

## Article

# A Genome-Wide Association Study of Nigerien and Senegalese Sorghum Germplasm of *Exserohilum turcicum*, the Causal Agent of Leaf Blight

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**Abstract:** In Senegal, sorghum ranks third after millet and maize among dryland cereal production and plays a critical role in the daily lives of millions of inhabitants. Yet, the crop's productivity and profitability are hampered by biotic stresses, including *Exserohilum turcicum*, causing leaf blight. A total of 101 sorghum accessions collected from Niger and Senegal, SC748-5 and BTx623, were evaluated in three different environments (Kaymor, Kolda, and Ndiagianiao) in Senegal for their reactions against the leaf blight pathogen. The results showed that 11 out of the 101 accessions evaluated exhibited 100% incidence, and the overall mean incidence was 88.4%. Accession N15 had the lowest incidence of 50%. The overall mean severity was 31.6%, while accessions N15, N43, N38, N46, N30, N28, and N23 from Niger recorded the lowest severity levels, ranging from 15.5% to 25.5%. Accession N15 exhibited both low leaf blight incidence and severity, indicating that it may possess genes for resistance to *E. turcicum*. Also, the accessions evaluated in this study were sequenced. A GWAS identified six novel single-nucleotide polymorphisms (SNPs) associated with an average leaf blight incidence rate. The candidate genes were found in chromosomes 2, 3, 5, 8, and 9. Except for SNP locus S05\_48064154, all five SNPs associated with the leaf blight incidence rate were associated with the plant defense and stress responses. In conclusion, the candidate genes identified could offer additional options for enhancing plant resistance against *E. turcicum* through plant breeding or gene editing.

**Keywords:** sorghum; *Exserohilum turcicum*; leaf blight; incidence; severity; GWAS



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## 1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most indispensable and versatile crops in terms of its uses and adaptability, especially in the arid and semi-arid regions of the world, where it plays a critical role in subsistence farming and the daily calorie needs of hundreds of millions of people [1–4]. In Senegal, sorghum is a vital commodity, food, and feed source for millions of inhabitants, and ranks third behind millet and maize in dryland cereal production [4,5]. In the last 5 years (2017/2018 to 2021/2022), a mean area of planted sorghum of 250,000 ha, as well as 302,000 tons of production, was recorded in Senegal [6]. During the same period, the mean yield in the country was 1.2 tons/ha [6], despite the fact that most of the sorghum crops planted were landraces with low yields; however, some of the advanced/improved varieties developed and released by Institut Sénégalais de Recherches Agricoles/Centre National de Recherches Agronomiques can

produce yields of up to 5 tons/ha in experimental stations with high farm inputs [4,7]. Nevertheless, sorghum production and profitability are constrained by biotic stresses in Senegal, including leaf blight, incited by *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs [syn. *Helminthosporium turcicum* (Pass.)] [8]. The pathogen also infects teosinte, Sudan grass, gamagrass, and johnsongrass, and the infection is most severe under heavy dew and temperatures ranging from 18 to 27 °C [8]. Studies have shown that *E. turcicum* is host-specific, meaning that the maize isolates of the pathogen are non-pathogenic to sorghum [8–10]. The reverse is true, resulting in two host-specific strains: *E. turcicum* f. sp. *zeae* attacks maize and *E. turcicum* f. sp. *sorghii* incites leaf blight on sorghum [9,10]. The host specificity is reported to be conferred by a single gene in the pathogen, *SorA*<sup>+</sup> in sorghum and *ZeaA*<sup>+</sup> in maize [10]. The leaf blight pathogen can survive on sorghum as mycelia, conidia in plant debris, weed hosts, or chlamydospores without host tissue in the soil [11]. The pathogen can infect both young and old plants, with symptoms characterized by large, elongated, spindle-shaped spots with a straw/grey color, surrounded by pigmented margins, and in older plants, the lesions may have yellowish-to-gray centers with reddish margins [8,11]. Sorghum leaf blight is widely distributed in areas where sorghum is planted, and under severe foliar infection, losses of up to 70% can occur [8,12–14]. Prom et al. [15] surveyed 206 sorghum farmers' production fields in Senegal during the 2019 growing season and noted that 198 fields had leaf blight-infected plants. Resistant sources of the sorghum leaf blight pathogen have been reported. Hepperly and Sotomayor-Ríos [16] evaluated sorghum germplasm in Isabel, Puerto Rico, and identified several sorghum lines, including IS12526, IS12576, IS12606, and IS12638, that possess high levels of resistance. Four lines, Gambella-1107, Seredo, 76TI#23, and Meko-1, were found to be resistant to *E. turcicum* f. sp. *sorghii* when evaluated in two locations in Southern Ethiopia [13].

However, the recent advances made using genomic resources (e.g., molecular markers, genomic mapping software) have made it possible to identify single-nucleotide polymorphisms (SNPs) to locate genes associated with economically important traits such as disease resistance [17]. For instance, Tomar et al. [18] identified eight QTLs associated with spot blotch disease resistance in the wheat genome using 14,063 polymorphic genotyping-by-sequencing markers. A total of 17 QTLs were identified at different genomic locations when wheat cultivars were evaluated in a greenhouse against four pathotypes of the stripe rust pathogen [19], while Liu et al. [20] documented 14 loci associated with stripe rust in multiple locations, as well as 37 loci with a significant association with the disease at all plant developmental stages. Further, Kawicha et al. [21] identified six unique significant SNPs for tomato fusarium wilt resistance located on chromosomes 2, 4, and 7 of the tomato genome. We hypothesized that SNPs associated with the leaf blight response could be identified, and that these SNPs might have a role in host defense. Thus, genome-wide association studies (GWASs) of the incidence and severity of the leaf blight pathogen against Nigerien and Senegalese sorghum germplasm, planted in three environments in Senegal, were conducted.

## 2. Results

The leaf blight incidence rate across the three locations in the Nigerien and Senegalese accessions was high, with the average rate =  $88.4 \pm 0.76$  (Table 1). The lowest incidence rates were observed in N15, N48, and S17. Likewise, Table 2 depicts the leaf blight severity levels from the highest to the lowest accessions. The accessions with the lowest severity levels are from Niger, including N15, N43, N38, N46, N30, N28, and N23. The highest leaf blight severity (75.5%) was recorded in Kaymor. In contrast, many susceptible accessions were from Senegal.

**Table 1.** The average leaf blight incidence rate across the three locations ordered from high to low. The standard error of the mean is listed next to the average value. Single dots indicate cultivars tested only once.

| Accessions | Incidence | SEM  | Accessions | Incidence | SEM   |
|------------|-----------|------|------------|-----------|-------|
| S47        | 100       | 0    | S31        | 88.14     | 7.88  |
| S42        | 100       | 0    | N49        | 88.14     | 7.88  |
| N5         | 100       | 0    | N4         | 88.13     | 6.31  |
| N7         | 100       | .    | N19        | 88.13     | 10.65 |
| N9         | 100       | 0    | S8         | 88        | 6.77  |
| S36        | 100       | 0    | S3         | 88        | 9.07  |
| N27        | 100       | 0    | S1         | 88        | 4.68  |
| N36        | 100       | 0    | N46        | 87.89     | 5.42  |
| S19        | 100       | 0    | N57        | 87.14     | 8.48  |
| N43        | 100       | 0    | N26        | 87.13     | 8.58  |
| S52        | 100       | 0    | S12        | 86.86     | 8.53  |
| N30        | 99.13     | 0.88 | N50        | 86.78     | 7.70  |
| S46        | 98.33     | 1.67 | S55        | 86.5      | 7.53  |
| S2         | 98.29     | 1.71 | S29        | 86.44     | 6.19  |
| S27        | 98        | 1.36 | S14        | 86.11     | 8.59  |
| N53        | 97.89     | 2.11 | S38        | 85.75     | 7.84  |
| S4         | 96.75     | 2.33 | S51        | 85.43     | 7.18  |
| S21        | 96.67     | 3.33 | S37        | 85.25     | 8.51  |
| S32        | 96.63     | 2.31 | N18        | 85.13     | 7.67  |
| S40        | 96        | 2.77 | S23        | 85.13     | 7.52  |
| N52        | 95.86     | 4.14 | N28        | 84.29     | 10.48 |
| SC748-5    | 95.86     | 4.14 | N55        | 84.29     | 10.33 |
| S11        | 94.71     | 3.43 | S39        | 83.67     | 8.92  |
| S30        | 94.5      | 5.5  | N8         | 83.63     | 10.85 |
| S58        | 94.5      | 5.5  | N29        | 83.57     | 8.55  |
| N25        | 94.44     | 3.91 | S20        | 82.75     | 9.61  |
| S59        | 94.13     | 4.24 | S50        | 82        | 10.21 |
| S49        | 94        | 3    | N44        | 81.88     | 8.68  |
| S60        | 94        | 3.98 | S33        | 81.29     | 12.13 |
| S57        | 93.89     | 4.55 | S5         | 80.86     | 11.69 |
| N23        | 93.44     | 5.52 | N39        | 80.71     | 12.59 |
| S34        | 93.25     | 4.44 | N58        | 80.13     | 9.66  |
| N54        | 93.22     | 5.02 | N42        | 80        | 10.38 |
| N20        | 92.22     | 5.21 | N41        | 80        | 11.55 |
| S44        | 92        | 3.98 | S43        | 79.86     | 11.2  |
| S18        | 92        | 5.89 | S6         | 79.38     | 9.55  |
| N34        | 91.44     | 4.76 | N51        | 79.22     | 9.88  |
| BTx623     | 91.14     | 7.06 | N40        | 79        | 10.83 |
| S22        | 91.11     | 8.89 | N6         | 78.75     | 9.42  |

**Table 1.** *Cont.*

| Accessions | Incidence | SEM   | Accessions | Incidence | SEM   |
|------------|-----------|-------|------------|-----------|-------|
| S56        | 90.89     | 5.33  | S41        | 78.5      | 16.4  |
| S48        | 90.86     | 6.22  | N2         | 78.5      | 12.84 |
| S10        | 90.38     | 5     | N45        | 78.13     | 11.83 |
| N24        | 90.33     | 7.1   | S15        | 77.86     | 7.95  |
| S35        | 90.25     | 5.6   | S45        | 77.78     | 12.38 |
| S28        | 89.38     | 4.2   | N59        | 76.5      | 9.72  |
| S16        | 89.13     | 10.88 | N56        | 76.13     | 10.89 |
| S7         | 89.13     | 6.63  | S13        | 75.88     | 12.41 |
| N60        | 89        | 7.2   | N38        | 72.86     | 12.58 |
| S9         | 88.89     | 7.35  | S17        | 70.83     | 11.36 |
| N22        | 88.71     | 9.44  | N48        | 67.5      | 12.62 |
| N3         | 88.67     | 5.8   | N15        | 50        | .     |
| S54        | 88.66     | 7.09  | Average    | 88.4      | 0.76  |

**Table 2.** The average leaf blight severity level across the three locations ordered from high to low. The standard error of the mean is listed next to the average value. Single dots indicate cultivars tested only once.

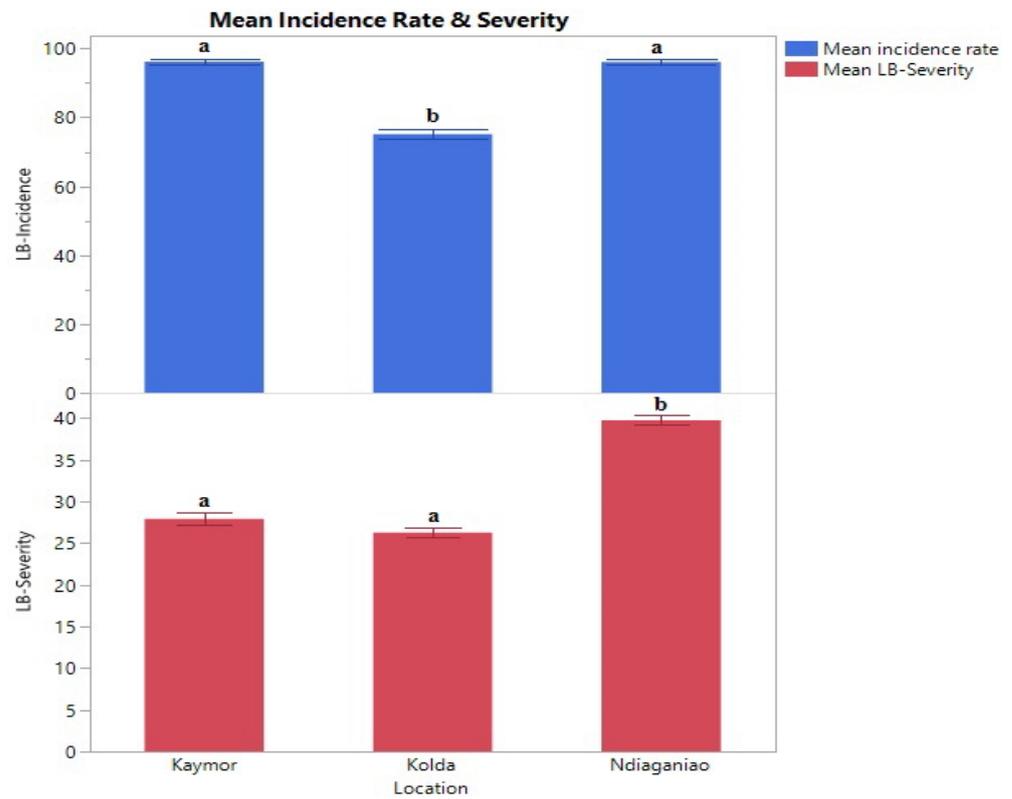
| Accessions | Severity | SEM  | Accessions | Severity | SEM  |
|------------|----------|------|------------|----------|------|
| S32        | 41.75    | 7.54 | S38        | 31.75    | 4.98 |
| S11        | 41.21    | 2.02 | S47        | 31.75    | 3.24 |
| S1         | 39.25    | 3.24 | S59        | 31.75    | 3.24 |
| S31        | 38.36    | 4.21 | N2         | 31.25    | 6.96 |
| S17        | 37.17    | 1.67 | S2         | 31.21    | 2.97 |
| S21        | 37.17    | 5.43 | S33        | 31.21    | 6.12 |
| N58        | 36.75    | 5.15 | SC748-5    | 31.21    | 4.81 |
| S18        | 36.75    | 3.98 | N3         | 31.06    | 4.12 |
| S34        | 36.75    | 3.98 | N20        | 31.06    | 4.12 |
| N40        | 35.51    | 3.33 | N25        | 31.06    | 4.44 |
| N7         | 35.5     | .    | N27        | 31.06    | 5.56 |
| N42        | 35.5     | 4.36 | N34        | 31.06    | 4.12 |
| S4         | 35.5     | 3.78 | N51        | 31.06    | 4.12 |
| S15        | 35.5     | 3.78 | S39        | 31.06    | 4.44 |
| S35        | 35.5     | 2.67 | N8         | 30.5     | 5.35 |
| S46        | 35.5     | 6.32 | N36        | 30.5     | 5.63 |
| S58        | 35.5     | 2.58 | N56        | 30.5     | 3.78 |
| S56        | 34.39    | 2.61 | S6         | 30.5     | 3.78 |
| N19        | 34.26    | 5.15 | S8         | 30.5     | 4.63 |
| N18        | 34.25    | 2.95 | S30        | 30.5     | 5.63 |
| S23        | 34.25    | 2.95 | S37        | 30.5     | 5    |

Table 2. Cont.

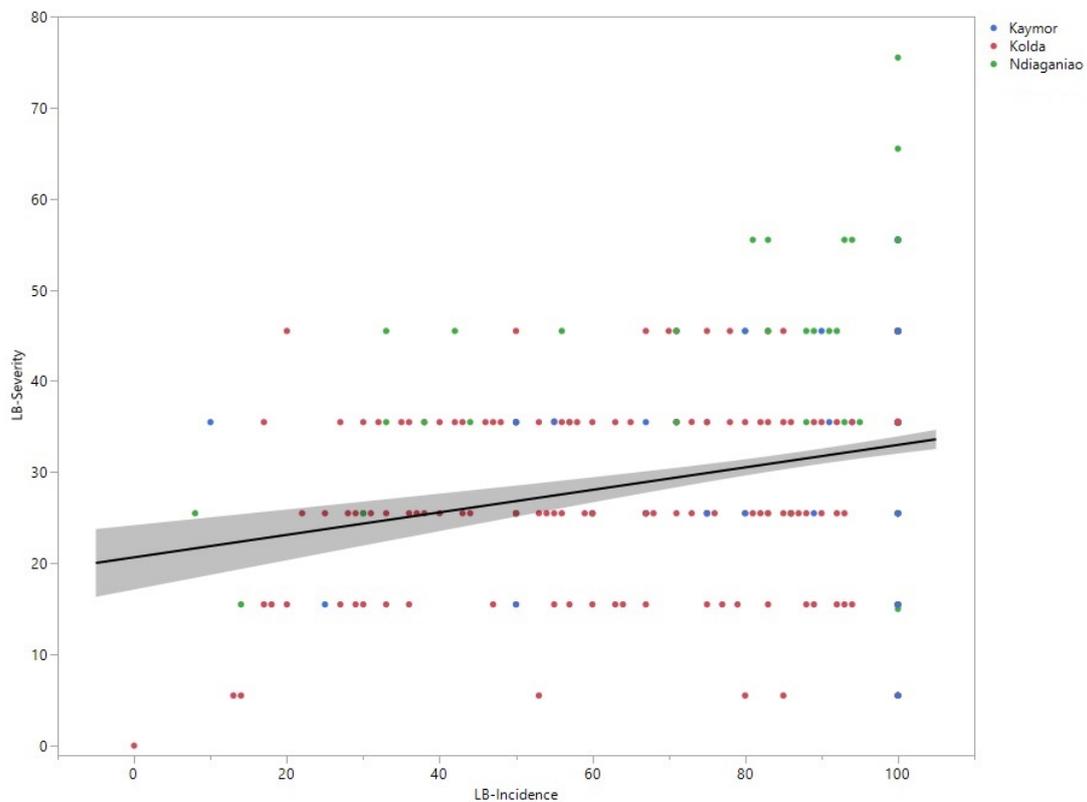
| Accessions | Severity | SEM  | Accessions | Severity | SEM  |
|------------|----------|------|------------|----------|------|
| S28        | 34.25    | 2.95 | S22        | 29.94    | 4.44 |
| S5         | 34.07    | 4.59 | S60        | 29.94    | 3.77 |
| S48        | 34.07    | 5.95 | N29        | 29.79    | 2.02 |
| S51        | 34.07    | 4.59 | N55        | 29.79    | 2.97 |
| S41        | 33.83    | 4.77 | S44        | 29.79    | 3.69 |
| S42        | 33.83    | 6.54 | S52        | 29.79    | 3.69 |
| S9         | 33.28    | 3.64 | N5         | 29.25    | 5.96 |
| S40        | 33.28    | 3.24 | N41        | 29.25    | 4.6  |
| S57        | 33.28    | 3.64 | N9         | 28.83    | 8.03 |
| N59        | 33       | 3.66 | N39        | 28.36    | 5.22 |
| S7         | 33       | 6.75 | N49        | 28.36    | 5.65 |
| S20        | 33       | 3.66 | N52        | 28.36    | 5.22 |
| S55        | 33       | 4.12 | S12        | 28.36    | 5.22 |
| N22        | 32.64    | 3.6  | N6         | 28       | 5.59 |
| N38        | 32.64    | 2.86 | N26        | 28       | 5.59 |
| N57        | 32.64    | 3.6  | N44        | 28       | 3.66 |
| S36        | 32.64    | 5.22 | N24        | 27.72    | 4.65 |
| S43        | 32.64    | 2.86 | S14        | 27.72    | 3.64 |
| N50        | 32.17    | 5.27 | BTx623     | 26.86    | 3.44 |
| N53        | 32.17    | 4.41 | S16        | 26.75    | 2.27 |
| N54        | 32.17    | 3.73 | S49        | 26.75    | 5.15 |
| S19        | 32.17    | 4.08 | S29        | 26.61    | 3.89 |
| S27        | 32.17    | 3.33 | S45        | 26       | 5.46 |
| S50        | 32.17    | 4.08 | N23        | 25.5     | 5.53 |
| S54        | 32.17    | 4.08 | N28        | 25.5     | 4.88 |
| N4         | 31.75    | 4.6  | N30        | 25.5     | 5    |
| N45        | 31.75    | 4.6  | N46        | 25.5     | 4.41 |
| N60        | 31.75    | 4.2  | N48        | 24.25    | 5.15 |
| S3         | 31.75    | 3.75 | N43        | 21.21    | 3.69 |
| S10        | 31.75    | 4.6  | N15        | 15.5     | .    |
| S13        | 31.75    | 4.2  | Average    | 31.57    | 0.43 |

The mean leaf blight incidence rate and severity were calculated based on three different locations: Kaymor, Kolda, and Ndiagianiao. The mean incidence rate in Kolda (mean  $\pm$  S.E. =  $75 \pm 1.5$ ) was statistically lower than the other two locations (Kaymor =  $96.2 \pm 12.8$  and Ndiagianiao =  $96 \pm 13.6$  for mean  $\pm$  S.E.) (Figure 1). Ndiagianiao showed a significantly high severity level (mean  $\pm$  S.E. =  $39.7 \pm 0.6$ ) compared to Kaymor (Mean  $\pm$  S.E. =  $27.9 \pm 0.8$ ) and Kolda (mean  $\pm$  S.E. =  $26.3 \pm 0.6$ ).

Pearson's correlation indicates a clear positive correlation between the leaf blight incidence rate and severity (Figure 2). As described in Figure 1, many accessions in Ndiagianiao showed the highest incidence rate and severity level.

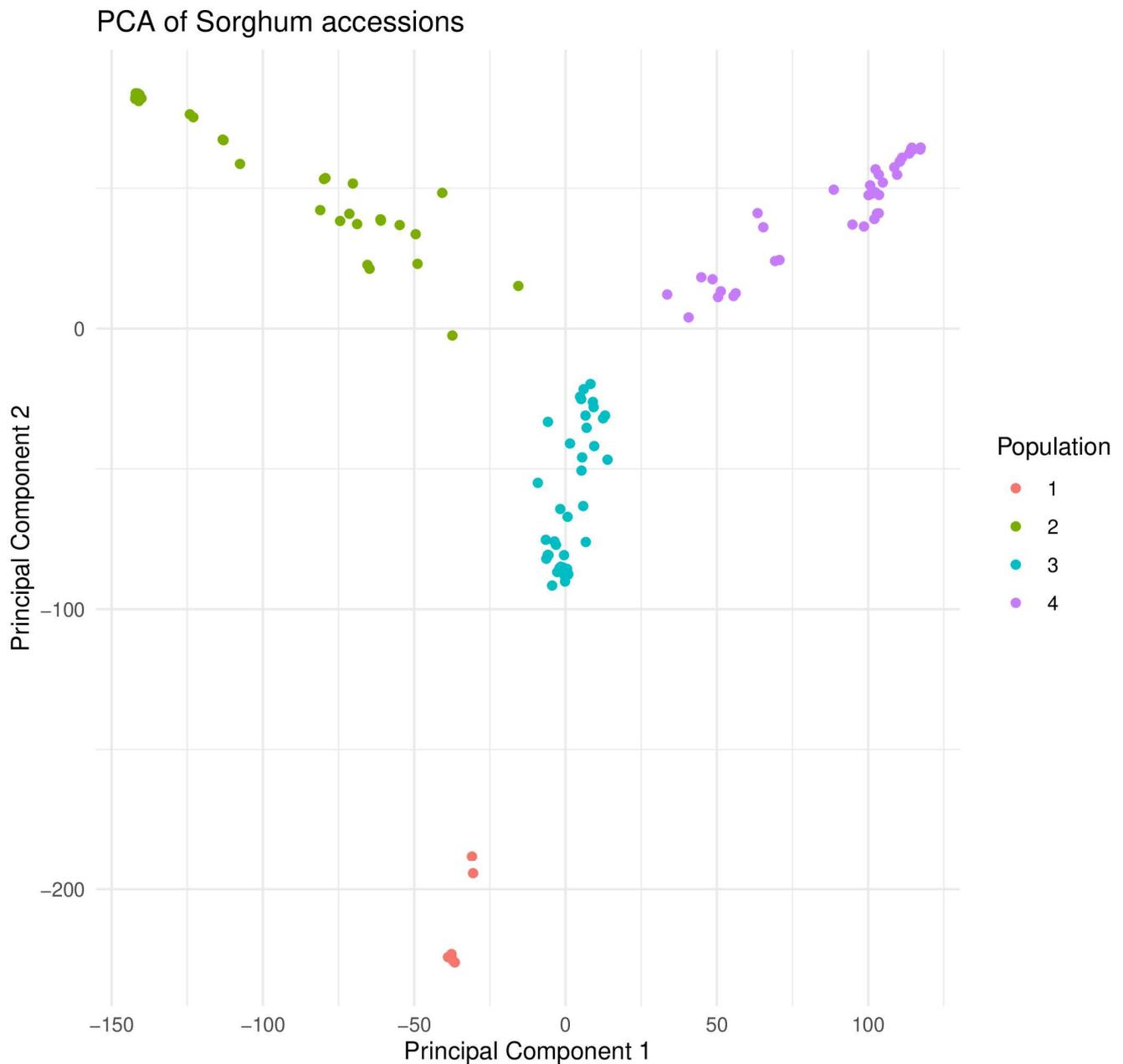


**Figure 1.** Comparisons of leaf blight incidence rate (blue) and severity level (red) based on three locations. Different letters indicate statistical significance.



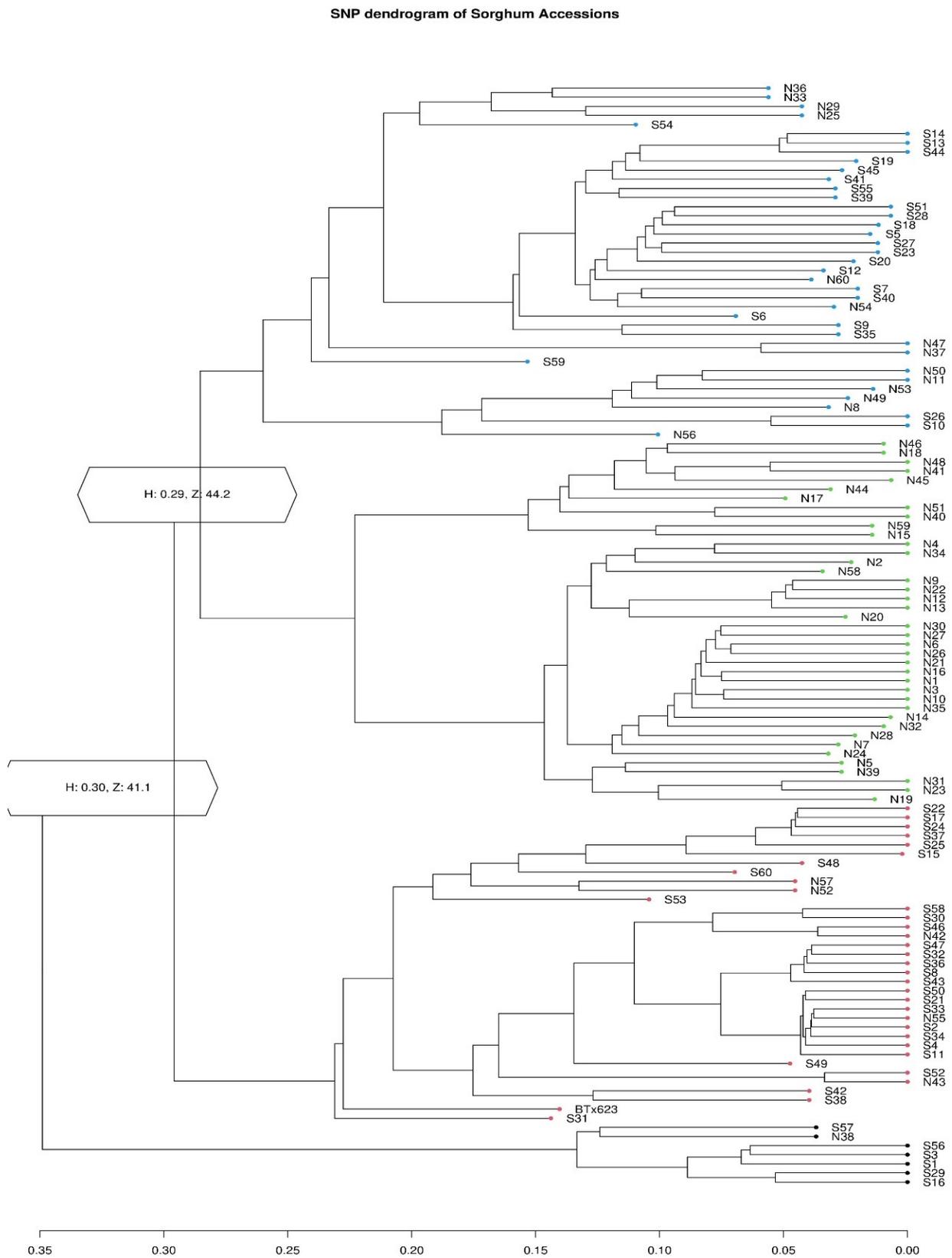
**Figure 2.** The correlation between leaf blight incidence rate and severity level. Pearson’s correlation showed a weak positive correlation of 0.22 with  $p < 0.0001$ . Different colors indicate surveyed locations in Senegal.

A PCA plot (Figure 3) and a dendrogram (Figure 4) generated based on SNP data agreed that four major groups exist among the Nigerien and Senegalese accessions. Figure 4 shows multiple accessions from the two countries that merge and form a group (blue).

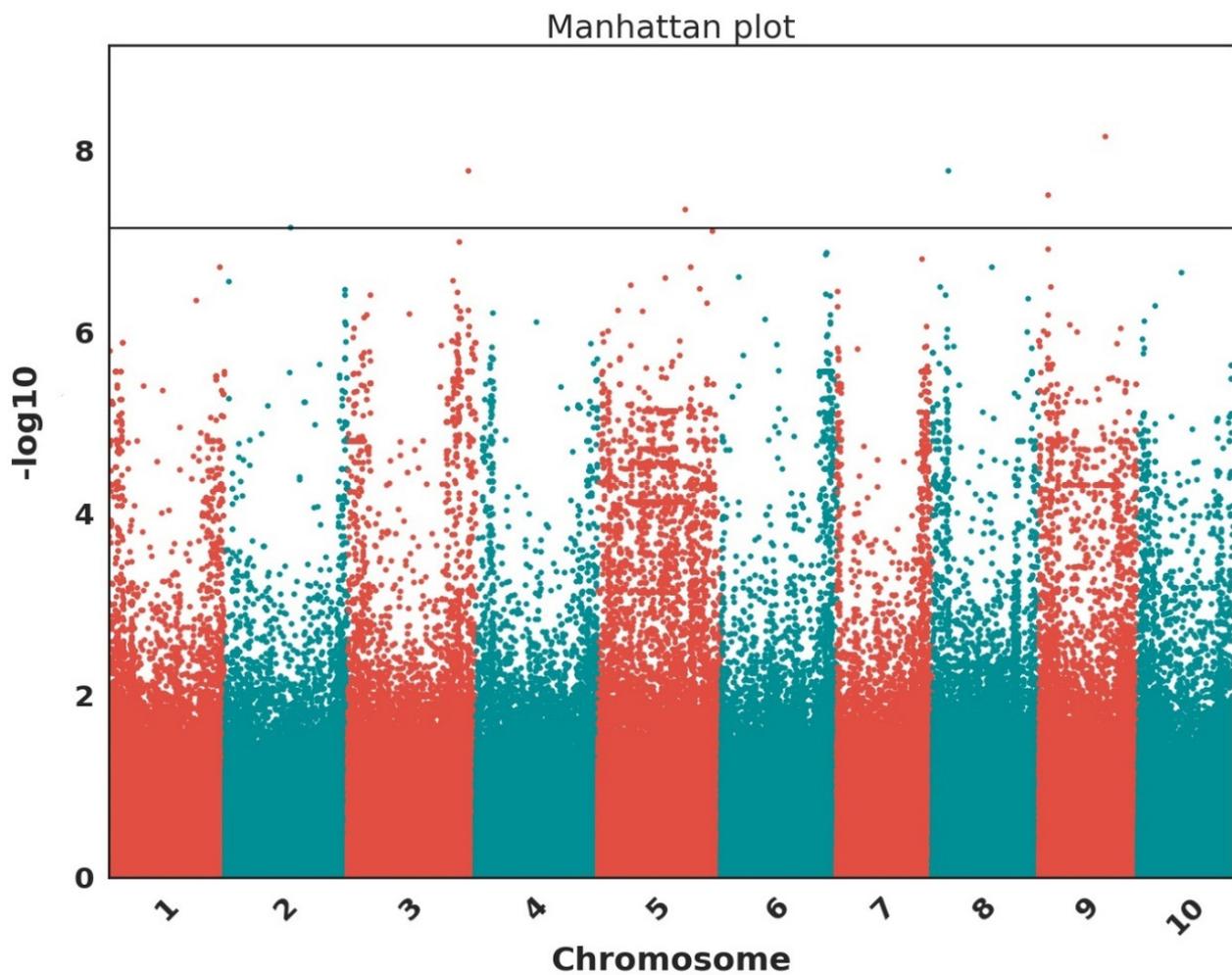


**Figure 3.** The principal component analysis of the Nigerien and Senegalese sorghum accessions. Four colors indicate different groups (red, green, cyan, and purple).

Figure 5 and Table 3 show the top SNPs passing the Bonferroni threshold, identified from the GWAS, and their associated genes for the leaf blight incidence rate. Based on the leaf blight severity level, the GWAS did not generate any SNP locus that passed the Bonferroni threshold. Six SNPs were found to be statistically significant. The LD heatmaps in Figure 6 show SNP locus S09\_38670567 and an LD around the locus, indicating a low LD.



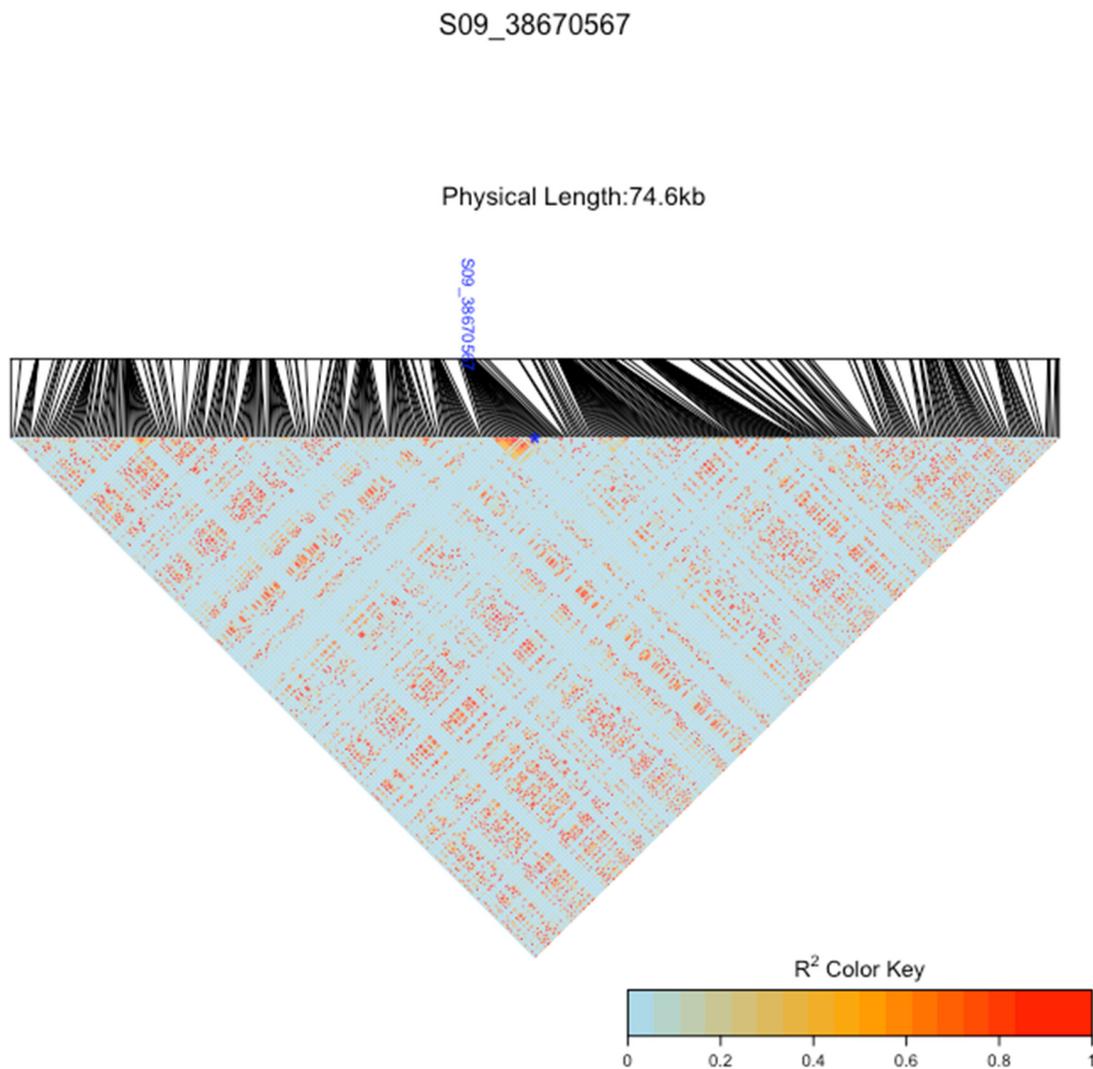
**Figure 4.** A dendrogram was constructed based on the genotype data of the accessions. Four major genetic groups were shown, indicated by the two main branches and different colors.



**Figure 5.** The genome-wide association for leaf blight incidence rate. Manhattan plot across ten chromosomes is shown with the Bonferroni threshold, indicating that six SNPs are statistically significant.

**Table 3.** Annotated genes nearest to the most significant SNPs are associated with leaf blight incidence rate. All six SNPs passed the Bonferroni threshold.

| Chr | Location | Candidate Gene and Function  | Base Pairs | Allele                       | <i>p</i> -Value |
|-----|----------|--|------------|------------------------------|-----------------|
| 9   | 38670567 | Sobic.009G099450<br>Zinc finger, CCHC-type   | 22,641     | Reference: C<br>Alternate: G | 0.000000007     |
| 3   | 67084062 | Sobic.003G351501<br>Homeobox-leucine zipper protein<br>anthocyaninless2-related  | 1083       | Reference: G<br>Alternate: A | 0.000000017     |
| 8   | 9250700  | Sobic.008G070700<br>Protein suppressor of gene silencing 3<br>XS domain-containing protein/XS zinc finger<br>domain-containing protein-related | 15,203     | Reference: A<br>Alternate: T | 0.000000017     |
| 9   | 5849212  | Sobic.009G057200<br>Electron transport oxidoreductase//Acyl-CoA<br>dehydrogenase 4, Peroxisomal  | 4543       | Reference: C<br>Alternate: T | 0.000000031     |
| 5   | 48064154 | No annotated gene nearby   | -          | Reference: T<br>Alternate: A | 0.000000044     |
| 2   | 37426140 | Sobic.002G147900<br>MYB/SANT-like DNA-binding domain   | 52,147     | Reference: G<br>Alternate: A | 0.000000007     |



**Figure 6.** LD heatmaps visualize the LD of SNP locus S09\_38670567. The blue stars indicate the exact location of the SNP locus.

### 3. Discussion

*E. turcicum* is an important pathogen of sorghum, causing sorghum leaf blight [8,17]. Figure 7 shows leaf blight-infected sorghum leaves characterized by long elliptical reddish-purple or yellowish-tan lesions. A survey of 206 sorghum farmers' fields in seven regions across Senegal during the 2019 growing season revealed that leaf blight was the most prevalent disease. Similarly, a total of 122 sorghum farmers' fields surveyed across the same seven regions in Senegal during the 2022 growing season recorded a 100% prevalence of leaf blight [22]. Among the accessions evaluated against the leaf blight pathogen, N15 from Niger exhibited the lowest incidence and severity, indicating that this accession may possess resistance genes to *E. turcicum*. One sorghum line, "Hageen Durra", was found to be resistant to leaf blight when evaluated in Central Sudan [12]. In India, many accessions, such as IS 13870, IS 18758, and IS 19670, exhibited high to moderate levels of resistance to leaf blight when evaluated in the field [23]. In this study, top SNPs passing the Bonferroni threshold, identified from the GWAS and their associated genes for a leaf blight incidence rate, were noted. However, based on the leaf blight severity level, the GWAS did not generate any SNP locus that passed the Bonferroni threshold. In a recent study, a transcriptional analysis revealed that rhamnogalacturonate lyase, plantacyanin, and zinc finger were expressed over a 70-fold change when inoculated with *E. turcicum* in sorghum [10]. In a maize GWAS study, multiple genes associated with SANT, WRKY,

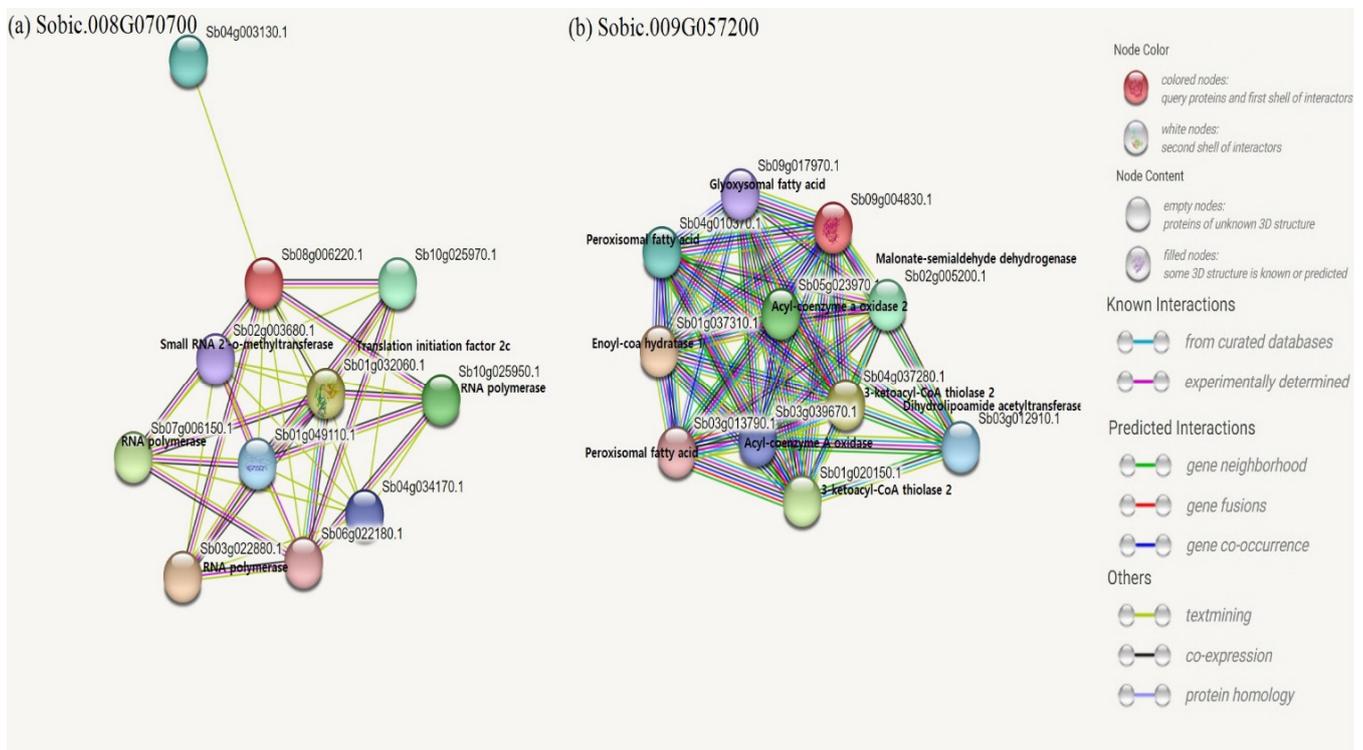
inositol-pentakis-phosphate 2-kinase, potassium uptake protein TrkA, and 4′phosphopante theinyl transferase were listed as top candidates against *E. turcicum* [24]. Lipps et al. [25] used two sorghum recombinant-inbred-line (RIL) populations for resistance to *E. turcicum* and identified a total of six quantitative trait loci (QTLs) across the two populations. In this study, we examined *E. turcicum* resistance to over one hundred accessions originally collected from Niger and Senegal and evaluated in three locations in Senegal. The accessions tested in Ndiaganiao showed high incidence and severity levels, indicating that Ndiaganiao provided a favorable environment for *E. turcicum* infection. Among the accessions, the lowest incidence rates were observed in N15, N48, and S17, while several accessions, including N15, N43, N38, N46, N30, N28, and N23, exhibited the lowest severity levels. As expected, the correlation test proved a clear positive correlation between the two traits.



**Figure 7.** Sorghum leaves infected with leaf blight.

A GWAS identified six novel SNPs associated with an average incidence rate. Sobic.009G099450, a gene annotated as a zinc finger, was 22,641 bp away from the top SNP locus S09\_38670567. As the SNP locus is in the LD with other SNPs in 2.2 Mb (SNPs in pairwise  $r^2 > 0.3$  with the significant SNP marker S09\_38670567 span a 2.21 Mb region, while SNPs in pairwise  $r^2 > 0.5$  span 194 bp), it is unsurprising that the nearest annotated gene is 22,641 bp away from the SNP. Zinc-finger-related SNPs were identified as top candidates in sorghum against fungal pathogens such as the Senegalese and sorghum mini-core collection [26–28]. CCHC-type zinc-finger proteins play key roles in both abiotic and biotic stress responses and plant growth and development [29]. S03\_67084062 was closely located to the homeobox-leucine zipper (HD-ZIP) protein. Homeodomain leucine zipper proteins are plant-specific transcription factors that contain a homeodomain and a leucine zipper domain and are highly associated with abiotic stress [30]. Gene silencing (S08\_9250700) is one of the critical defense mechanisms that protect plants from pathogens, and it controls the sequence-specific regulation of gene expression [31]. The closest annotated gene from S09\_5849212 is associated with Acyl-CoA dehydrogenase 4, Peroxisomal. The biosynthesis of jasmonic acid in plant peroxisomes requires the action of acyl-coenzyme A oxidase [32]. The nearest gene of SNP locus S02\_37426140 is the MYB/SANT-like DNA-binding domain. MYB transcription factor genes are well known to be involved in controlling various processes, like responses to biotic and abiotic stresses, development, differentiation, metabolism, and defense [33]. Except for SNP locus S05\_48064154, all five SNPs associated with a leaf blight incidence rate were associated with plant defense and stress responses.

Among the top candidate genes, Sobic.008G070700 and Sobic.009G057200 were available for the predicted protein network nodes (Figure 8). Sobic.008G070700 was associated with RNA polymerases, translation initiation factor 2c, and small RNA 2'-o-methyltransferase. Sobic.009G057200 was linked to 3-ketoacyl-CoA thiolase 2, enoyl-coa hydratase, acyl-coenzyme an oxidase 2, malonate-semialdehyde dehydrogenase, peroxisomal fatty acid, dihydrolipoamide acetyltransferase, and glyoxysomal fatty acid. Among the genes linked to our candidates, Arabidopsis 3-ketoacyl-CoA thiolase 2 is involved in abscisic acid (ABA) signal transduction, which plays a major role in plant defense [34]. In *Magnaporthe oryzae*, methylmalonate-semialdehyde dehydrogenase regulates conidiation, polarized germination, and pathogenesis [35]. Peroxisomal fatty acid  $\beta$ -oxidation is reported to negatively impact plant survival under salt stress [36]. Another study concluded that peroxisomal-CoA synthetase is involved in fatty acid  $\beta$ -oxidation and pathogenicity in rice blast fungi [37]. Likewise, many genes linked to our top candidate genes through network nodes are also associated with plant stress and defense responses, indicating the importance of the identified genes in this study. Overall, limited GWAS work has been conducted on sorghum leaf blight, compared to Northern corn leaf blight (NCLB) in Maize [24,25,37,38]. A GWAS on NCLB caused by *Setosphaeria turcica* (perfect stage of *E. turcicum*) identified 22 SNPs significantly associated with NCLB in three tropical maize germplasm mapping panels [38]. Zhang et al. [17] detected 113 unique candidate genes in sorghum lines evaluated against *E. turcicum*, and when these genes were compared to those found in NCLB in maize, they concluded that they play a role in resistance to both crops. Using a GWAS on NCLB in maize, 81 genes were revealed, and 12 of these genes were shown to play a role in plant defense [24]. Recently, Lipps et al. [25] detected six QTLs across two sorghum recombinant inbred line populations when evaluated for resistance against the leaf blight pathogen. All these GWAS on sorghum and maize against *E. turcicum* will continue to enhance our knowledge of the resistance mechanisms in these two economically important crops, thereby increasing our food security globally.



**Figure 8.** Available predicted protein network nodes of top candidate genes. (a) Sobic.008G070700. (b) Sobic.009G057200. Red beads indicate the candidate genes. Genes lacking annotated functions were shown, but no function was listed right next to each gene.

#### 4. Materials and Methods

*Study area:* The research fields were established in Senegal, West Africa, during the 2022 growing season. Senegal is located between latitudes 12°30' and 16°30' N and longitudes 11°30' and 17°30' W, with the drier northern part lying in the Sahelian zone and the southern part with more rain in the Sudanian zone [39]. The three fields were in Kaymor (13°58'33.7'' N and 15°05'03.2'' W) in the Kaolack region, Kolda (12°51'14.7'' N and 14°48'15.4'' W) in the Kolda region, and Ndiaganiao (14°35'58.7'' N and 16°44'10.7'' W) in the Thies region. The annual rainfall is 752 mm in Kaolack, 1072 mm in Kolda, and 377 mm in Thies, while the minimum and maximum temperatures range from 25 to 35 °C, 24 to 35 °C, and 23 to 33 °C, respectively [15]. These sites were selected because of their history of a high incidence of leaf blight, ranging from 88% to 100% [15,22]. A total of 120 sorghum accessions with bright non-diseased seeds were randomly collected from farmers' fields in Niger and Senegal, and with SC748-5 and BTx623 were planted in these three different environments. Seeds of each accession were planted in 1.8 m rows with 0.8 m row spacing in each field environment. The accessions were planted in a randomized complete block design, and each accession was replicated thrice. The fields were kept weed-free with occasional hand-hoeing. Plants were evaluated for leaf blight incidence at the soft-to-early-hard-dough stage of development. The leaf blight incidence was based on the formula below [14,15]:

$$\text{Incidence} = \frac{\text{Number of plants with the disease in a row}}{\text{Total number of plants in a row}} \times 100$$

*Disease severity scale:* The severity scale was previously described by Prom et al. [14] and spanned from 0 to 11 with mid-points, where 1 = 5.5, 2 = 15.5, 3 = 25.5, 4 = 35.5, 5 = 45.5, 6 = 55.5, 7 = 65.5, 8 = 75.5, 9 = 85.5, 10 = 95.5, and 11 = 100, used to calculate the mean severity.

*Statistical Analysis:* Student's *t*-test for all possible comparisons was performed across the population with JMP Pro 15 (SAS Institute, Cary, NC, USA). In addition, the incidence rate and severity were compared across the three experimental locations using Student's *t*-test in JMP Pro 15. A Pearson correlation coefficient was computed to test the correlation between the incidence rate and severity of the disease as well.

#### GWAS

DNA extraction of fresh sorghum leaves sampled from 120 genotypes from Niger and Senegal (60 from each country) was conducted using either a NucleoSpin Plant II kit (Macherey-Nagel, ref. 740770) or a modified 2% Cethyl Trimethyl Ammonium Bromide (CTAB) method [40,41]. The DNA was then purified using 7.5 M ammonium acetate and isopropanol. The OD ratios (260/230, 260/280) were checked using a SpectraMax® Quick-Drop™ Micro Volume spectrophotometer (Molecular Devices), and the samples were run on a 1% agarose gel stained with ethidium bromide for quality control. The Genomics and Bioinformatics Service performed the sequencing at Texas A&M (TxGen, 1500 Research Pkwy Suite 250, College Station, TX 77845, USA) using an Illumina NovaSeq 6000 (2.6X average coverage). All the samples were repurified before library preparation. The sequencing raw data were processed using the GATK (Genome Analysis Tool Kit) best practices for variant calling, implemented in the DRAGEN platform (Illumina, San Diego, CA, USA). The sequences were aligned against Sorghum bicolor v3.1.1, available at Phytozome ([https://phytozome-next.jgi.doe.gov/info/Sbicolor\\_v3\\_1\\_1](https://phytozome-next.jgi.doe.gov/info/Sbicolor_v3_1_1), accessed on 17 October 2023), as the reference genome for SNP calling. The calls were filtered using the following parameters: minimum coverage depth = 3 and minimum genotype quality = 9 on a Phred scale. The resulting variants were then filtered to only accept SNPs with a minimum minor allele frequency of 0.05 and a maximum rate of missing data of 0.5. Variant filtration was performed using bcf tools. The SNP data were finally inputted using Beagle (Beagle 8.7e1.jar). The output was split by chromosome in a VCF format and contained over  $5 \times 10^3$  SNPs. PLINK v1.9 [42] was used for the VCF file conversions and a random

selection of 50,000 SNPs from the genotypic data. An analysis of the population structure was performed in R studio v1.4.1717 [43], using the FactoMineR v2.8 [44] and Factoextra v1.0.7 [45] packages for a principal component analysis (PCA) and k-means clustering with the average silhouette method. For the validation and assignment of the accessions to genetic groups, a dendrogram was generated using SNPRelate v1.28.0 and gdsfmt v1.30.0 [46] and visualized with ggtree v3.2.1 [44]. The linkage disequilibrium (LD) of a local variant around the statistically significant SNP was plotted using LDheatmap v1.0-6 [47]. For the GWAS, we utilized GEMMA v0.98.3 [48]. Association tests were performed using a univariate linear mixed model, accounting for the relatedness matrix as a covariate term and employing the Wald test for determining statistical significance. To generate phenotype and genotype distribution, we utilized the software package vcf2gwas v0.8.3 [49]. Top candidate SNPs that surpassed the Bonferroni test were tracked to identify their exact location using the reference sorghum genome sequence, version 3.1.1, accessed through the JGI Phytozome 13 website. All available predicted protein network nodes from the top candidate genes were searched through the STRING database version 11.5 website (<https://string-db.org/>, accessed on 17 October 2023).

## 5. Conclusions

In Senegal, sorghum ranks third after millet and maize among dryland cereal production, while in Niger, sorghum ranks second to pearl millet in cereal production [4,5,50]. In both countries, sorghum plays a critical role in the daily lives of hundreds of millions of inhabitants for human food, drinks, baked food, animal feed, commercial ventures, building materials, and other uses. An increase in sorghum production will be critical in ensuring food security, especially in semi-tropical and tropical countries, including these two countries, due to the expected increase in global population and the impact of climate change. A robust and integrated management of sorghum diseases such as leaf blight and pests are components that must be addressed to realize the goal of increasing production by 2050, and any information that contributes to our understanding of the host–pathogen interaction will be of value. This work is significant because it suggests that the N15, N48, and S17 lines identified may be excellent sources for use in breeding for leaf blight resistance in both countries. In addition, the candidate genes identified in this study are anticipated to offer additional options for enhancing plant resistance against fungal pathogens through plant breeding or gene editing. Finally, the utilization of GWAS on sorghum accessions is one way to identify loci (SNPs) associated with leaf blight resistance, incited by *E. turcicum*, a widespread and devastating disease in West Africa, which is essential for breeding strategies aimed at predicting germplasm with disease resistance and high-yielding germplasm to ensure future food security in the region.

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