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Assessing recombinant inbred sorghum lines for resistance to shoot fly (*Atherigona soccata*): Focus on glossiness, vigor, and infestation traits

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Abstract
The sorghum shoot fly (*Atherigona soccata* Rondani) is a devastating pest affecting sorghum production worldwide. This study aimed to identify shoot fly-resistant sorghum lines by evaluating 210 recombinant inbred lines (RILs) for seedling vigor, leaf glossiness, egg percent and oviposition rates. While no significant differences were found in seedling vigor or leaf glossiness among the RILs, substantial variation in oviposition rates was observed. Several RILs demonstrated lower egg-laying preference compared to susceptible checks, highlighting their potential as valuable genetic resources for breeding shoot fly-resistant sorghum varieties.

Keywords: *Atherigona soccata*, resistance, seedling vigour, leaf glossiness, egg percentage, oviposition

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a crucial cereal crop cultivated in diverse climates globally. However, its productivity is severely threatened by the sorghum shoot fly (*Atherigona soccata* Rondani), a pest causing significant yield losses in both grain and fodder sorghum [1-2]. This pest targets young seedlings, leading to "dead heart" formation and reduced yields [3-4]. The economic impact of shoot fly infestation in India alone is substantial, with reported grain losses reaching 80-90% and fodder yield reductions of 68% [5]. The development of shoot fly-resistant sorghum varieties is imperative for sustainable pest management. However, resistance to shoot fly is a complex trait governed by multiple factors, including antixenosis (Non-preference for oviposition), antibiosis, and tolerance [6]. Understanding the contributions of various plant traits to shoot fly resistance is crucial for effective breeding programs. Previous research has suggested seedling vigor and leaf glossiness as potential indicators of resistance, but findings have been inconsistent. This study focused on evaluating a large population of sorghum RILs for their resistance to shoot fly. We investigated the relationship between seedling vigor, leaf glossiness, egg percent and oviposition rates, aiming to identify promising lines with reduced susceptibility to this destructive pest.

Materials and Methods

Plant Material and Experimental Design: The study utilized 210 recombinant inbred lines (RILs) derived from a cross between a shoot fly susceptible parent (27B) and a resistant parent (IS 2122). Six standard check varieties (SPV1616, SSG-59-3, Pant Chari-5, Pant Chari-6, IS18851 (resistant), and Swarna (susceptible)) were included for comparison. The field experiment was conducted at the Instructional Dairy Farm of Govind Ballabh Pant University of Agriculture and Technology in Pantnagar, Uttarakhand, India. The experimental material was planted during the 2018 monsoon season in the Tarai region of the Indian Himalayan foothills, an area with a semi-humid subtropical climate. This region's soil, categorized as medium to heavy-textured Tarai soil, is rich in organic matter, has high cation exchange capacity and nutrient retention, but suffers from poor drainage and a high-water table. The planting utilized an augmented block design [7], with each RIL planted in two rows and six check varieties replicated in each block.

Screening Procedure and Agronomic Practices
Shoot fly infestation was induced using the "Interland fishmeal technique," known to attract shoot flies [8]. Four "infector rows" of sorghum were sown 15 days before the main RIL rows to enhance pest pressure. All standard agronomic practices except for pesticide application were followed to ensure optimal crop growth and accurate assessment of natural resistance levels.

Data Collection: Five competitive plants were randomly selected from each recombinant inbred line. All genotypic lines were tagged, and observations were recorded on these plants. The average of these five plants with respect to four traits was used for statistical analysis.
Data collection focused on four traits:

1. **Seedling vigor:** Scored on a 1-5 scale at 7 days after emergence (DAE), with 1 being the most vigorous.
2. **Leaf glossiness:** Scored on a 1-5 scale at 14 DAE, with 1 being completely glossy.
3. **Egg percent:** A number of plants with shoot fly eggs were calculated at 14 DAE and number of total plants per line were calculated.

Plants with eggs (percent) = (Number of plants with eggs)/(Total number of plants in a line) ×100

Oviposition (Eggs per plant): Numbers of shoot fly eggs on all plants in a line were recorded at 21 DAE and number of eggs per plant was calculated. Number of eggs were counted randomly on five plants per genotype and taken as average. It was discontinued on the plants showing deadhearts.

Oviposition (Eggs per plant) = (Total no. of eggs on all plants in a line)/(Plant stand per line)

Statistical Analysis
Due to the ordinal nature of the seedling vigor and leaf glossiness data, a Kruskal-Wallis test [9] was employed to assess potential differences among the recombinant inbred lines (RILs) and check varieties. Descriptive statistics, including means and standard deviations, were computed for each parameter: seedling vigor, leaf glossiness, percentage of plants with eggs, and oviposition rate. To explore potential relationships among these traits, a Spearman rank correlation analysis was performed on all four traits.

Results
The Kruskal-Wallis test revealed no significant differences in seedling vigor (H = 209.00, p = 0.487) or leaf glossiness (H = 209.00, p = 0.487) among the 210 recombinant inbred lines (RILs). However, there was variation within each trait. Seedling vigor scores (Table 1) ranged from 1.0 (most vigorous) to 5.0 (Least vigorous), with a mean of 3.34 and a median of 3.40. Approximately 23.8% of RILs exhibited greater vigor than the susceptible check 'Swarna' (score of 2.86). The resistant check 'IS18851' had a score of 1.9. Leaf glossiness scores (Table 1) ranged from 1.2 (most glossy) to 4.2 (Least glossy), with a mean of 2.64 and a median of 2.60. Notably, 44.8% of RILs surpassed the glossiness of the susceptible check 'Swarna' (score of 2.6). The resistant check 'IS18851' had a score of 1.6. Results indicated that seedling vigor and glossiness can to be considered as important traits associated with shoot fly resistance. The descriptive statistics (Table 2) of shoot fly infestation traits revealed significant variability among the 210-sorghum recombinant inbred lines (RILs). There are 210 observations for both 'Plants with eggs (%)' and 'Oviposition (Number of eggs per plant)'. This suggests that the data includes measurements from 210 different recombinant inbred lines. On average, approximately 15.49% of plants had eggs, and the average number of eggs per plant was 34.45. The standard deviation for 'Plants with eggs (%)' is 7.442, indicating moderate variability in the percentage of plants infested with eggs across the different lines. Similarly, the standard deviation for 'Oviposition (Number of eggs per plant)' is 11.186, suggesting moderate variability in the number of eggs laid per plant. The minimum and maximum values provide the range of observed values for each parameter. The percentage of plants with eggs ranged from 1.71% to 40.54%, while the number of eggs per plant ranged from 11.11 to 65.52. The percentage of plants with eggs ranged from 1.71% to 40.54%, with a mean of 15.49%, indicating a wide range of susceptibility to oviposition. Similarly, the number of eggs per plant varied from 11.11 to 65.52, with an average of 34.45, highlighting the diverse oviposition preferences of shoot flies across the RILs. The heatmap (Figure 1) titled 'Spearman Rank Correlation Matrix' provides a visual representation of these correlations. Spearman Rank Correlation revealed moderate positive correlation (0.577) between leaf glossiness and oviposition (%).

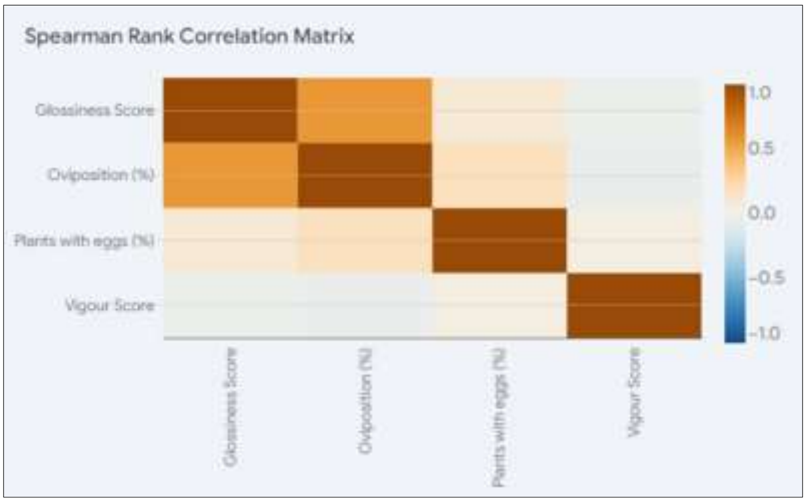

Fig 1: Spearman Rank Correlation Matrix

Discussion

The non-significant differences among RILs for seedling vigor and leaf glossiness in this sorghum population suggest that these traits alone may not be the significant determinants of shoot fly resistance, this finding is in accordance with one of the earlier findings [10] indicating that shoot fly resistance in sorghum is highly complex, with low heritability and high environmental influence, emphasizing the need for marker assisted selection. Although some of the earlier works also revealed that, leaf glossiness and plant vigor are significant morphological traits associated with shoot fly resistance in sorghum [11-13]. The wide variability of observed values on both traits implies that the variability is due to genetic components and, hence can be exploited for breeding purposes. There was considerable variation in the percent of plants with eggs and the number of eggs per plant among the RILs, which was also evident by the descriptive statistics of shoot fly infestation traits. This kind of variation is an indication of the complexity of shoot fly resistance. Earlier researches revealed that resistant sorghum genotypes recorded lowest shoot fly oviposition and incidence compared to susceptible genotypes [14]. Variation in egg number per plant among RILs indicates the complexity of shoot fly resistance in sorghum [15] More than one factor, such as genetic, environmental, and plant-insect interaction, may influence this variation [16-17].

There was a moderate positive correlation between leaf glossiness and oviposition. Some of the earlier studies suggest leaf glossiness in sorghum has a high positive correlation with shoot fly oviposition [18-20], while other studies indicate a negative association [21-22]. The reason for these discrepancies might lie within the differences in sorghum genotypes, environmental conditions, or shoot fly populations. From the low degree of association of most trait pairs with other correlated traits, it does not always hold that seedling vigor and percentage of plants with eggs indicate leaf glossiness or susceptibility to oviposition in this particular RIL population. This variability in the shoot fly infestation traits, as well as the complexity of relationships existing within these traits, definitely pinpoints the need for further investigations leading to a better understanding of mechanisms of shoot fly resistance in sorghum. Comprehensive knowledge about these mechanisms is crucial to developing effective strategies for the management of this devastating pest toward better sorghum productivity.

Table 1: Seedling vigour and glossiness score for 210 RILs and checks.

S. No.	Genotype	Vigour Score (Scale 1-5)	Glossiness Score (Scale 1-5)
1.	SFRIL1	3	1.8
2.	SFRIL22	2.8	3.8
3.	SFRIL4	3	4
4.	SFRIL5	5	2.4
5.	SFRIL9	3	3.2
6.	SFRIL10	2.8	2.6
7.	SFRIL11	3	1.8
8.	SFRIL12	2.2	3.6
9.	SFRIL13	1.8	1.8
10.	SFRIL14	1	3
11.	SFRIL15	3.4	3.8
12.	SFRIL16	3.8	2.6
13.	SFRIL18	3	3.4
14.	SFRIL19	3.2	3.6
15.	SFRIL20	4	1.6
16.	SFRIL21	5	2.2
17.	SFRIL23	3	3.4
18.	SFRIL24	2.6	2.4
19.	SFRIL27	3	3.6
20.	SFRIL28	3	2.2
21.	SFRIL33	3.2	2
22.	SFRIL35	3.2	3.2
23.	SFRIL36	3	1.8
24.	SFRIL37	3.6	3
25.	SFRIL38	3	4
26.	SFRIL40	3.2	3.4
27.	SFRIL43	3.8	2.4
28.	SFRIL44	3.4	3.6
29.	SFRIL45	3.8	2.8
30.	SFRIL49	3.2	2.2
31.	SFRIL56	3	3.4
32.	SFRIL57	4.8	3
33.	SFRIL63	3.4	3.6
34.	SFRIL64	4.2	2.6
35.	SFRIL68	4.6	1.8
36.	SFRIL70	3.8	3
37.	SFRIL71	5	3.4
38.	SFRIL73	5	1.4
39.	SFRIL77	2.8	3.2

40.	SFRIL78	3.2	2.2
41.	SFRIL79	1.8	2.6
42.	SFRIL80	4.6	3.2
43.	SFRIL82	3.2	4
44.	SFRIL83	3.8	4
45.	SFRIL90	1.8	2.2
46.	SFRIL91	2.6	1.8
47.	SFRIL97	4.8	2.2
48.	SFRIL100	3.6	3.4
49.	SFRIL105	5	4
50.	SFRIL106	3	3.2
51.	SFRIL107	3.6	2
52.	SFRIL112	3.2	2
53.	SFRIL120	5	3.4
54.	SFRIL121	2.8	1.4
55.	SFRIL122	1.4	1.6
56.	SFRIL123	3.8	1.2
57.	SFRIL125	2.8	2.4
58.	SFRIL126	2.8	2.4
59.	SFRIL127	3.2	2.6
60.	SFRIL128	3	2.8
61.	SFRIL129	2.2	3
62.	SFRIL130	2.6	3.6
63.	SFRIL131	3	1.6
64.	SFRIL132	2.8	1.2
65.	SFRIL133	4.4	2.2
66.	SFRIL134	3.8	2
67.	SFRIL135	2.4	3
68.	SFRIL136	3.4	3.8
69.	SFRIL117	4	2
70.	SFRIL137	1.6	1.6
71.	SFRIL140	2.2	2
72.	SFRIL141	4.8	2.6
73.	SFRIL142	1.6	2.4
74.	SFRIL143	4	2.6
75.	SFRIL144	3.6	3.8
76.	SFRIL146	5	2.2
77.	SFRIL147	4.4	3.6
78.	SFRIL149	3.4	1.6
79.	SFRIL154	2.4	3.6
80.	SFRIL155	4	3.2
81.	SFRIL159	3	1.2
82.	SFRIL160	3.2	3.6
83.	SFRIL161	3	3.2
84.	SFRIL163	3.6	3.4
85.	SFRIL164	2.6	2.6
86.	SFRIL166	2.6	3.6
87.	SFRIL168	3.6	4
88.	SFRIL170	1	3.2
89.	SFRIL173	4.8	1.8
90.	SFRIL176	2	2.2
91.	SFRIL177	3	3.2
92.	SFRIL179	3.6	3
93.	SFRIL180	3.8	2.4
94.	SFRIL182	4.6	2.4
95.	SFRIL184	5	1.8
96.	SFRIL185	4.6	1.6
97.	SFRIL188	4.6	1.8
98.	SFRIL189	3	2.4
99.	SFRIL193	5	2.4
100.	SFRIL194	3.2	2.8
101.	SFRIL196	4.6	1.8
102.	SFRIL198	3.6	1.2
103.	SFRIL200	3	2.2
104.	SFRIL202	3.4	2
105.	SFRIL204	3.4	3.4
106.	SFRIL205	3.8	3
107.	SFRIL206	3.4	1.2

108.	SFRIL207	3	3
109.	SFRIL208	3.8	3.6
110.	SFRIL210	4.8	1.8
111.	SFRIL211	4.4	2.8
112.	SFRIL214	3	3
113.	SFRIL217	1	1.8
114.	SFRIL218	2.6	1.4
115.	SFRIL219	2.4	1.6
116.	SFRIL220	3.4	3.4
117.	SFRIL221	3.8	3.6
118.	SFRIL223	3.8	3.6
119.	SFRIL224	3.8	3
120.	SFRIL227	5	3.4
121.	SFRIL228	1.4	2.8
122.	SFRIL231	3.4	2
123.	SFRIL232	3.4	3.2
124.	SFRIL234	3	3
125.	SFRIL237	3	2.6
126.	SFRIL240	2.8	3
127.	SFRIL241	1	2.4
128.	SFRIL242	2.2	3
129.	SFRIL243	3	3.4
130.	SFRIL244	2	3
131.	SFRIL245	3	2.6
132.	SFRIL248	3.2	3
133.	SFRIL249	4	3.2
134.	SFRIL250	2	3
135.	SFRIL253	3.6	2.8
136.	SFRIL254	3.8	2.4
137.	SFRIL256	4.2	2
138.	SFRIL257	3.4	2.4
139.	SFRIL258	3.4	2.6
140.	SFRIL261	2.6	4.2
141.	SFRIL262	3.2	3
142.	SFRIL263	2.6	2.4
143.	SFRIL264	3.4	2.6
144.	SFRIL265	3.4	2.4
145.	SFRIL267	3.6	1.4
146.	SFRIL268	3.6	1.6
147.	SFRIL272	3.4	3
148.	SFRIL273	3.8	3.4
149.	SFRIL274	3	3.2
150.	SFRIL280	3.4	3.6
151.	SFRIL281	3.2	2
152.	SFRIL286	3.6	2
153.	SFRIL288	3.4	2.6
154.	SFRIL289	2.8	2.4
155.	SFRIL290	2.8	4
156.	SFRIL292	3	2.8
157.	SFRIL293	2	3.6
158.	SFRIL302	2	4
159.	SFRIL303	4.6	3
160.	SFRIL307	4	3.2
161.	SFRIL308	3.2	2.4
162.	SFRIL313	5	3.6
163.	SFRIL314	2	2.6
164.	SFRIL315	3.6	2
165.	SFRIL316	3.2	2.4
166.	SFRIL317	3.2	3.4
167.	SFRIL318	3.8	3.2
168.	SFRIL319	3.2	1.6
169.	SFRIL322	3	1.6
170.	SFRIL324	2.8	2.4
171.	SFRIL325	5	2.6
172.	SFRIL326	3	1.2
173.	SFRIL328	2	3.8
174.	SFRIL329	3.6	4.2
175.	SFRIL330	4	2.6

176.	SFRIL331	3.6	2
177.	SFRIL332	3.4	2
178.	SFRIL333	3	3.8
179.	SFRIL335	2.2	2.6
180.	SFRIL338	3.4	4.2
181.	SFRIL341	5	3
182.	SFRIL342	4.2	2.6
183.	SFRIL343	3.8	1.8
184.	SFRIL344	2	3.2
185.	SFRIL346	1.4	2.2
186.	SFRIL347	2.2	2.6
187.	SFRIL352	5	3
188.	SFRIL354	3.8	2.8
189.	SFRIL355	3.4	2.2
190.	SFRIL359	3.4	1.4
191.	SFRIL371	3.2	2.6
192.	SFRIL372	3.4	3.6
193.	SFRIL374	3.8	2.4
194.	SFRIL376	3.4	1.4
195.	SFRIL380	4.6	1.8
196.	SFRIL383	3.4	3.4
197.	SFRIL389	3.4	2
198.	SFRIL390	2	1.4
199.	SFRIL391	3.8	1.4
200.	SFRIL392	3.4	2.8
201.	SFRIL393	3	2.2
202.	SFRIL394	3.2	1.6
203.	SFRIL395	3.6	1.6
204.	SFRIL397	5	2.4
205.	SFRIL399	4	1.6
206.	SFRIL408	4.4	2.2
207.	SFRIL412	3	1.4
208.	SFRIL414	4.4	3.8
209.	SFRIL415	2.8	1.4
210.	SFRIL418	4.4	1.6
211.	Pant Chari-5	3.29	2.5
212.	Pant Chari-6	3.00	2.5
213.	SPV1616	2.63	2.8
214.	SSG-59-3	2.49	2.5
215.	IS18551	1.89	1.6
216.	Swarna	2.86	2.6

Table 2: Descriptive Statistics of Shoot Fly Infestation Traits in Sorghum Recombinant Inbred Lines

Statistic	Plants with eggs (%)	Oviposition (Number of eggs per plant)
count	210	210
mean	15.492	34.446
std	7.442	11.186
min	1.71	11.11
25%	10.09	25.822
50%	15.01	33.82
75%	19.947	42.415
max	40.54	65.52

Conclusion

This study, re-emphasizes the complexity of the trait of resistance in sorghum shoot flies and thus warrants attention to more than one trait while framing a breeding program. Seedling vigor and leaf glossiness could not be a differentiator for resistance in this RIL population, but oviposition rates were the key indicator. Notably, the positive correlation of leaf glossiness with egg infestation makes this trait less likely to serve as a reliable marker for resistance, necessitating the need to know the mechanisms involved. Future research should probe the interactions of genetic and environmental factors in the expression of shoot-fly resistance, as shoot fly resistance mechanism is highly complex, with high environmental influence, emphasizing the need for marker assisted selection.

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