

Article

Durum Wheat Mediterranean Landraces: A Valuable Source for Resistance to Tan Spot Disease

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Abstract: Tan spot (TS), caused by *Pyrenophora tritici-repentis* (*Ptr*), has gained significant importance in Tunisia. In this study, a Mediterranean durum wheat collection of 113 accessions were evaluated under field conditions, during the 2018–2019 cropping season, for resistance to *Ptr* at Koudia experimental station in Bou Salem (Tunisia). The disease progress curve (AUDPC) was used to screen this collection, and the effect of days to heading (DH) and plant height (PH) were evaluated in relation to TS resistance. No significant correlation of PH with AUDPC was found, yet a significant correlation ($r = 0.212$, $p \leq 0.05$) was established between DH and AUDPC scores, suggesting that DH may have an effect on TS development. Moreover, correlation between seedling and adult reactions was significant ($r = 0.695$, $p \leq 0.001$). Although susceptible accessions clustered separately from resistant accessions, the clustering was independent of the country of origin and the status of improvement of the wheat accessions. In total, 67% and 80% of resistant and moderately resistant accessions, respectively, were landraces, suggesting therefore the possible presence of novel sources of resistance to *Ptr* in some landraces, which can be used to establish a breeding program for resistance to tan spot disease.

Keywords: tan spot; durum wheat; landrace; diversity; plant height; days to heading; resistance



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1. Introduction:

Durum wheat (*Triticum turgidum* L. subsp. *durum*) has a significant agricultural importance and represents the third most important cereal crop worldwide, accounting for 8% of the wheat planted area and provides 5% of the total wheat production worldwide [1,2]. It originated in the Fertile Crescent and spread west of the Mediterranean basin to North Africa 9000 years ago. It was also introduced to the latter region mainly from Egypt, Greece and Italy, hence, making it one of the secondary centers of diversification of durum wheat [3,4]. Most of the durum wheat is grown in the Mediterranean region (75% of the world's durum wheat production) because of its adaptation to the environment and its end-use products such as semolina, pasta, couscous, and burghul [5,6]. In Tunisia, durum wheat is cultivated in over half a million hectares, representing 87% of the area dedicated to wheat production [7].

Climate change, abiotic and biotic stresses and the decreasing area for suitable farmland constitute considerable threats to wheat production. In addition, wheat production is threatened by the emergence of high-yielding elite cultivars grown in large areas that led to the loss of genetic diversity that are more vulnerable to newly emerging strains of pathogens [8–12]. The adoption and widespread of reduced or no-till practices in the 1970s

led to the increased level of fungal primary inoculum in many wheat production areas, thus the establishment of stubble-borne destructive foliar diseases such as tan spot disease [13–15]. In Tunisia, tan spot has gained importance over the last few years on durum wheat [16]. Increased adoption of minimum, no till agronomic practices, and monoculture of limited durum wheat varieties led to disease outbreaks that often caused important yield losses. Tan spot caused by the necrotrophic fungus *Pyrenophora tritici-repentis* (*Ptr*) (anamorph: *Drechslera tritici-repentis*) infects both durum and bread wheat. The pathogen overwinters on wheat infested stubble as pseudothecia, which releases ascospores the following cropping season that are considered to be the primary source of inoculum infecting wheat seedlings [17,18]. Symptoms appear as tan-brown flecks that can expand into lens-shaped, tan lesions with a yellow halo [19]. Conidia produced in maturing lesions on leaves serve as secondary inoculum, leading therefore to the spread of the disease to the upper leaves and adjacent plants. Several studies discussed and confirmed the possible effect of agronomic traits such as plant height, days to heading and days to maturity on the severity of diseases such as Septoria tritici blotch (STB), spot blotch and tan spot. As a matter of fact, the flag leaves and glumes, important in grain filling, are infected with conidia earlier on short and early heading and early maturing plants than on tall and later heading and maturing plants [20–22]. Nevertheless, other studies reported the opposite conclusion or that these traits do not affect disease severity in any way [23–25].

Under favorable conditions for disease development, tan spot can cause up to 50% yield losses and reduce grain quality by the formation of red or pink smudge, particularly in durum wheat [17,26–28]. Several management strategies such as the use of non-host plants in the crop rotation, chemical control, and wheat variety genetics are available. However, one single strategy is not sufficient to control the disease. In Tunisia, tan spot management has basically relied on chemical control in conjunction with other diseases such as STB and yellow rust. However, the high pathogenic variability and high genome plasticity of *P. tritici-repentis* may limit the effectiveness of resistance due to the rapid and frequent emergence of new virulent strains [29–31]. An integrated control that includes cultivar mixtures, gene pyramiding and crop rotation represents the most effective and environmentally friendly alternatives providing control of tan spot [17,32–34]. Accordingly, the identification of wheat genotypes that have novel resistance genes and that yield well under a wide range of climatic conditions is crucial [35]. Despite that tan spot is a threat to durum wheat production worldwide, limited sources of resistance to *Ptr* have been identified so far, compared to common wheat.

Tunisia, situated on the Mediterranean coast of North Africa, is considered as one of the main secondary centers of diversification of durum wheat [4]. The earliest cultivated forms of wheat were essentially landraces that are heterogeneous and farmer-developed populations having a wide adaptation. These wheat landraces conceal a broad, diverse genetic base of cultivated wheat [36–39]. The increased prevalence of tan spot urged breeders to explore novel sources of resistance, particularly in landraces that represent an important group of genetic resources for the improvement of commercially valuable traits. Several studies using morphological, physiological and agronomic traits have shown that genetically diverse local germplasm well adapted to the environmental conditions can be considered as an important reservoir of useful genes for exploitation in wheat breeding programs [40–42]. Hence, screening durum wheat landraces for resistance to *Ptr* could represent an alternative source of resistance.

The objective of the present study was to look for potentially new sources of resistance to tan spot by screening a diverse collection of durum wheat germplasms that mostly included landraces. The responses of these accessions to tan spot were assessed in the field at seedling and adult plant growth stages to characterize the resistance to *Ptr* at seedling and adult-plant. This study also looks at correlations between disease development, plant height and days to heading.

2. Materials and Methods

2.1. Plant Materials

A durum panel consisting of 113 accessions provided by USDA-Gene bank (Aberdeen, ID, USA), including accessions from 12 different Mediterranean countries (Algeria (2), Egypt (7), Greece (9), Israel (2), Italy (10), Jordan (7), Lebanon (1), Morocco (26), Portugal (15), Spain (14), Syria (2) and Turkey (18)) was tested for resistance to *Ptr* under field conditions. These materials were at different improvement statuses, including landraces (78), cultivars (12), breeding material (7) and uncertain improvement status (16) (Table S1, Figure 1).

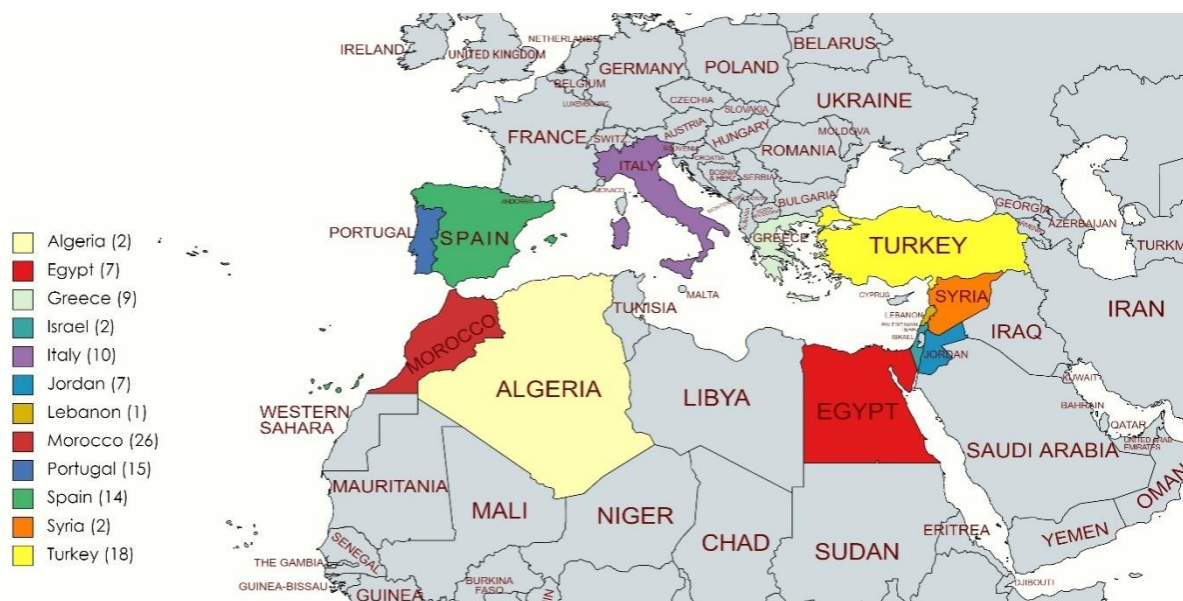


Figure 1. Geographical distribution of durum wheat accessions used in the present study.

2.2. Experimental Design and Inoculation Method

Durum wheat accessions were planted on 13 November, 2018, in a wheat-after-wheat production system, at the experimental station of the Septoria precision phenotyping platform of Tunisia based at INGC- Koudia research station, (Bou Salem, Governorate of Jendouba). This region is known to be a hot spot for tan spot disease where infection occurs annually. In fact, a recent study by Kamel and Cherif [16] showed that over 80% of tan spot incidence and severity were found in the region of Jendouba (Tunisia) in 2018 and that over a survey of 3 years, the atmospheric relative humidity was not a limiting factor for disease development and the temperatures recorded were favorable for tan spot development.

The experiment was conducted in an augmented design with unreplicated entries. These entries were sown in plots of two rows with 1 m length. Local checks, ‘Nasr’ and ‘Salim’, known for their susceptibility and resistance to tan spot disease, respectively, were also sown in two rows and replicated four times among blocks and the average of these replicates was used to classify the different accessions. The replicated checks were also used as a tool to verify the uniformity of the infection among and within plots. Spacing between plots and blocks was 50 cm and 1 m, respectively. The middle of the block was planted with the susceptible variety ‘Nasr’, which served as a disease spreader.

In October, before sowing, straw from the previous cropping season was incorporated into the soil with a rotary harrow. The inoculum density was approximately 1 kg m⁻². Additional inoculation was performed when the tested material reached the GS14 stage [43] by evenly spreading freshly cut infected wheat straw over the experimental plots and disease spreader rows, using susceptible cv, to ensure optimal disease development. The infected debris were obtained from the same experimental station of the previous cropping

season, which was highly infected by the disease. Irrigation was applied in the evenings creating favorable conditions for disease infection and standard wheat agronomic practices were followed during the trial period.







2.3. Agronomic Traits

Plants were characterized by the following traits: Days to heading (recorded for all genotypes when approximately 50% of the plants in a plot had spikes fully emerged from the boot (GS 55)) [43] and plant height (each plot was measured using a yardstick (in cm) from the ground level to the tip of the spikes at the maturity stage).

2.4. Disease Severity Rating

The collection of 113 accessions, which mostly included Mediterranean durum wheat landraces, were evaluated for tan spot resistance under field conditions. The inoculated plants were evaluated for the severity of their reaction to *P. tritici-repentis* infection at GS30 [43] using a slightly modified 0–5 lesion rating scale [19], as outlined in Table 1. Scores equal to 5 indicated susceptibility, while those equal to 4 indicated a moderately susceptible reaction of the genotype. Scores equal to 3 indicated a moderately resistant reaction of the genotype, while those equal or less than 2 indicated resistance.

Table 1. Disease score (0–5 scale) based on lesion appearance used to assess the severity of *Pyrenophora tritici-repentis* lesions.

Resistance		
		
Score 0: No lesion present	Score 1: Small, dark brown to black singular spots lacking any distinctive chlorotic or necrotic halo	Score 2: Small dark brown to black spots with small chlorotic halo. Some necrosis
Moderate resistance	Moderate susceptibility	Susceptibility
		
Score 3: Small to medium oval shaped lesion surrounded by a chlorotic halo	Score 4: Lesions with distinctive necrotic or chlorotic halo. Main lesion coalescing with surrounding singular lesions	Score 5: Large oval shaped lesions with the main lesion coalescing with most surrounding singular lesions

Resistance expressed at the seedling stage in growth chamber/greenhouse may or may not be expressed at the adult stage in the field. It was, therefore, highly important to compare seedling resistance with adult plant resistance under field conditions. Disease assessment was also carried out at three consecutive time points at GS55 [43] with a 7-day interval between each evaluation. Subsequently, tan spot progression was evaluated by measuring the incidence and severity based on the double-digit scale (00–99) [44] where the first digit indicates disease incidence on the infected plants, and the second digit refers

to the severity of infection. The area under disease progress curve (AUDPC) was calculated according to the formula shown below [45]:

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i) \quad (1)$$

where:

Y_i = disease severity at time t_i

$t_{(i+1)} - t_i$ = time interval (days) between two disease scores

n = numbers of scoring events

2.5. Statistical Analysis

R software version 4.0.5 [46] was used for all data analysis. Principal Components Analysis (PCA) was performed using the 'prcomp' function from the R package 'MASS' [47]. The determination and visualization of the optimal number of clusters were performed using the 'fviz_nbclust' function from the R package 'factoextra' using the method «within cluster sums of squares» [48]. The 'clusGap' function from R package 'cluster' was used to calculate gap statistic based on number of clusters [49]. The 'kmeans' function from the R package 'stats' was used to perform k-means clustering with the optimal k [46]. Finally, the coefficient of correlation between variables was performed with the 'cor.test' function from the R package 'stats' [46].

3. Results and Discussion

3.1. Reaction of Durum Wheat Accessions to Tan Spot

All Mediterranean durum wheat accessions evaluated against tan spot responded differentially to *Ptr* under field conditions at both seedling and adult stages, exhibiting reactions that ranged from susceptible (S) to moderately susceptible (MS) and moderately resistant (MR) to resistant (R). As AUDPC is inversely proportional to the degree of resistance, accessions having low AUDPC are resistant while those with high AUDPC are susceptible (Table S1). Subsequently, the AUDPC scores of accessions were classified in comparison to the AUDPC of the resistant and susceptible checks implemented in the experimental design. The average AUDPC of the resistant and the susceptible checks ('Salim' and 'Nasr') were estimated as 492.901 and 721.605, respectively (Table S2). Therefore, resistant accessions (R) have an AUDPC that is ranged between 268.518 and 487.654 and between 497.530 and 598.765 for moderately resistant (MR) accessions. Accessions with AUDPC scores from 617.901 to 711.111 were considered moderately susceptible (MS), while those with AUDPC scores greater than 725.925 were considered susceptible (S).

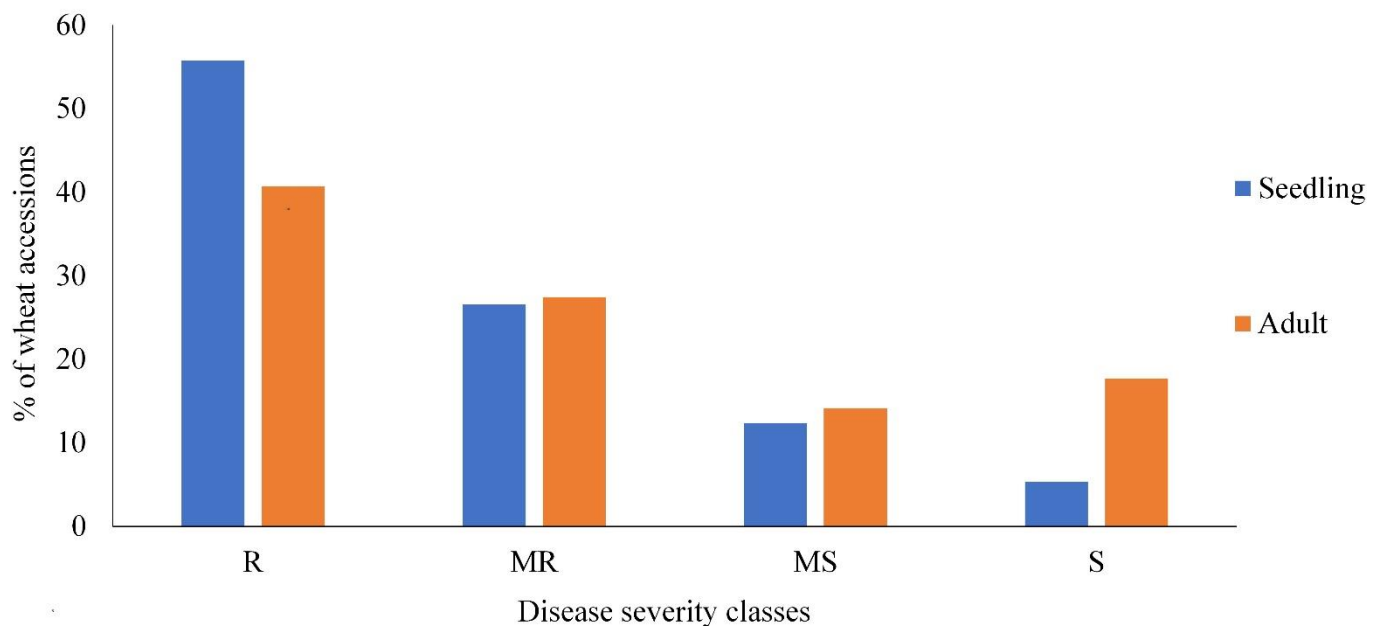
3.2. Comparison of Seedling and Adult Plant Resistance

Tan spot infection was uniform among and within all inoculated plots. Disease symptoms are illustrated in Figure 2.

On average, 56% of the accessions were resistant (R), 27% moderately resistant (MR), 12% moderately susceptible (MS) and 5% susceptible (S) under field conditions at the seedling stage whilst at the adult stage, 41, 27, 14 and 18% were classified as R, MR, MS and S, respectively (Figure 3). Among the 46 accessions resistant at the adult stage, 31 (67%) were landraces. In total, 80% of MR accessions were landraces. Table S1 shows the list of accessions, their country of origin, improvement status, plant height (PH), days to heading (DH) and disease scores at seedling and adult stages (AUDPC).



Figure 2. Typical tan spot symptoms in the field at the adult stage corresponding to GS55 at the Septoria Precision Phenotyping Platform (Experimental station of Koudia, Bou Salem, Tunisia).



R: Resistant; MR: Moderately Resistant; MS: Moderately susceptible; S: Susceptible

Figure 3. Frequencies of resistance levels (%) of the 113 durum wheat accessions to tan spot disease in the field at seedling and adult stages in the 2018–2019 cropping season.

Among the 63 accessions that were R at the seedling stage, 40 showed similar resistance levels at the adult stage, then 17 were MR, 5 were MS and one accession was classified as S at the adult stage. Among 30 accessions having an MR reaction at the seedling stage, 12 remained MR, 4 were MS, 6 were R and 8 were classified as S at the adult stage. Overall, 63% and 40% of accessions maintained the same level of resistance (R-MR) at both seedling and adult stages. Among 14 accessions having an MS reaction at the seedling stage, 5 remained MS, 2 were MR, and 7 were S at the adult stage. Among 6 accessions of S type at the seedling stage, two were MS and four were S at the adult stage.

At the seedling stage, the collection from Spain had the largest number of resistant accessions (12), followed by those from Turkey (10). The accessions from Greece and Portugal had eight resistant accessions at seedling stages, followed by six accessions from Egypt and Italy (Figure 4). The same trend was observed at the adult stage with accessions from Spain having the largest number of resistant accessions (9), followed by Greece (8), Egypt (5), Portugal (5) and Turkey (5) (Figure 5). The accession from Lebanon showed MS and S reactions at seedling and adult stages, respectively (Figures 4 and 5) and thus have no effective resistance at both stages. Durum wheat from Morocco had the greatest number of susceptible accessions at both seedling and adult stages with three and five genotypes, respectively (Figures 4 and 5).

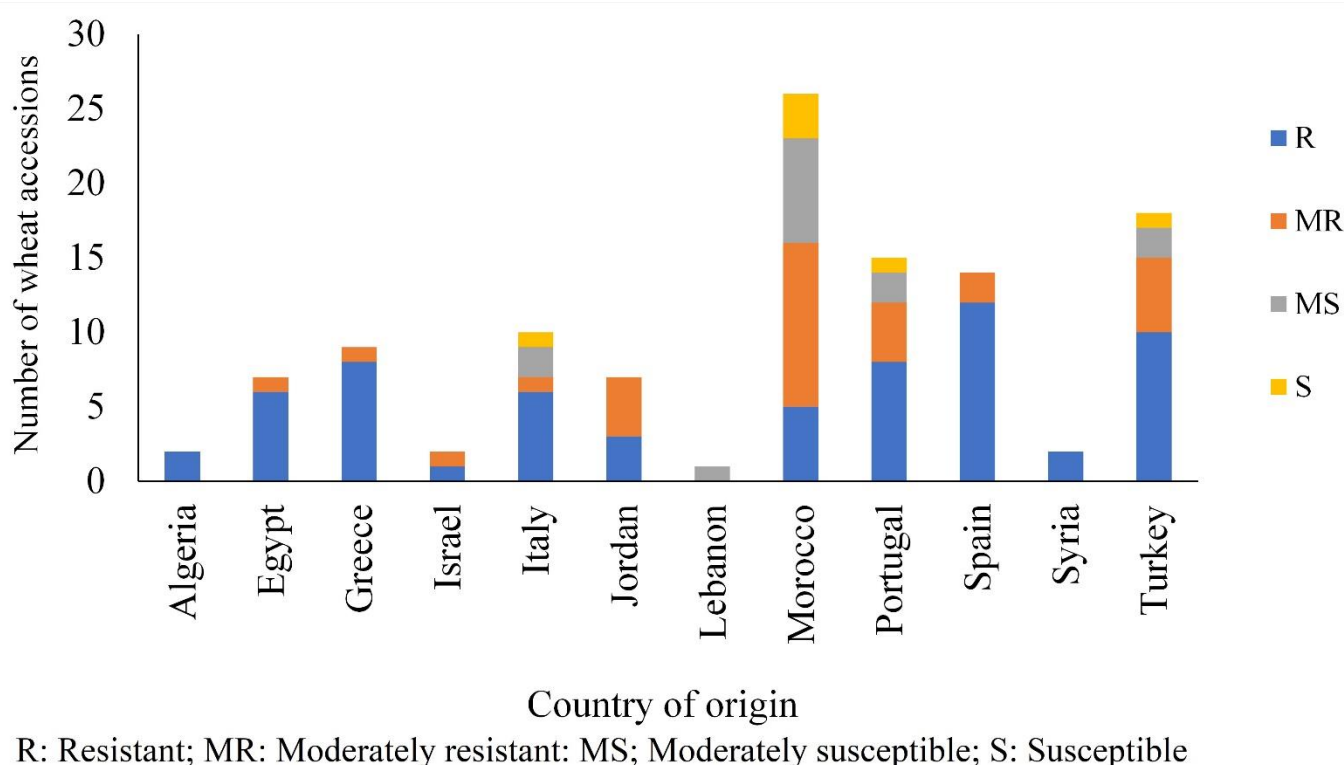
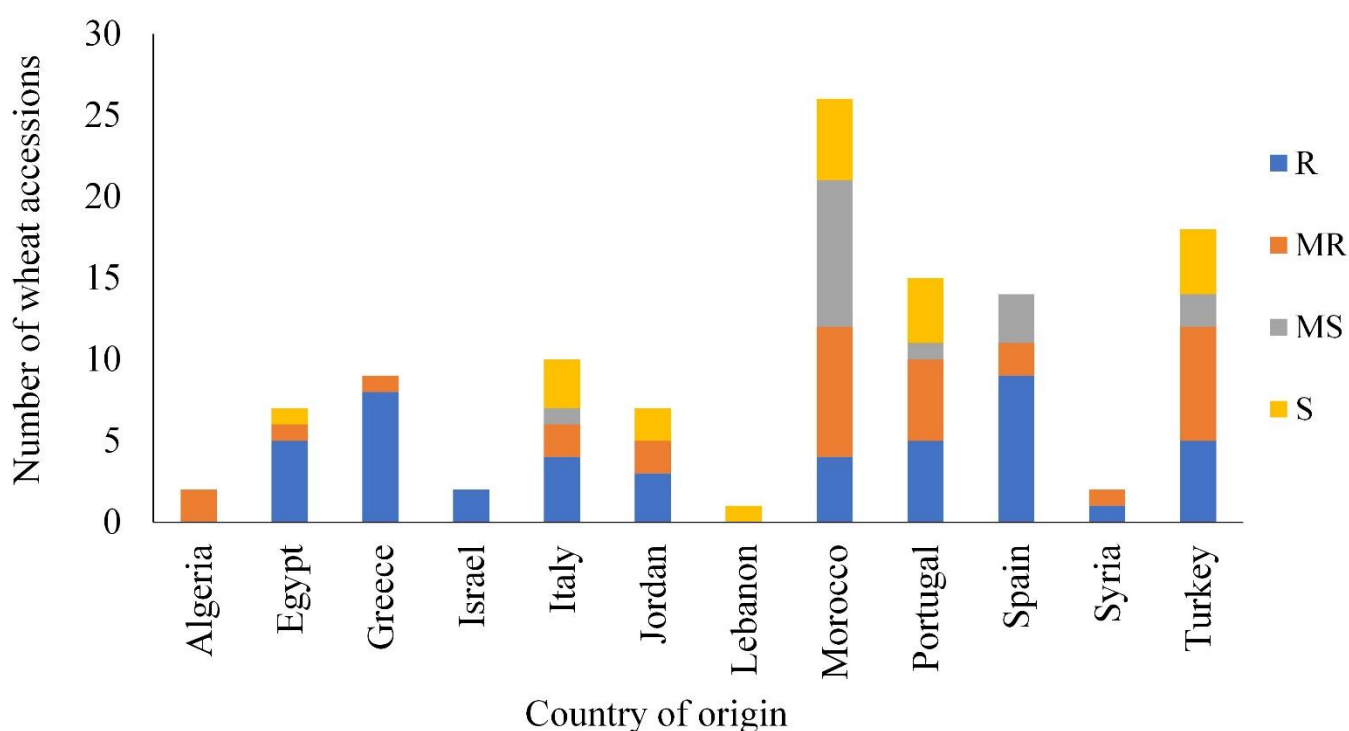


Figure 4. Resistance levels of 113 durum wheat accession's reactions to tan spot disease in-field at the seedling stage among countries of origin in the 2018–2019 cropping season.

At the seedling stage, 82% of durum wheat landraces had a good level of resistance (R-MR). While at the adult stage, 71% maintained their level of resistance, suggesting that these landraces may be an important source of useful genes for *Ptr* resistance as previously reported in accessions of spring wheat landraces (Table S1) [38,39].

Correlation between seedling and adult reactions was significant (Pearson's correlation $r = 0.695$, $p\text{-value} \leq 0.001$). Positive correlation has been reported between assessments of resistance at the seedling stage and of adult plants in the field in several studies. Greenhouse evaluation based on lesion length and field assessments of the AUDPC for tan spot resistance in Oklahoma (USA) were found to be significantly correlated [50]. Likewise, a significant correlation was observed between field disease ratings and greenhouse disease ratings in North Dakota (USA) even though the correlation coefficient ranged from $r = 0.29$ to 0.50 depending on the observer and the type of disease severity rating [23]. Similarly, in a study of the resistance to tan spot disease in winter wheat cultivars at different growth stages, a significant correlation ($r = 0.86$) was reported for greenhouse and field tan spot reactions in winter wheat [51]. Therefore, screening for tan spot at the seedling stage is often preferred to allow the most susceptible materials to be discarded before confirmation of field resistance. Although significant correlation was often established between seedling

and adult stages' reaction types [50,52], there were some inconsistencies in infection type between different growth stages [53]. The difference between seedling and adult reactions is likely due to the presence of APR factors in some genotypes that confer incomplete or partial resistance in the field [54]. Hence, adult plant resistance (APR) may serve as an effective tool in the search of broad-spectrum resistance as it is mostly polygenic and provides more resistance than the seedling. In a study of relationship of seedling and adult plant resistance to *Ptr*, most of the cultivars were found to have slightly higher levels of susceptibility at the adult stage under field conditions than at the seedling stage in greenhouse conditions, even though there was a similar trend between the two resistance levels across cultivars [52]. This was attributed to the interaction of different *Ptr* isolates; the amount of inoculum that resulted from wheat monoculturing over several years under field conditions was higher in comparison to the growth chamber conditions where accessions were tested using a single monoconidial *Ptr* isolate [52].



R: Resistant; MR: Moderately resistant; MS: Moderately susceptible; S: Susceptible

Figure 5. Resistance levels of 113 durum wheat accession's reactions to tan spot disease in-field at the adult stage among countries of origin in the 2018–2019 cropping season.

Moreover, *Ptr*-Wheat interaction follows an inverse gene-for-gene model where susceptibility results from the unique interaction between Necrotrophic effectors (NEs) termed *Ptr* ToxA, *Ptr* ToxB and *Ptr* ToxC, and specific receptors in the host termed *Tsn1*, *Tsc2*, and *Tsc1*, respectively, while the lack of NE recognition by the host leads to resistance [13,34]. The inheritance of resistance to tan spot is known to be both qualitative and quantitative [13,18,39,55–59]. To date, nine major *Tsr* genes (*Tsr1*, *Tsr2*, *Tsr3*, *Tsr4*, *Tsr5*, *Tsr6*, *Tsr7*, *TsrHar* and *TsrAri*) have been identified [60–66]. Numerous studies have reported quantitative resistance to *Ptr* that is effective against multiple races of the pathogen (non-race-specific resistance) [13,18]. In the case of *Ptr*, breeding strategies include the elimination of susceptibility genes, or the introgression of broad-spectrum resistance genes [13]. However, the removal of these gene can result in undesirable effects such as susceptibility to other pathogens as well as the loss of genetic diversity and useful genes that may be affecting other important traits. Many studies suggested the involvement of multiple NE–host

interaction(s), which may depend on plant growth stages [52,67–69]. In a recent study, Kokhmetova et al. [70] attributed resistance to tan spot to the known toxin insensitivity genes with major effects as well as broad-spectrum and race-non-specific genes, which have minor effects.

Recent studies conducted on *Ptr* in Tunisia by Laribi et al. [71,72], Kamel and Cherif [16] and Kamel et al. [73] revealed the presence of six *Ptr* races (2, 4, 5, 6, 7, 8) as well as ‘atypical’ isolates that were able to cause necrosis on the differential line ‘Glenlea’ but lacked the expected *ToxA* gene, suggesting therefore the involvement of additional NEs in the *Ptr*/wheat interaction in the North Western region (NWR) of Tunisia where the experiment was conducted. The predominant races in the latter region were races 5, 7 and ‘atypical’ isolates, while *Ptr* ToxB was found to be predominant. A recent study by Laribi et al. [71] showed that the *Ptr* population from (NWR) was genetically highly diverse. Hence, screening for resistance in the latter region may provide information not only on resistance to the prevalent races in Tunisia but also on broad-spectrum resistance to multiple NEs. Adult plant resistance (APR) is often preferred over seedling resistance as it has the potential to provide non-race specific resistance to TS [13]. Therefore, the identification of accessions with non-race specific resistance to *Ptr* can be a major step towards the development of lines that are resistant to *Ptr*. The resistance response of the tested accessions in this study may be associated with the diversity of *Ptr* in Tunisia similarly to the study of Dinglasan et al. [68] where they hypothesized that the prevalence of diverse *Ptr* populations from Russia, India, Pakistan and India was a main factor influencing wheat selection pressure. In the latter study, a number of accessions carrying all-stage resistance genes (ASR) mainly originated from Russia and India, while those with APR were from Pakistan and India. Similarly, Liu et al. [74] found that the number of resistant lines decreased when inoculated with more than one isolate. Hence, screening the 113 accessions against multiple races/isolates in fact increased the chances of these accessions having sources of durable resistance. Disease response was also found to differ between *ToxA* insensitive and sensitive accessions at seedling but not at adult-plant stages suggesting that other genetic factors or interactions contribute to disease response at the adult-plant stage [68].

Adult plant resistance (APR) occurs in mature plants and provides partial resistance. APR is a quantitative form of disease resistance that is considered more durable and has been deployed effectively in other host pathosystems such as leaf rust and stripe rust. For instance, some APR genes have been proven to provide resistance to all isolates of a pathogen species (broad spectrum) and/or resistance to multiple pathogen species, such as, the *Lr67* and *Lr34* genes, respectively [75–77]. As APR genes are mostly race non-specific, they most likely provide similar levels of resistance to different *Ptr* races. It is most often desired by wheat breeders as it allows effective selection for resistance to *Ptr*, whereas seedling resistance, even though time consuming for breeders, is important as it reduces primary inoculum spread.

As established by other researchers, durable resistance gene pyramiding represents an alternative to ensure an effective resistance against fungal pathogens for a longer time in a conducive environment favorable to disease development [33,34]. This requires the availability of resistant genetic resources and a better understanding of the host–pathogen interaction. The identification of genetically diverse lines with respect to tan spot resistance is of extreme relevance to select parents for breeding programs so as to pyramid genes from different resistant resources into a single genotype. In fact, it has been widely agreed that partial resistances to fungal pathogens are effective and durable [78–80]. The genetic complexity of the resistance at multiple scales is a guarantee to ensure a successful and long-lasting resistance, unlike resistance based on a single gene, which can be effective in the short term [57]. The majority of genetic studies have observed that resistance to tan spot is controlled by multiple major genes and the rare interaction between different genes imply that all genes need to be incorporated to develop durable and effective resistant cultivars [55–59].

Many accessions in this study displayed an immune response or a high level of resistance under field conditions at both seedling and adult stages (40 and 14 accessions maintained a resistant and moderate response), probably as a result of synergy between APR genes or between APR and seedling resistance genes. Therefore, these accessions may provide all-stage resistance genes (ASR) functional at all stages of plant development. Hence, they can be deployed as potential sources for improvement of *Ptr* resistance. The results of this study also highlight the potential of durum wheat landraces for *Ptr* resistance and the importance of gene pyramiding to provide sustainable resistance. The transfer of these probable additional novel genes into currently commercialized cultivars would provide a more effective and durable resistance.

3.3. Correlation of Tan Spot Infection with Plant Height and Days to Heading

In addition to the disease response, we also recorded the plant height (PH) and days to heading (DH) in order to identify any association to tan spot infection. We observed a significant variation within the collection in relation to these traits, as indicated in Table S1. PH ranged from 85 to 165 cm and from 138 to 164 days for DH, respectively. PH for local checks 'Nasr' and 'Salim' were 100 and 95 cm, respectively. The shortest accession was 85 cm for PI numbers 371822, 404588 and 517188 while the tallest height was 165 cm for PI 487293. DH for local checks 'Nasr' and 'Salim' were 132 and 134 days, respectively. The greatest DH, among the 113 accessions, was 164 days for PI numbers 525387, 341453 and 341612 while the lowest DH was 138 for PI 517188. To further understand the effect of PH and DH on tan spot infection, a principal component analysis (PCA) was conducted [81,82] using PH, DH and AUDPC as parameters. Results showed two dimensions of PCA explaining 79.7% of data variance (Figure 6). The first dimension accounted for 46.5% of the variances while the second dimension accounted for 33.2% of variances.

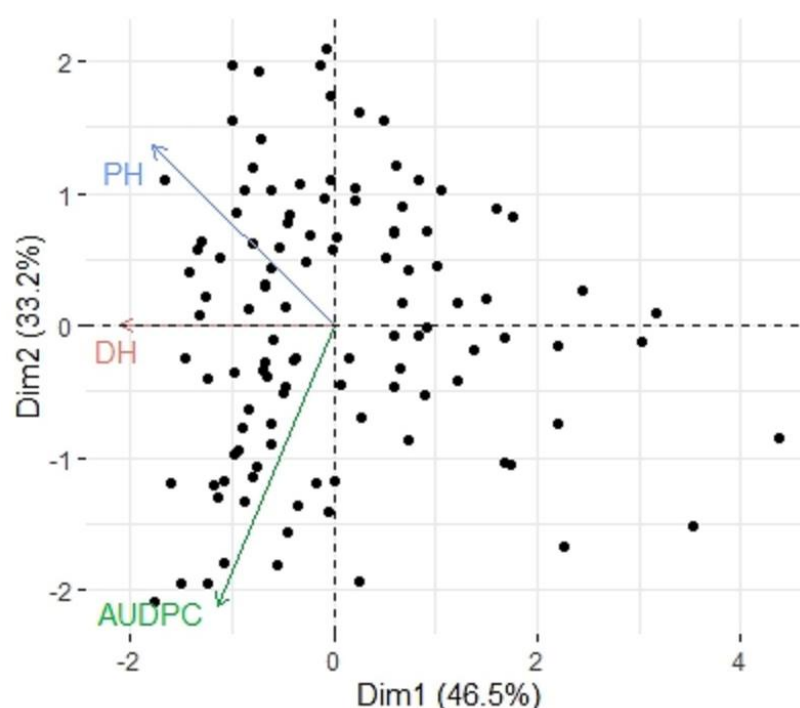
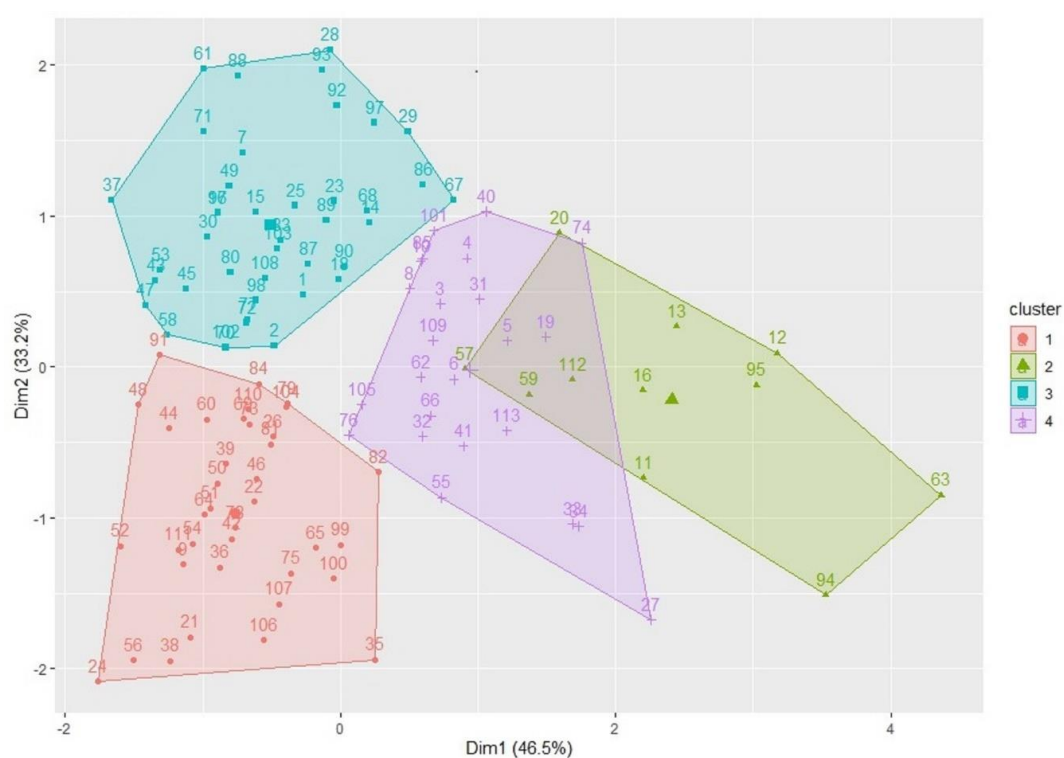


Figure 6. Principal component analysis (PCA) representing the contribution of three factors: plant height (PH), days to heading (DH) and AUDPC scores in 113 accessions.

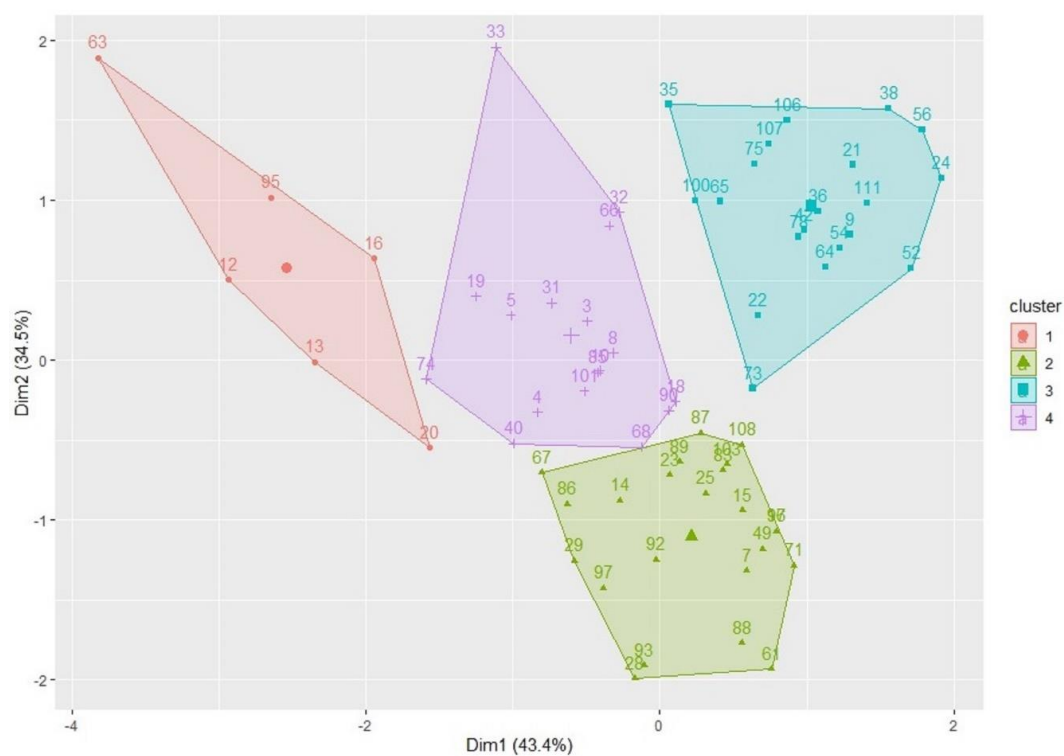
In an attempt to classify the different accessions, two clusters were conducted using the R function “K-means”. The first cluster (Figure 7A) included all 113 accessions and allowed the classification of wheat accessions into four different clusters. The first cluster (red) consisted of 37 accessions. Cluster 2 (green) contained 11 accessions. Cluster 3

(blue), which is the largest cluster, contained 41 accessions. Finally, cluster 4 (purple) consisted of 24 accessions. Among 78 landraces, 24, 8, 30 and 16 belonged to clusters 1, 2, 3 and 4, respectively. Among 16 accessions with uncertain improvement status, 6, 8 and 2 belonged to clusters 1, 3 and 4, respectively. Among seven breeding lines; one belonged to cluster 1, two belonged to cluster 2, one belonged to cluster 3 and three to cluster 4. Among 12 cultivars, six belonged to cluster 1, one belonged to both clusters 2 and 3 and three belonged to cluster 4 (Figure 7A, Table S1). The second cluster (Figure 7B) included 66 accessions (46 R and 20 S) and allowed the classification of wheat accessions into four different clusters. Results showed two dimensions explaining 77.9% of data variance (Figure 7B). The first dimension accounted for 43.4% of the variances, while the second dimension accounted for 34.5% of variances. The first cluster (red) consisted of six accessions. Cluster 2 (green), which is the largest cluster, contained 23 accessions. Cluster 3 (blue) contained 20 accessions. Finally, cluster 4 (purple) consisted of 17 accessions. Clusters 1, 2 and 4 included all resistant accessions while susceptible accessions clustered separately in cluster 3 (Figure 7B). PH and DH ranged from 85 to 150 cm and from 138 to 164 days, respectively, for resistant accessions (Clusters 1, 2 and 4) and from 120 to 150 cm and from 152 to 164 days, respectively, for susceptible accessions (Cluster 3).

The correlation between PH and AUDPC was $r = 0.004$ ($p = 0.97 > 0.05$), while the correlation between DH and AUDPC was $r = 0.212$ ($p = 0.02 \leq 0.05$). Thus, no consistent relationship between PH and AUDPC was found. Conversely, there is an association between DH and AUDPC. Likewise, in a study of heritability of resistance to tan spot in durum wheat and its association with other agronomic traits in North Dakota (USA), no significant correlation between plant height and the measurements of disease severity were reported [23]. In addition, in a recent study of tan spot resistance in a hexaploid wheat collection from Kazakhstan, correlation of PH with AUDPC scores was not found to be significant, further suggesting that PH does not significantly affect tan spot resistance [70]. Contrarily to our results, the latter study suggested no significant effect of DH on TS resistance. Similarly, in a study of tan spot resistance in the bread wheat variety Ernie, no significant correlations were detected between tan spot severity with either DH or PH [83]. However, many other studies reported a negative correlation between DH or PH with disease resistance; indeed, in a recent study of resistance of 372 European wheat varieties to tan spot disease in Germany, a highly significant negative correlation of tan spot infection with PH and a moderate negative correlation with DH were reported, indicating that plant height and late heading might be potential tan spot disease escape traits, therefore, indicating a role of plant height in disease expression [84]. In another study that characterized 358 European winter wheat cultivars and 14 spring wheat cultivars for their resistance to tan spot in Germany, the authors showed that shorter plants showed a tendency to be more susceptible while no significant correlation between *Ptr* resistance and DH was established [85]. The different outcome of these studies may be partially explained by the use of different germplasm and the conduction of these experiments in different environments. Similar contradictory conclusions have been reported in other diseases such as Fusarium head blight (FHB), Septoria nodorum blotch (SNB), and Septoria tritici blotch (STB) [86–88]. For instance, studies based on the relationship between leaf spot disease severity of wheat and PH and DH have shown negative associations for SNB and STB diseases in wheat [21,89,90]. Conversely, in a study that investigated the relationships between plant stature, maturity class, and susceptibility to STB, a low correlation was found between plant height and STB severity, suggesting that there is probably no linkage between shortness and susceptibility [91].



(A)



(B)

Figure 7. (A) Clustering of 113 wheat genotypes representing the contribution of 3 factors: Plant height (PH), days to heading (DH) and the area under disease progression curve (AUDPC); (B) clustering of 66 wheat genotypes (46 R and 20 S) representing the contribution of 3 factors: plant height (PH), days to heading (DH) and the area under disease progression curve (AUDPC).

4. Conclusions

In this study, field evaluations for tan spot resistance were conducted on a panel of 113 durum wheat accessions. The collection showed vast phenotypic diversity. We found that resistance and susceptibility reactions varied within and among countries and between seedling and adult stages. Our results showed no significant correlation between PH and AUDPC scores, whereas a significant correlation between DH and AUDPC was found, indicating that this trait may have an effect on disease resistance or susceptibility. Highly significant correlation between seedling and adult reactions was observed ($r = 0.695$, $p\text{-value} \leq 0.001$), suggesting that screening accessions at the adult stage only or at the seedling stage can be sufficient for evaluating disease resistance. In this study, among 113 accessions, 40 and 14 accessions maintained resistant and moderately resistant reactions to tan spot disease at both seedling and adult stages under Tunisian field conditions, respectively, indicating most likely the presence of complete resistance. More in-depth research is required, as the latter accessions may harbor valuable all-stage resistance genes (ASR)/QTLs, hence, providing high levels of resistance to *Ptr* at all development stages that can be used in breeding programs. In addition to the importance of gene pyramiding to provide sustainable resistance, our results also emphasize the potential of durum wheat landraces as a new genetic solution to tan spot in Tunisia.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agriculture11111148/s1>. Table S1: The list of accessions, their country origin, improvement status, plant height (PH), Days to heading (DH) and disease scores at seedling stage and adult stage (AUDPC). Table S2: The area under disease progress curve (AUDPC) of checks implemented in the experimental design.

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