery during the rainy season (June–September) in 2008 and 2009 at ICRISAT, Patancheru, India. Each accession was grown in 1 row of 2 m length with three replications in a randomized complete block design (RCBD). Screening was done without artificial inoculation since sufficient natural spore load of mold fungi are present during the rainy season over sorghum fields at ICRISAT for natural field epiphytotic conditions (Bandyopadhyay et al. 1988, Thakur et al. 2007). The lines were sown in the first half of June so that grain maturing stages coincided with periods of frequent rainfall in August–September. To enhance mold development, high humidity (>90% RH) was provided through sprinkler irrigation of test plots twice a day for 30 min each between 10 AM and 12 noon, and between 4 and 6 PM on rain-free days from flowering to physiological maturity (when most grains in the middle of the panicle develop a black layer at the hilum). Ten uniform flowering plants were tagged in each row. The visual panicle grain mold rating (PGMR) was taken on each of the tagged plants at the prescribed physiological maturity using a progressive 1 to 9 scale, where 1 = no mold infection, 2 = 1–5%, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75% and 9 = 76–100% molded grains on a panicle (Thakur et al. 2006).

Screening for downy mildew resistance. The parental lines were evaluated in a greenhouse along with a susceptible check H 112 using a sandwich inoculation technique (Thakur et al. 2007). The experiment was conducted in a complete randomized design (CRD) with two replications and 45–50 seedlings per replication. Seed of the test accessions were incubated in moist Petri dish chambers (Petri dish lined with wet blotting paper) for 24 h at 35°C. Sprouted seeds (with 0.5- to 1.0-mm long plumules and radicles) were placed on the adaxial surface of a piece of systemically infected sorghum leaf, and covered with another piece of infected leaf (adaxial surface touching the seeds), thus 'sandwiching' the sprouted seeds in moist Petri dishes. The Petri dishes were incubated in darkness at 20°C for 24 h for infection of the seedlings. The seedlings were transplanted in 15-cm-diameter pots (45–50 seedlings/pot) filled with sterilized soil-sand-farmyard manure mix (2:1:1 by volume) and placed in a greenhouse maintained at 25±1°C. Downy mildew incidence was recorded 14 days after transplanting as the percentage of plants with downy mildew symptoms.

Screening for anthracnose resistance. The test lines along with susceptible check (H 112) were evaluated in the anthracnose nursery during the rainy season in 2008 and 2009 at ICRISAT, Patancheru. Plants were inoculated by placing 3–4 sorghum grains colonized by *C. graminicola* in the whorl of each plant 30 days after seedling emergence and high humidity was maintained through overhead sprinklers twice a day on rain-free days till physiological maturity of the grain (Thakur et al. 2007). The anthracnose severity was recorded on 10 uniformly flowered plants at the soft dough stage using a progressive 1–9 scale, where 1 = no disease and 9 = 76–100% leaf area covered with lesions (Thakur et al. 2007).

Screening for leaf blight resistance. The test lines along with susceptible check (H 112) were evaluated in leaf blight nursery planted during 3rd week of September in 2008 and 2009 at ICRISAT, Patancheru. Plants were inoculated by placing 3–4 sorghum grains colonized by *E. turcicum* in the whorl of each plant 30 days after seedling emergence, and high humidity was maintained through overhead sprinklers twice a day on rain-free days till physiological maturity of the grain. The leaf blight severity was recorded on 10 uniformly flowered plants at the soft dough stage using a progressive 1–9 scale (Thakur et al. 2007).

Results and discussion

Resistance to anthracnose. Disease pressure was adequate as indicated by disease score of 9.0 in the susceptible check H 112 in both screens of 2008 and 2009 (Table 1). High level of resistance was observed in B-lines to anthracnose and 21 of the 29 lines screened were found resistant (score ≤3.0 on 1–9 scale) in both 2008 and 2009 screens. One of the B-lines, ICSB 474 showed high susceptibility to anthracnose (score >6.0). Anthracnose scores of R-lines ranged from 2.0 (64 DTN) to 8.0 (M 35-1) in 2008 and 2009 (Table 2). Seven of the 19 lines screened showed resistance (score ≤3.0 on 1–9 scale) in both the years. Generally, sweet sorghum R-lines/varieties were found to be more susceptible to anthracnose than B-lines.

Resistance to grain mold. None of the 29 B-lines showed resistance to grain mold in both years. However, ICSB 321 (2.7) and ICSB 401 (2.3) showed resistant reaction in 2008 screen compared to 3.7 on resistant check SSV 84 and 9.0 in the susceptible check H 112 (Table 1). Generally, higher level of resistance to grain mold was observed in R-lines than in B-lines. Of the 19 R-lines, seven were resistant during 2008, whereas three

Table 1. Disease reaction of 29 B-lines for anthracnose, grain mold, downy mildew and leaf blight.

Line	Anthracnose[1] 2008	Anthracnose[1] 2009	Grain mold[2] 2008	Grain mold[2] 2009	Leaf blight[1] 2008	Leaf blight[1] 2009	Downy mildew incidence[3] (%)
ICSB 73	2.0	2.0	6.3	6.7	3.3	2.3	100
ICSB 94	2.0	2.0	6.0	6.7	3.3	3.0	98
ICSB 258	3.0	2.0	8.3	4.3	4.0	2.0	6
ICSB 271	2.3	2.0	7.7	5.7	3.3	2.0	99
ICSB 309	2.3	2.0	7.7	6.3	3.0	2.3	30
ICSB 319	2.0	2.0	7.3	6.0	5.7	3.3	33
ICSB 321	4.0	2.0	2.7	–[4]	5.7	–	100
ICSB 401	2.0	2.0	2.3	4.7	3.0	2.0	39
ICSB 428	2.0	2.0	6.7	4.7	4.3	3.3	59
ICSB 468	2.3	2.0	7.3	6.3	6.0	3.3	97
ICSB 474	6.0	6.7	8.0	–	4.7	–	47
ICSB 479	3.7	2.0	8.0	–	6.0	–	83
ICSB 480	4.0	2.6	6.0	6.3	5.7	4.0	94
ICSB 487	3.0	2.1	7.0	5.3	7.3	6.3	99
ICSB 514	2.0	2.0	8.0	5.7	5.0	2.7	90
ICSB 545	2.3	2.0	5.7	6.3	4.0	3.3	10
ICSB 597	3.0	2.0	8.0	6.7	4.7	3.3	100
ICSB 652	5.3	3.0	8.0	–	5.3	–	88
ICSB 690	3.7	3.1	6.3	–	4.0	–	0
ICSB 722	2.7	2.0	5.0	6.0	4.0	3.7	100
ICSB 729	2.3	2.0	7.3	5.3	5.0	4.0	92
ICSB 766	2.3	2.0	6.7	6.0	5.0	3.7	100
ICSB 279	2.0	2.0	5.7	5.3	4.7	3.3	92
ICSB 311	2.0	2.0	6.7	6.0	5.0	2.7	100
ICSB 453	2.7	2.0	5.0	5.0	2.7	2.7	99
ICSB 565	2.7	2.0	7.7	6.7	4.0	3.3	3
ICSB 584	3.7	3.6	7.0	–	4.3	–	97
ICSB 38	2.3	2.3	5.7	6.0	5.0	3.3	100
SSV 84 (breeding check)	5.0	5.3	3.7	–	4.7	3.7	97
H 112 (susceptible check)	9.0	9.0	9.0	–	9.0	8.0	100
SE(m) ±	0.28	2.2	0.82	0.4	0.5	2.5	3.6

1. Mean of 3 replications on 1–9 scale under artificial inoculation.
2. Mean of 3 replications on 1–9 scale at physiological maturity stage under natural infection.
3 Mean of 2 replications under sandwich inoculation.
4. Data not available.

lines (ICSR 93034, NSS 254 and RSSV 106) were resistant in 2008 and 2009. Sweet sorghum breeding check SSV 84 was moderately resistant (mold score <5.0) to grain mold.

Resistance to leaf blight. Leaf blight score ranged from 2.7 (2008–09) in ICSB 453 to 7.3 (2008) and 6.3 (2009) in ICSB 487 compared to 9.0 (2008) and 8.0 (2009) in the susceptible check H 112. Sweet sorghum breeding check SSV 84 recorded 4.7 score in 2008 and 3.7 in 2009 (Table 1). Of the 29 B-lines, six were resistant both in 2008 and 2009. Among R-lines, 104GRD, ICSV 700, M 35-1 and Moulee were resistant in both the years.

Resistance to downy mildew. Generally high level of susceptibility was observed both in B- and R-lines to downy mildew. Among B-lines, ICSB 690 was found free from downy mildew, while ICSB 258, ICSB 545 and ICSB 565 were resistant with ≤10% disease incidence. Susceptible check H 112 and sweet sorghum check SSV 84 recorded >90% incidence. Only one R-line ICSR 93034 showed resistance (<10% incidence) to downy mildew.

Resistance to multiple diseases. None of the B- or R-lines was resistant to all the four diseases, however, resistance to three diseases (anthracnose, grain mold and

Table 2. Disease reaction of 19 R-lines/varieties for anthracnose, grain mold, downy mildew and leaf blight.

Line/variety	Anthracnose[1]		Grain mold[2]		Leaf blight[1]		Downy mildew incidence[3] (%)
	2008	2009	2008	2009	2008	2009	
104GRD	4.7	6.3	2.3	–[4]	3.0	3.0	–
E 36-1	3.0	4.3	3.7	2	5.0	7.0	93
64 DTN	2.0	2.0	4.0	2	5.7	6.3	100
ICSR 93034	2.7	2.0	3.0	2	5.7	4.7	5
ICSV 700	3.7	3.7	3.0	–	3.3	3.0	99
ICSV 93046	2.3	2.0	2.7	–	4.7	3.3	80
IS 23526	2.7	2.0	3.0	–	5.7	4.0	100
M 35-1	6.7	8.0	4.3	6	3.3	3.0	52
Moulee	6.3	7.3	6.3	7	3.3	3.0	52
NSS 254	2.0	2.0	2.3	2	5.7	5.0	99
RSSV 106	2.3	2.0	3.3	2	5.7	4.7	95
RSSV 9	4.0	4.7	5.7	–	4.3	4.0	–
S 35	2.0	3.0	4.3	6	5.7	4.0	98
SP 4484-2	4.0	5.7	5.0	6.5	5.0	6.0	90
SP 4495	3.7	4.3	7.3	5	5.3	5.0	98
SP 4511-2	4.0	5.0	3.0	6	6.3	6.3	96
SP 4511-3	5.0	5.0	4.7	6.5	6.7	6.0	83
SPV 1411	7.3	7.3	4.7	–	3.7	4.7	54
SSV 84 (breeding check)	5.0	4.3	3.7	2	4.7	7.0	97
H 112 (susceptible check)	9.0	9.0	9.0	9	9.0	8.0	100
SE(m) ±	0.37	3.1	0.4	0.8	0.6	2.9	3.6

1. Mean of 3 replications on 1–9 scale under artificial inoculation.
2. Mean of 3 replications on 1–9 scale at physiological maturity stage under natural infection.
3. Mean of 2 replications under sandwich inoculation.
4. Data not available.

downy mildew) was observed in ICSR 93034. Among B-lines, ICSB 73, -94, -271, -309, -401 and -453 were resistant to leaf blight and anthracnose, and ICSB 258, -545 and -565 to anthracnose and downy mildew. Among R-lines, NSS 254 and RSSV 106 were resistant to anthracnose and grain mold. Resistance to individual diseases is reported in germplasm accessions and grain hybrid parental lines (Thakur et al. 2007), but this is probably the first report of resistance in sweet sorghum hybrid parental lines.

Agronomic performance of promising disease resistant lines. Significant differences existed among the parental lines for all the agronomic traits recorded indicating sufficient variability existed among the parental lines (Table 3). Time to 50% flowering ranged between 60 and 74 days among the promising maintainer lines (B-lines) while it varied between 81 and 83 days among the restorer lines (R-lines). The B-line ICSB 565 flowered significantly earlier than others in 60 days. Plant height varied from 1.7 m to 3.6 m; grain yield from 3.4 t ha^{-1} to 9.4 t ha^{-1}; stalk yield from 15.4 t ha^{-1} to 61.2 t ha^{-1}; and juice weight from 2.8 t ha^{-1} to 25.7 t ha^{-1}. Brix at physiological maturity varied from 11.5 to 16.8% in B-lines and 17.3 to 18% among the three R-lines. However, the sugar yield ranged between 0.6 to 1.5 t ha^{-1} among B-lines while it was 3.3 to 3.6 t ha^{-1} among R-lines. The B-lines, ICSB 565 and ICSB 94 were low in sugar yield while the rest of them had comparatively better sugar yield. Sweet sorghum hybrid CSH 22SS was superior to the breeding check variety SSV 84 for grain yield, stalk yield, juice weight and sugar yield. Large variability for the candidate traits in these advanced B-lines and varieties for ethanol yield would be helpful in identifying

Table 3. Agronomic performance of selected sweet sorghum hybrid parental lines[1].

Line/variety	Time to 50% flowering (days)	Plant height (m)	Grain yield (t ha^{-1})	Stalk yield (t ha^{-1})	Juice weight (t ha^{-1})	Brix (%)	Sugar yield (t ha^{-1})
ICSB 73	66	2.1	6.7	28.4	9.9	14.3	1.4
ICSB 94	65	1.7	6.7	17.8	5.5	11.5	0.6
ICSB 258	74	1.7	7.3	25.1	7.4	12.2	0.9
ICSB 271	68	1.8	8.9	27.2	10.2	13.7	1.4
ICSB 309	71	1.9	8.5	28.5	9.0	13.3	1.2
ICSB 401	67	2.0	8.9	27.3	7.6	14.7	1.1
ICSB 453	74	1.9	6.5	24.0	9.0	16.7	1.5
ICSB 545	73	2.1	9.4	29.7	9.4	14.5	1.4
ICSB 565	60	1.7	6.3	15.4	2.8	16.8	0.5
RSSV 106	81	3.4	4.0	55.5	19.9	17.3	3.4
NSS 254	83	3.3	4.8	56.9	20.4	17.8	3.6
ICSR 93034	82	3.0	3.4	52.5	18.5	18.0	3.3
CSH 22SS	81	3.6	6.4	61.2	25.7	16.0	4.1
SSV 84 (breeding check)	83	3.0	1.2	60.6	17.7	21.0	3.7
Mean	73	2.4	6.4	36.4	12.4	15.6	2.0
CV	1.9	3.5	11.0	18.3	24.2	7.6	22.4
LSD (*P* <0.05)	2.3	0.1	1.2	11.2	5.0	2.0	0.8

1. Data calculated on 10 plants per plot and extrapolated to hectare considering 80000 plants ha^{-1}.

heterotic hybrid combinations for sugar yield and grain yield coupled with multiple disease resistance.

Literature on disease reaction of sweet sorghum lines is very limited and this is probably the first report on identification of promising disease resistant sweet sorghum hybrid parental lines.

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