Deciphering resistance to \textit{Zymoseptoria tritici} in the Tunisian durum wheat landrace accession ‘Agili39’

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Abstract

\textbf{Background:} Septoria tritici blotch (STB), caused by \textit{Zymoseptoria tritici} (\textit{Z. tritici}), is an important biotic threat to durum wheat in the entire Mediterranean Basin. Although most durum wheat cultivars are susceptible to \textit{Z. tritici}, research in STB resistance in durum wheat has been limited.

\textbf{Results:} In our study, we have identified resistance to a wide array of \textit{Z. tritici} isolates in the Tunisian durum wheat landrace accession ‘Agili39’. Subsequently, a recombinant inbred population was developed and tested under greenhouse conditions at the seedling stage with eight \textit{Z. tritici} isolates and for five years under field conditions with three \textit{Z. tritici} isolates. Mapping of quantitative trait loci (QTL) resulted in the identification of two major QTL on chromosome 2B designated as \textit{Qstb2B_1} and \textit{Qstb2B_2}. The \textit{Qstb2B_1} QTL was mapped at the seedling and the adult plant stage (highest LOD 33.9, explained variance 61.6%), conferring an effective resistance against five \textit{Z. tritici} isolates. The \textit{Qstb2B_2} conferred adult plant resistance (highest LOD 32.9, explained variance 42%) and has been effective at the field trials against two \textit{Z. tritici} isolates. The physical positions of the flanking markers linked to \textit{Qstb2B_1} and \textit{Qstb2B_2} indicate that these two QTL are 5 Mb apart. In addition, we identified two minor QTL on chromosomes 1A (\textit{Qstb1A}) and chromosome 7A (\textit{Qstb7A}) (highest LODs 4.6 and 4.0, and explained variances of 16% and 9%, respectively) that were specific to three and one \textit{Z. tritici} isolates, respectively. All identified QTL were derived from the landrace accession Agili39 that represents a valuable source for STB resistance in durum wheat.

\textbf{Conclusion:} This study demonstrates that \textit{Z. tritici} resistance in the ‘Agili39’ landrace accession is controlled by two minor and two major QTL acting in an additive mode. We also provide evidence that the broad efficacy of the resistance to STB in ‘Agili 39’ is due to a natural pyramiding of these QTL. A sustainable use of this \textit{Z. tritici} resistance source and a positive selection of the linked markers to the identified QTL will greatly support effective breeding for \textit{Z. tritici} resistance in durum wheat.

\textbf{Keywords:} Durum wheat, Landraces, \textit{Zymoseptoria tritici}, \textit{Mycosphaerella graminicola} quantitative trait loci (QTL), Gene efficacy, QTL epistasis, Gene pyramiding
The genus *Triticum* L. comprises several wheat species with various ploidy levels, but global wheat production is almost entirely based on bread wheat, *T. aestivum* L. var. Thell. (2n = 6x = 42, sub-genomes AABBDD), and durum wheat, *T. turgidum* var. *durum* (2n = 4x = 28, sub-genomes AABB), also known as pasta wheat [4]. Durum wheat accounts for about 8% to the global wheat production, and its cultivation is concentrated in latitudes ranging from 55°N to 40°S [5, 6], corresponding mostly to the Mediterranean Basin, the North American Great Plains, India and the former USSR [6]. Durum wheat is also produced in sub-Saharan Africa (SSA) where Ethiopia is a leading country and considered as one of the biggest durum wheat producers with approximately 0.6 million ha [7], and a center of diversity for tetraploid wheat [8].

Northern Africa has also been the cradle of wheat production for centuries and was the breadbasket for the Roman Empire [9, 10] with locations such as Dougga in Tunisia, as exquisite trading zones for wheat and other commodities until the late 500’s AD [11]. In Tunisia, durum wheat occupies 725 Mha which approximates represent commodities until the late 500’s AD [11]. In Tunisia, durum wheat was identified in many countries such as Tunisia [12], Ethiopia [8], Iran [32] and Spain [33].

Until now, up to 22 septoria tritici blotch (*Stb*) resistance genes have been identified and mapped [34, 35]. However, due to the apparent dichotomy in natural *Z. tritici* populations for either bread wheat or durum wheat [36–38], the presence of these mapped *Stb* genes in durum wheat cannot be determined using well characterized *Z. tritici* strains originating from bread wheat. Thus far, the substantial research progress is mainly based on the *Z. tritici* – bread wheat pathosystem [34, 39]. Therefore, and albeit the growing efforts to dissect the durum wheat – *Z. tritici* interactions [12, 33, 40, 41], resistance breeding to *Z. tritici* in durum wheat has slowly progressed over the last 25 years compared to bread wheat [34]. This affects many small growers who produce this wheat as a staple crop in an area that is severely struck by septoria tritici blotch.

In this study, we have embarked on increasing the understanding of the *Z. tritici* – durum wheat pathosystem. Here, we have first screened eight durum wheat Tunisian landrace accessions for *Z. tritici* resistance. Subsequently, we developed a mapping population between the resistant landrace accession ‘Agili39’ and the susceptible modern cv. Khiar to identify the genetic basis of resistance to *Z. tritici* and to map the underlying genes under greenhouse and field conditions.

**Results**

### Phenotyping of RILs, landrace accessions and modern cultivars at the seedling stage

The 20 *Z. tritici* isolates grew successfully under laboratory conditions enabling appropriate inoculum production and phenotyping. None of the tested durum landraces and cultivars was resistant to the entire suite of *Z. tritici* isolates (Table 1), but the landrace accessions showed a broader efficacy compared to the cvs. Khiar and Karim, resulting in a significant ‘line x isolate’ interaction, indicating specific gene action (Additional Table 1). Interestingly, necrosis values were high and ranged...
between 72 and 97% (data not shown). The parents of the developed RILs, ‘Agili39’ and cv. Khiar showed highly significantly different pycnidia values of 6% and 36%, respectively (Table 1), and henceforward, we selected a set of eight *Z. tritici* isolates that discriminated between ‘Agili39’ and cv. Khiar for subsequent phenotyping of the developed F6 RILs population (Table 2).

The seedling screening of the RILs with the selected eight *Z. tritici* isolates resulted in non-symmetric frequency distributions skewed towards the resistance phenotype for all tested isolates (Fig. 1 panel A, Additional Fig. 1). Subsequent analyses of variance of the split-plot design seedling experiment revealed that the ‘RIL’ term was highly significant for necrosis and pycnidia AUDPC scores at $p=0.0001$ (Table 3). This result indicates that the observed variation in the data is accounted for the variable genetic make-up of the tested lines.

Differentiation between the isolates was observed for necrosis and pycnidia scores in the RIL population tested at the seedling stage (Table 4). The highest population mean necrosis coverage (60.60%) was registered for RILs inoculated with IPO92003 isolate (Table 4). However, the least population mean necrosis coverage (34.5%) was observed for RILs inoculated with Tun1 isolate. Pycnidia population mean scores were also variable and were high for lines inoculated with Tun6 isolate (23.9%), but relatively low for lines inoculated with IPO95052 isolate (9.4%). For all tested isolates, necrosis scores ranged between 0 and 100%, however a maximum of 90% of pycnidia score was registered for lines inoculated with IPO95052 isolate (Table 4). All seedling phenotypic traits were repeatable with the highest repeatability registered for isolates IPO92003 and Tun6 for necrosis and pycnidia scores, respectively and with an equal repeatability of 0.96 for both seedling phenotypic traits and isolates (Table 4).

### Phenotyping of RILs at the adult plant stage

During all field trials, STB developed well after the inoculations, but only pycnidia coverage was assessed. The susceptible parent cv. Khiar showed high disease severities throughout the trials, whereas ‘Agili39’ remained free of disease (0 pycnidia) (Fig. 1 panel B).

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**Table 1** Percentage of pycnidia on the sprayed-inoculated primary leaves of durum wheat landraces and cultivars with 20 *Zymoseptoria tritici* isolates at 21 days-post inoculations. Coloured cells indicate least significant differences (LSDs; $p=0.05$) with resistant in green (not significantly different from 0% Pycnidia), intermediate in yellow (significantly different from 0% Pycnidia and 100% Pycnidia) and susceptible in red (not significantly different from 100% Pycnidia)
The three-way analysis of variance revealed that the 'genotype', 'isolate' and their interaction 'genotype x isolate' terms are highly significant at $p = 0.0001$ (Table 5). This result indicates a 'genotype x isolate' specificity at the adult plant. The term 'year' was significant at $p = 0.05$. However, the term 'block', the two-way interaction terms 'genotype x block' and 'genotype x year', and the three-way interaction term 'genotype x year x isolate' were not significant, indicating no variation in the micro-environment and the homogeneity of the field inoculation (Table 5).

Overall, adult plants F9 showed the highest average of pycnidia coverage compared to the F7 inoculated with the Tun1 isolate (21.5% of pycnidia for the F9 compared to 15.4% for the F7), and to the F6 and the F8 generations inoculated with the Tun6 isolate (29.9% of pycnidia for the F9 compared to 19.6% and 19.0% for the F6 and the F8, respectively) (Table 4, Fig. 1 panel B). The field disease severity on the F9 generation inoculated with Tun1 and Tun6 isolates was also variable with a higher severity for Tun6 isolate (29.9%) compared to the Tun1 isolate (21.5%), which indicates once more a specific 'genotype x isolate' interaction. Interestingly, average pycnidia coverage was relatively low at the F10 generation inoculated with the IIB-123 isolate (6.5% of pycnidia), with a maximum pycnidia coverage of 19.4% (Table 4).

Adult pycnidia coverage heritability ($H^2$) was high for the Tun1 and the Tun6 isolates, with a higher heritability for the adult pycnidia coverage caused by the Tun6 isolate ($H^2 = 0.98$) compared to the heritability of the pycnidia coverage caused by the Tun1 isolate ($H^2 = 0.88$) (Table 4). Field data generated by the inoculation of the F10 RIL with the IIB123 isolate were also repeatable ($0.97$) (Table 4).

Correlations between the seedling and the adult plant assays

Low to high correlations were observed between the different traits (Fig. 2, Additional Table 2). Phenotypic scores obtained by the IPO92003 isolate were the least correlated with all phenotypic scores obtained by the tested isolates at the seedling and the adult plant stages (Fig. 2, Additional Table 2). Nonetheless, necrosis and pycnidia AUDPC scores generated by the IPO92003 isolate on the tested RILs at the seedling stage were highly correlated ($r = 0.6$). *Z. tritici* isolates IPO95052,
Fig. 1 Frequency distributions of the disease severity assessed as pycnidia percentage in seedlings and adult plants of the F6-F10 recombinant inbred lines of Agili39/Khiar with three Zymoseptoria tritici under field and controlled conditions. ‘A’ and ‘K’ are referring to ‘Agili39’ and cv. Khiar parents, respectively.

Table 3 Analysis of variance of necrosis and pycnidia AUDPC (N-AUDPC and P-AUDPC) seedling data from the ‘Agili39’/Khiar recombinant inbred lines (RILs) tested following a split-plot design

| Source of variation | df* | Mean of Square | F value     | Pr(> F)    |
|---------------------|-----|---------------|-------------|------------|
|                     |     | N-AUDPC       | P-AUDPC     |            |
| Replicate           | 2   | 5,303,251     | 834,974     | 2.2345     | 2.8532     | 0.14966 | 0.09689 |
| Isolate             | 7   | 2,092,146     | 167,005     | 0.8815     | 0.5707     | 0.54841 | 0.7668  |
| Error a (Main plot) | 12  | 2,373,376     | 292,643     |            |            |         |
| RIL                 | 160 | 217,971       | 125,635     | 12.3639    | 13.2979    | <2.00E-16*** | <2.00E-16*** |
| Isolate x RIL       | 1099| 16,572        | 8909        | 0.94       | 0.943      | 0.87956 | 0.86706 |
| Error b (Sub-plot)  | 2183| 17,630        | 9448        |            |            |         |

Significance codes: 0 **** 0.001 *** 0.01 ** 0.05 * 0.1 . 1

* Degrees of freedom
IPO91009, Tun6, IIB-123 and IPO91004 tested at the seedling stage were correlated between each other for necrosis and pycnidia AUDPC scores (0.3 < \( r \) > 0.5) (Fig. 2, Additional Table 2). The highest correlation coefficient (\( r \)) of 0.9 was registered between pycnidia AUDPC scores generated on lines inoculated by the IPO91004 and the II-B123 \( Z. tritici \) isolates at the seedling stage. In contrast, necrosis and pycnidia scores were rather moderately correlated between IPO91018, IIB-123, IPO95052 and IP91004 isolates with a maximum \( r \) of 0.5 (Additional Table 2). Field data generated by Tun6 isolate were highly correlated across the F6 – F9 generation (\( r = 0.8 \)) (Additional Table 2). Interestingly, positive correlations were also observed between adult and seedling disease scores induced by Tun6 isolate (Additional Table 2). Similarly, positive correlations were registered between field scores generated by Tun6, and II-B123 isolates, with a correlation coefficient \( r = 0.4 \) (Fig. 2, Additional Table 2).

The ‘Agili39’/Khiar linkage genetic map and the identification of QTL for \( Z. tritici \) resistance

The ‘Agili39’/Khiar genetic linkage map consisted of 1959 SNP markers assigned to 30 linkage groups representative of the 14 durum wheat chromosomes (Additional Table 3). The total length of the genetic map was

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**Table 4** Mean, range (minimum and maximum) and repeatability / heritability (\( H^2 \)) of Necrosis and Pycnidia values of the durum parents ‘Agili39’ and cv. Khiar and their derived recombinant inbred lines at the seedling and the adult plant stages

| Isolate | Parent | Mean (± Std)* | RILs Physiological stage | Population mean (± Std)* | Range (Min–Max) | Repeatability / \( H^2 \) |
|---------|--------|---------------|---------------------------|--------------------------|-----------------|--------------------------|
|         |        | Necrosis %    | Pycnidia %                | Necrosis %               | Pycnidia %      | Necrosis %              |
|         |        |               |                           |                          |                 |                          |
| Tun1    | Agili39| 0.0±0.5       | 0.0±0.8                   | 15.40±1.7               | 0.00–51.60      | 0.88 b                   |
|         | Khiar | 7.0±5.0       | 46.7±3.5                  | 21.50±2.2               | 0.00–82.40      |                          |
| Tun6    | Agili39| 6.7±4.2       | 0.0±1.8                   | 34.50±2.2               | 0.00–90.00      | 0.85 b                   |
|         | Khiar | 9.0±0.5       | 0.0±2.5                   | 19.60±1.9               | 0.00–55.3       | 0.98 b                   |
| IIB123  | Agili39| 1.7±3.0       | 0.0±3.5                   | 7.0±1.5                 | 0.00–7.00       | 0.97                     |
|         | Khiar | 9.0±5.0       | 46.7±3.5                  | 35.30±3.0               | 0.00–93.30      | 0.93                     |
| IPO91004| Agili39| 23.3±0.6      | 0.0±2.3                   | 40.5±2.7                | 0.00–86.70      | 0.91                     |
|         | Khiar | 8.0±1.5       | 80.0±5.3                  | 30.5±2.4                | 0.00–80.00      | 0.88                     |
| IPO91009| Agili39| 1.7±0.5       | 0.0±3.0                   | 30.5±2.4                | 0.00–93.30      | 0.88                     |
|         | Khiar | 6.6±3.5       | 56.7±1.5                  | 6.0±0.94                | 0.00–19.40      | 0.97                     |
| IPO91018| Agili39| 11.7±5.3      | 0.0±0.2                   | 42.10±2.9               | 0.00–100.00     | 0.92                     |
|         | Khiar | 8.0±3.2       | 70.0±3.5                  | 48.20±2.9               | 0.00–100.00     | 0.92                     |
| IPO92003| Agili39| 6.6±3.3       | 13.3±0.2                  | 35.0±3.0                | 0.00–93.00      | 0.93                     |
|         | Khiar | 5.0±3.4       | 5.0±1.8                   | 22.0±1.8                | 0.00–87.30      | 0.93                     |
| IPO90502| Agili39| 5.3±2.6       | 0.0±1.5                   | 36.40±3.1               | 0.00–80.00      | 0.88                     |
|         | Khiar | 7.0±1.6       | 80.0±2.5                  | 9.40±2.1                | 0.00–90.00      | 0.89                     |

* Standard deviation

**Table 5** Analysis of variance for adult plant disease severity scores in the F6-F9 ‘Agili39’/Khiar recombinant inbred lines (RILs) that were inoculated with \( Zymoseptoria tritici \) isolates Tun1 and Tun6

| Source of Variance | Df  | Mean of Square | F value | Pr(> F) |
|--------------------|-----|----------------|---------|---------|
| Genotype           | 174 | 4283           | 9.439   | <2e-16 *** |
| Isolate            | 1   | 20,847         | 45.94   | 2.18e-09 *** |
| Year               | 3   | 1721           | 3.793   | 0.013581 * |
| Block              | 4   | 300            | 0.661   | 0.620963 |
| Genotype x Block   | 51  | 262            | 0.578   | 0.980775 |
| Genotype x Isolate | 167 | 955            | 2.105   | 0.000165 *** |
| Genotype x Year    | 465 | 162            | 0.356   | 1       |
| Genotype x Year x  | 653 | 365            | 0.89    | 0.80147 |

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

* Degrees of freedom
4220 cM. In average, linkage groups consisted of 80.83 SNP markers spanning an average length of 140.7 cM, in which adjacent loci were in average 2.3 cM distant (Additional Table 3). Linkage group sizes ranged from 28.7 cM and 338.0 cM, corresponding to linkage groups 12 and 7, respectively, both representative of chromosome 1B (Additional Table 3).

For the QTL analysis, the permutation test used to define the significant threshold LOD resulted in a LOD value of 3.5, hence only QTL with a LOD ≥ 3.5 were considered, which excluded all detected QTL with isolate IPO92003. In total, we identified four significant QTL on three chromosomes 1A, 7A and 2B. None of these QTL was mapped with every tested Z. tritici isolate, which underscores specificity of the interaction between Z. tritici and durum wheat. Two QTL were identified on chromosome 2B (Table 6; Fig. 3) designated as Qstb2B_1 and Qstb2B_2. Qstb2B_1 was effective in both the seedling stage—particularly against isolates Tun6, IIB123, IPO91009, IPO95052 and IPO91004, but not for isolates Tun1, IPO91018 and IPO92003—and the adult plant stage, where it provided resistance to Z. tritici isolate Tun6, but not to Tun1 and IIB-123 isolates. Qstb2B_1 was mapped between the two SNP markers Tag_1056626 and Tag_111757 (Additional Table 4) with a confidence interval ranging between 69.5 and 75.5 cM and explained up to 61.6% of the phenotypic variance at the adult plant stage for Tun6 isolate, and up to 38.0% for IPO91004 isolate at the seedling stage (Table 6). LOD values of the Qstb2B_1 QTL were variable depending on the isolate (Table 6). The highest LOD for the Qstb2B_1 QTL was registered for Tun6 at the F9 generation (33.9) at the adult plant stage, and for IPO91004 isolate for the necrosis score (17.3) at the seedling stage (Table 6).

A second QTL, designated as Qstb2B_2, was mapped on chromosome 2B. This QTL was solely mapped at the adult plant stage for isolates Tun1 and Tun6, but not for the IIB123 isolate. This QTL is flanked by the two SNP...
markers Tag_100031118 and Tag_3027184 (Additional Table 4) at a confidence interval of 106.5 and 123 cM (Table 6). The highest LOD and explained variance were registered for the Tun6 isolate at the F9 generation with 32.8 and 54.3%, respectively (Table 6). Comparing the physical positions of the flanking markers linked to the Qstb2B_1 and Qstb2B_2 QTL indicates that these two QTL are 5 Mb apart (Additional Table 4).

Two additional QTL with lower LODs and explained variances were mapped on chromosomes 1A and 7As designated as Qstb1A and Qstb7A, respectively and showed specificity for Tun1, Tun6 and IPO91009 isolates for Qstb1A, and for IIB-123 isolate for Qstb7A (Table 6, Fig. 3). These QTL were uniquely detected at the seedling plant stage. Qstb1A was flanked by the two SNP markers Tag_1694925 and Tag_1127081 (62.5—63 cM), and explained up to 16.0% of the phenotypic variance with a maximum LOD of 4.6 for Tun1 isolate (Table 6, Additional Table 4). Qstb7A, mapped on chromosome 7A at 53 cM, was solely detected with IIB-123 isolate at the seedling plant stage for necrosis scores (Table 6). This QTL is flanked by the SNP markers Tag_2277193 and

### Table 6 Detected quantitative trait loci (QTL) for Zymoseptoria tritici necrosis and pycnidia resistance in the seedling and the adult plant stages in the ‘Agili39’/Khiar recombinant inbred lines

| Chromosome | QTL ID | Isolate | Trait – Physiological stage | LOD | Peak position (cM) | Flanking markers | Confidence Interval (cM) | PVE (%) | Additive effect |
|------------|--------|---------|-----------------------------|-----|-------------------|----------------|-------------------------|---------|----------------|
| 1A         | Qstb1A | Tun1    | Necrosis-Seedling           | 4.6 | 63                | Tag_1694925—Tag_1127081 | 62.5—63                  | 16.0    | 36.6           |
|            |        | Tun6    | Necrosis-Seedling           | 4.1 | 63                | Tag_1694925—Tag_1127081 | 62.5—63                  | 9.1     | 50.9           |
|            |        |         | Pycnidia-Seedling           | 4.4 | 63                | Tag_1694925—Tag_1127081 | 62.5—63                  | 9.9     | 54.1           |
|            |        | IPO91009| Necrosis-Seedling           | 4.1 | 63                | Tag_1694925—Tag_1127081 | 62.5—63                  | 9.8     | 38.0           |
| 7As        | Qstb7A | IIB123  | Necrosis-Seedling           | 4.0 | 53                | Tag_2277193—Tag_100009953 | 52.5—60.5                | 9.0     | 46.2           |
| 2B         | Qstb28_1 | Tun6 | Necrosis-Seedling           | 11.6| 74                | Tag_1056626—Tag_111757 | 72.5—75.5                | 29.3    | 91.4           |
|            |        |         | Pycnidia-Seedling           | 11.9| 74                | Tag_1056626—Tag_111757 | 72.5—75.5                | 29.6    | 93.7           |
|            |        |         | Pycnidia-Adult (F7:8)       | 23.4| 75                | Tag_1056626—Tag_111757 | 74.5—75.5                | 52.9    | 1.5            |
|            |        |         | Pycnidia—Adult (F8:9)       | 33.9| 74                | Tag_1056626—Tag_111757 | 72.5—75.5                | 61.6    | 24.4           |
|            |        | IIB123  | Necrosis-Seedling           | 12.9| 74                | Tag_1056626—Tag_111757 | 72.5—75.5                | 33.7    | 87.6           |
|            |        |         | Pycnidia-Seedling           | 5.4 | 74                | Tag_1056626—Tag_111757 | 70.5—75.6                | 17.5    | 38.2           |
|            |        | IPO91004| Necrosis-Seedling           | 17.3| 75                | Tag_1056626—Tag_111757 | 72.5—75.6                | 38.0    | 96.9           |
|            |        |         | Pycnidia-Seedling           | 7.2 | 74                | Tag_1056626—Tag_111757 | 69.5—75.6                | 21.2    | 52.5           |
|            |        | IPO91009| Necrosis-Seedling           | 9.5 | 74                | Tag_1056626—Tag_111757 | 73.5—75.6                | 24.6    | 60.1           |
|            |        | IPO95052| Necrosis-Seedling           | 7.2 | 75                | Tag_1056626—Tag_111757 | 71.5—75.6                | 21.1    | 77.2           |
|            |        |         | Pycnidia-Seedling           | 3.2 | 74                | Tag_1056626—Tag_111757 | 72.5—75.6                | 10.0    | 36.0           |
| Qstb28_2   | Tun1   |         | Pycnidia-Adult (F6:7)       | 4.8 | 117               | Tag_100031118—Tag_3027184 | 107.5—123              | 14.9    | 0.6            |
|            |        |         | Pycnidia-Adult (F8:9)       | 5.5 | 113               | Tag_100031118—Tag_3027184 | 106.5—118.5            | 17.1    | 9.1            |
|            | Tun6   |         | Pycnidia-Adult (F5:6)       | 32.8| 116               | Tag_100031118—Tag_3027184 | 114.5—118.5            | 54.3    | 1.9            |

* Phenotypic Variance Explained

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**Markers** 

- **Tag_100031118** and **Tag_3027184** (Additional Table 4) at a confidence interval of 106.5 and 123 cM (Table 6). The highest LOD and explained variance were registered for the Tun6 isolate at the F9 generation with 32.8 and 54.3%, respectively (Table 6). Comparing the physical positions of the flanking markers linked to the Qstb2B_1 and Qstb2B_2 QTL indicates that these two QTL are 5 Mb apart (Additional Table 4).

Two additional QTL with lower LODs and explained variances were mapped on chromosomes 1A and 7As designated as Qstb1A and Qstb7A, respectively and showed specificity for Tun1, Tun6 and IPO91009 isolates for Qstb1A, and for IIB-123 isolate for Qstb7A (Table 6, Fig. 3). These QTL were uniquely detected at the seedling plant stage. Qstb1A was flanked by the two SNP markers Tag_1694925 and Tag_1127081 (62.5 cM—63 cM), and explained up to 16.0% of the phenotypic variance with a maximum LOD of 4.6 for Tun1 isolate (Table 6, Additional Table 4). Qstb7A, mapped on chromosome 7A at 53 cM, was solely detected with IIB-123 isolate at the seedling plant stage for necrosis scores (Table 6). This QTL is flanked by the SNP markers Tag_2277193 and...
Tag_100009953 (Additional Table 4), with a LOD of 4.0 and explained 9% of the phenotypic variance (Table 6).

Overall, Qstb2B_1 and Qstb2B_2 had the widest efficacy compared to Qstb1A and Qstb7A (Fig. 3, Table 6). We also noticed that only Qstb1A and Qstb2B_1 QTL were effective to both necrosis and pycnidia, whereas Qstb7A was only effective to necrosis (Table 6). All four QTL detected for resistance to Z. tritici in the ‘Agili39’/Khiar population were derived from the ‘Agili39’ parent (Table 6).

Identification of epistatic QTL
The QTL interaction analysis has disclosed an epistatic QTL mapped on chromosome 5B, designated as Qstb5B.
more studies are required for an effective breeding strategy in durum wheat for STB resistance. This observation could be explained by the minor effect of the Qstb5B QTL in controlling pycnidia development, compared to the major effect of the Qstb2B_1 and Qstb2B_2 QTL in reducing pycnidia development.

Discussion

Zymoseptoria tritici is a major threat to European and Mediterranean bread and durum wheat production [44]. Despite the increasing efforts to elucidate the genetic basis of tetraploid wheat resistance to STB [8, 40, 45], more studies are required for an effective breeding strategy in durum wheat for STB resistance.

In our study, all data indicated and confirmed significant 'isolate' and 'line x isolate' interactions as determined in earlier studies [37, 38, 46, 47]; and recently proven in the bread wheat – Z. tritici pathogens where both Stb6 and AvrStb6 genes were cloned [48–50]. Moreover, and comparably to other cereal diseases, namely to rust [51–54], we determined QTL that are detected for either seedling (Qstb1A and Qstb7A) or adult plant stage (Qstb2B_2), as well as a QTL that was detected at both stages (Qstb2B_1). Our findings confirm that specific plant physiological stage resistances are commonly observed in the Z. tritici – wheat pathosystem [34]. Specific plant physiological stage resistances were also confirmed for other fungal diseases such as the powdery mildew and the leaf rust diseases [55, 56]. In fact, some Z. tritici resistance genes are uniquely effective at the seedling stage, such as the Stb7 gene mapped in the spring wheat cultivar ST6 [57], or at the adult plant stage, such as the Stb17 gene [58, 59]. In contrast, other resistance genes have proven to be effective at both seedling and adult plant stages alike the Stb4 and Stb5 qualitative genes [60, 61].

Subsequently, we compared the identified QTL to formerly identified Z. tritici genes using the reported literature. This comparison has revealed that a putative QTL for resistance to Z. tritici was mapped on chromosome 1A at 68 cM at the adult plant stage by Kidane et al. [8] through a genome-wide association study conducted on an Ethiopian durum wheat landrace population. Two other QTL mapped on chromosome 1A were also revealed by Goudeemand et al. [62] in the bread wheat Apache/Balance population, and by Risser et al. [63] named as QStb1a_1a_fb-1A in the bread wheat bi-parental mapping population Floret/Biscay. These QTL were mapped at the adult plant stage between 56 and 69 cM and could thus co-localize with the QTL mapped in the ‘Agili39’/Khiar population. However, and in contrast to the above-mentioned studies, the Qstb1A QTL mapped in the ‘Agili39’/Khiar population was solely detected at the seedling plant stage.

The Qstb7A QTL particularly conferred reduced necrosis values to Z. tritici isolate IIB123 and co-localizes with the major Stb3 gene that was mapped in the bread wheat cultivar Israel 493 [64, 65]. Two QTL for STB resistance were mapped on chromosome 2B in the ‘Agili39’/Khiar population. Other studies have also revealed genomic regions on chromosome 2B associated with the STB resistance [8, 62, 66–69].

The Qstb2B_1 QTL identified in the ‘Agili39’/Khiar population likely co-localized with the known major gene Stb9 that was mapped in the French bread wheat
Table 7  Epistatic analysis between quantitative traits loci in the Agili39/Khiar bi-parental mapping population using the inclusive composite interval mapping of digenic epistatic QTL (ICIM-EPI) method in QTL the IciMapping software

| Isolate-Trait-Physiological Stage | Chromosome 1 | QTL ID | Position 1 (cM) | Flanking markers (Position 1) | Chromosome 2 | QTL ID | Position 2 (cM) | Flanking markers (Position 2) | LOD c | PVE (%) f | Add 1 g | Add 2 h | AddbyAdd i |
|----------------------------------|-------------|--------|----------------|-------------------------------|-------------|--------|----------------|-------------------------------|------|----------|--------|--------|-----------|
| IPO95052-Pycnidia-Seedling       | 1A          | Qrb1A  | 60             | Tag_1123459—Tag_1694925       | 58          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 19.1 | 0.9      | 74.7   | 70.5   | 76.6      |
| IIB 123-Pycnidia-Seedling        | 7A          | Qrb7A  | 55             | Tag_2277193Tag_100009953       | 58          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 12.9 | 2.0      | 56.4   | 49.8   | 62.9      |
| IPO91004-Pycnidia-Seedling       | 5B          | Qrb7A  | 75             | Tag_4411932—Tag_4005129        | 65          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 9.0  | 1.9      | -0.1   | 0.0    | 0.8       |
| IPO95052-Pycnidia-Seedling       | 1B          | Qrb7A  | 80             | Tag_4411932—Tag_4005129        | 55          | Qrb5B  | 50             | Tag_1125523—Tag_4909926       | 19.5 | 1.1      | 59.4   | -59.0  | -56.8     |
| Tun6-Necrosis-Seedling           | 2B          | Qrb2B1 | 75             | Tag_1056626—Tag_1117572        | 58          | Qrb5B  | 60             | Tag_1125523—Tag_4909926       | 19.8 | 0.9      | 74.3   | 73.1   | 75.0      |
| Tun6-Pycnidia-Seedling           | 7B          | Qrb2B1 | 75             | Tag_1056626—Tag_1117572        | 55          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 15.8 | 10.6     | 104.2  | -83.8  | -69.0     |
| IPO91004-Pycnidia-Seedling       | 1B          | Qrb2B1 | 70             | Tag_100012058—Tag_1056626      | 45          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 17.1 | 2.9      | 66.8   | 58.4   | 60.2      |
| Tun6-Pycnidia-Adult (F5:6)       | 5B          | Qrb2B2 | 115            | Tag_100031118—Tag_3027184      | 55          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 6.8  | 5.4      | 1.9    | -0.4   | -0.4      |
| IPO91009-Pycnidia-Seedling       | 1B          | Qrb2B2 | 115            | Tag_100031118—Tag_3027184      | 55          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 15.2 | 1.8      | -26.8  | 54.4   | 57.3      |
| IPO95052-Pycnidia-Seedling       | 1B          | Qrb2B2 | 120            | Tag_100031118—Tag_3027184      | 55          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 23.0 | 1.1      | 56.5   | -57.7  | -60.0     |
| IPO95052-Pycnidia-Seedling       | 1B          | Qrb2B2 | 100            | Tag_1077600—Tag_100008169      | 55          | Qrb5B  | 55             | Tag_1125523—Tag_4909926       | 29.1 | 1.0      | 49.6   | 78.7   | 79.7      |

a Chromosome at the first scanning position
b Scanning position in cM of the first flanking marker pair
c Chromosome at the second scanning position
d Scanning position in cM of the second flanking marker pair
e LOD score caused by epistatic effects
f Phenotypic variation explained by epistatic effects
g Estimated additive effect of position 1
h Estimated additive effect of position 2
i Additive by additive epistatic effect at the two scanning positions. A positive value indicates that the effect of the parents’ effect is larger than the recombinant effect, and a negative value means that the recombinant effect is larger than the parents’ effect.
cv. Courtot [66]; with the qSTB.2 QTL mapped in the Ethiopian durum wheat landrace population [8]; with the QStb.ihar-2B.2 QTL mapped in the Liwilla/Begra bread wheat doubled-haploid population [68]; and with the QStb.lfl-2B.1 mapped in the eight-founder MAGIC population of winter wheat [69]. However, the Qstb2B_1 QTL is different from the QTL mapped at the long arm of chromosome 2B in the Nimbus/Stigg bread wheat mapping population [67].

The Qstb2B_2 QTL likely co-localized with the QTL identified on chromosome 2B in the mapping populations Apache/Balance and FD3/Robigus [62] associated with both necrosis and pycnidia resistance in the adult plant stage. However, due to the unavailability of marker sequences, we cannot conclude that Qstb2B_2 derived from ‘Agili39’ is the same locus that was mapped in the aforementioned bread wheat mapping populations [67].

Thus far, in durum wheat, only partial resistance to Z. tritici was reported [16, 71]. Here, we derived Qstb2B_1 from ‘Agili39’ that provides resistance to five Z. tritici isolates at the seedling stage, and to two isolates at the adult plant stage. Qstb2B_1 explains up to 61.6% of the observed phenotypic variance and was characterized by a high heritability (0.98) with a dual action at the seedling and the adult plant stages. ‘Agili39’ is also the origin of Qstb2B_2, QTL providing a major adult resistance explaining up to 54.3% of the observed phenotypic variance. Our findings confirm that Tunisian durum landraces harbor highly effective Z. tritici resistance QTL.

In fact, the initial screening of the Tunisian landrace accessions showed a remarkable genetic diversity for STB resistance, as claimed also by Ouaja et al. [12] proving that Tunisian durum wheat landraces encompass diverse and valuable sources of resistance to Z. tritici. Eight landrace accessions (Agili37; Agili38; Agili39, Sbei99; Dersbessi 12, Mahmoudi101, JK85 and Azizi27) were highly resistant and one landrace showed an intermediate response (‘Agili41’). The different ‘Agili’ landrace accessions reacted differently to the deployed Z. tritici isolates, suggesting a different genetic background, which is in accord with Ferjaoui et al. [72] study hypothesizing that the tested ‘Agili’ accessions most likely carry different Stb genes.
genes combinations. Hence, and alike other durum wheat landrace populations [8], the Tunisian durum wheat landraces uncover untapped allelic diversity that is of a great value to support effective breeding strategies to enhance STB resistance in durum wheat.

Our data demonstrated that the broad efficacy of the observed STB resistance in ‘Agili39’ is due to several stacked QTL, both at seedling as well as adult plant stages, which was also commonly observed in inheritance studies in bread wheat [34, 58]. Pyramiding genes for disease resistance has been an effective strategy in preventing boom-and-bust cycles, and is now amenable through marker assisted breeding as a strategy to maintain disease resistance durability, such as for wheat stem rust where various resistance gene combinations have well controlled the disease since the mid-1950s and more recently to the devastating Ug99 race [56, 73–75]. A concrete illustration for Z. tritici is the effective resistance to a wide range of isolates in the bread wheat germplasm ‘KK4500’ and ‘TE11’ which is conferred by stacking several known Stb genes [76–78] and also in other germplasm several QTL have contributed to broad efficacy of resistance [59]. Our data also confirm that stacking QTL in durum wheat results in broad efficacy of STB resistance. This study has identified genotypes harboring diverse resistance loci entailing dual actions at the different physiological stages constituting thus potential effective sources for Z. tritici resistance and will thus support sustainable breeding approach for Z. tritici resistance in durum wheat.

Finally, we explored QTL epistasis and identified four significant pairwise interactions of the identified QTL with an epistatic QTL mapped on chromosome 5B, designated as Qstb5B. Hence, the epistasis analysis has revealed other QTL that affects the expression of Z. tritici resistance in the ‘Agili39’/Khiar population. In fact, epistatic interactions between QTL are an important factor that affects the phenotypic expression of genes and genetic variation in populations [79–81]. Similarly, to many other studies [82–84], our data demonstrated interaction between QTL having main effect (Qstb2B_1 and Qstb2B_2) that are involved in epistasis with the Qstb5B QTL affecting the same trait. The epistasis analysis showed an additive-by-additive effect between the Qstb2B_1/Qstb5B QTL and the Qstb2B_2/Qstb5B QTL, with a major effect of QTL mapped on chromosome 2B (Qstb2B_1 and Qstb2B_2) over the Qstb5B QTL. Interestingly, the epistasis analysis showed that the Qstb2B_2 QTL, proven to control pycnidia development at the adult plant stage by QTL analysis, has also an effect in controlling pycnidia development at the seedling stage when interacting with the epistatic Qstb5B QTL. Nonetheless, the epistasis analysis did not indicate an interaction between the two major QTL Qstb2B_1 and Qstb2B_2 mapped on chromosome 2B.

Conclusion
Our study deciphered ancient broad-based resistance to Z. tritici in a durum wheat landrace accession. A positive selection for the QTL linked markers may result in new high yielding durum wheat cultivars with wide resistance to Z. tritici reminiscent of the durable resistance to STB in landraces. Given the overall high susceptibility to STB in modern durum wheat cultivars, our data shed new light on disease resistance breeding in durum wheat.

Methods
Plant materials and study layout
Eleven durum wheat accesses (Table 8) and a bi-parental recombinant inbred (RIL) population derived from a single seed descent cross between the Tunisian landrace accession ‘Agili39’ and the high yielding commercial cv. Khiar, were screened for resistance to septoria tritici blotch. The RIL population was generated by crossing the resistant parent ‘Agili39’ to the susceptible Khiar following a single seed descent approach (SSD). The F1 plants were selfed to generate the F2 seeds. One head row of each F2 plants were then randomly selected and sown in one row to produce the F2 derived F3 plants (F3). This procedure was followed for all subsequent generations up to the F10 plants. Thirteen recombinant population (Agili39'/ Khiar) is maintained and available upon request at the National Institute of Agronomy -Tunisia (INAT) at and the CIMMYT gene banks.

For STB resistance screening, we performed three experiments (Table 2). The first experiment was repeated three times and comprised the screening of the 11 Tunisian landraces at the seedling stage (Z13.3/21) with a panel of 20 Z. tritici isolates (Table 2). The first experiment was performed to understand overall resistance patterns to STB and to select potential isolates for further screening of the RILs derived from the ‘Agili39’/Khiar cross, which consisted of the second experiment (experiment 2), performed thrice at the seedling stage. Finally, in the third experiment we tested the ‘Agili39’/Khiar population under field conditions in Oued-Bejá, located in North-Western Tunisia, over a period of five years, 2011–2014 and 2016 with three different Z. tritici isolates.

Screening landraces and RILs population at the seedling stage for resistance to Zymoseptoria tritici
Experimental design, Plants management and growth conditions
A first experiment that consisted of the pre-screening of 11 Tunisian landrace accessions with 20 Z. tritici isolates...
isolates was conducted at the seedling stage. The pre-screening assay followed a complete block design in three replicates and included the susceptible parent cv. Khiar, the susceptible cv. Karim and the resistant parent ‘Agili39’ as checks (Table 2 and 8). This experiment enabled the selection of eight Z. tritici isolates that discriminated between the ‘Agili39’ and the cv. Khiar parents and were subsequently used to screen the ‘Agili39’/Khiar derived recombinant inbred lines (RILs) at the seedling stage.

For the seedling assay of the RIL population (experiment 2), we followed a split plot design with isolates as whole plots. Each whole plot consists of three neighbouring trays of fifty-four pots. The tested isolates were randomly allocated to the whole plots. Individual pots were the experimental units, and they were randomly arranged for each isolate/replicate combination on separate parallel greenhouse tables. ‘Replicate’, ‘isolate’, ‘line’ and the ‘isolate x line’ interaction were the fixed terms of our split plot model. However, the random term of the model consists of the ‘replicate x whole plot’ interaction. In all three replicates, eleven checks several checks were included with both parents ‘Agili39’ and cv. Khiar (Table 8).

Five seeds per pot per accessions were grown in VQB 7 × 7×8 cm plastic pots (TEKU®, Lohne, Germany), whereas 157 F6 RILs of the ‘Agili39’/Khiar mapping population were planted in round peat pots (Jiffy, Moerdijk, Netherlands), also five seeds per pot, using a special mixture for growing seeds (Substraat Zaaia) provided by the greenhouse facility Unifarm of Wageningen University and Research (WUR), The Netherlands.

### Table 8 Nine Tunisian durum wheat landraces and two cultivars that were investigated for resistance to Zymoseptoria tritici

| Name       | Habitus | Source   | Empiric evaluation of septoria tritici blotch under field conditions |
|------------|---------|----------|---------------------------------------------------------------------|
| Agili 37   | landrace| INATb    | Resistant                                                           |
| Agili 38   | landrace| INAT     | Resistant                                                           |
| Agili 39 (P) | landrace| INAT     | Resistant                                                           |
| Agili 41   | landrace| INAT     | Resistant                                                           |
| Azziz 27   | landrace| INAT     | Resistant                                                           |
| Derbessi 12| landrace| INAT     | Resistant                                                           |
| Jneh Khotifa 85 | landrace| INAT     | Resistant                                                           |
| Mahmoudi 101 | landrace| INAT     | Resistant                                                           |
| Sbei 99    | landrace| INAT     | Resistant                                                           |
| Khiar (P)  | cultivar| INRATc   | Susceptible                                                         |
| Karim      | cultivar| INRAT    | Susceptible                                                         |

* Parents of the recombinant inbred population
b National Institute of Agronomy-Tunis, Tunisia
c National Institute of Agronomical Research-Tunis

**Zymoseptoria tritici isolates and inoculation procedures**

Pre-cultures of each isolate were prepared in an autoclaved 100 ml Erlenmeyer flask containing 50 ml yeast glucose (YG) liquid medium (30 g glucose, 10 g yeast per litre distilled water). The flasks were inoculated with frozen isolate samples that were selected from different durum wheat growing countries and maintained at -80 °C in a Z. tritici isolate collection at WUR. The inoculated flasks were subsequently placed in an incubated rotary shaker (Innova 4430, New Brunswick Scientific, USA) set at 125 rpm and 15 °C for 5–7 days. These pre-cultures were then used to inoculate two 1 l Erlenmeyer flasks containing 500 ml YG media per isolate that were incubated under the aforementioned conditions to provide sufficient inoculum for the seedling inoculation assays at growth stage (GS) 11 [85]. Spores were subsequently collected after overnight settling in static cultures, concentrated by decanting the supernatant medium, adjusted to 10⁷ spores ml⁻¹ in a total volume of 40 ml for a set of 18 plastic pots or 24 Jiffy® pots and supplemented with two drops (µl/ml) of Tween 20 surfactant (MERCK®, Nottingham, UK).

Prior to the inoculation, plant development was allowed for 10 days in a greenhouse adjusted at a temperature of 18/16 °C (day/night rhythm) and relative humidity (RH) of 70%. Inoculations were conducted by spraying the inoculum over the seedlings that were placed in an inoculation cabinet on a rotary table, adjusted at 15 rpm, which is equipped with interchangeable atomizers and a water cleaning device to avoid cross-contamination. Infected plants were incubated in transparent plastic bags for 48 h under 100% RH. Post-inoculation conditions were set at a temperature of 22 ± 2 °C and RH of ≥ 95%. Light intensity (son-T Agro 400 W lamps) and day length (16/8 h light/dark) were similar during pre- and post-inoculation conditions. Ten days after inoculation, seedlings were trimmed for the second and subsequent leaves to enable sufficient light on the inoculated primary leaves for appropriate disease development. Fertilizer (Sporumix PG®, Rotterdam, Netherlands; 0.5 g.l⁻¹) was applied to support plant growth.

**Disease severity scoring in the seedling assay**

In the seedling assays, disease severities were evaluated at 15, 18 and 21 days post-inoculation (dpi). These multiple observations enabled Area Under the Disease Progress Curve (AUDPC) calculations for quantitative analyses of temporal differences in disease progress. We estimated the quantitative presence of necrosis and pycnidia on the inoculated seedling leaves in percentages [36, 37, 86]. AUDPC calculations for seedling scores followed the trapezoidal method, which approximates the
time variable and calculates the average disease intensity between each pair of adjacent time points [87].

**Screening RILs population at the adult plant stage for resistance to Zymoseptoria tritici**

**Experimental design and inoculation procedures**

Adult plant assays of the 'Agili39'/Khiar population were conducted at Oued-Bejá, located in North-Western Tunisia (36° 46′ 27.516″ N, 10° 3′ 36.432″ E), for five years, 2011 – 2014 and 2016, with Tun1 – Tun6 and IIB-123 Z. tritici isolates, respectively. This region belongs to the sub-humid bioclimatic zone of Tunisia with an average rainfall ranging from 500 to 850 mm and a daily mean temperature between 10–28 °C [72].

For the field trials, we adopted an augmented randomized complete block design. Five blocks with 1.5 m width and spaced 1.5 m were linearly drilled with 30 to 35 RILs, parents and four modern durum cultivars per block. Each line was sown as one spike per row of 1.5 m length and spaced 25 cm. We randomized all RILs, the parents and four additional checks modern durum wheat cvs. Karim, Nasr, Maali and Salim, important in Tunisian breeding programs and showing different levels of susceptibility in each block. For the 2016 field trial, we used a complete random block design with three replicates with both parents 'Agili39' and 'Khiar' as checks.

Field inoculations were conducted with three isolates (Tun1, Tun6 and IIB123) across the F5:6-F9:10 RILs generations. We used Z. tritici isolate Tun6 for three years to screen the F5:6 (N = 164), the F7:8 (N = 158) and the F8:9 (N = 157) RILs in 2011, 2013 and 2014, respectively and isolate Tun1 to screen the F6:7 (N = 158) in 2012 and the F8:9 (N = 157) in 2014. In 2016, we screened the F9:10 (N = 155) with Z. tritici isolate IIB123. In all field trials and across all generations, plants were inoculated twice. The first inoculation occurred at the tillering stage (GS 21–26) in order to initiate the disease infection, and the second inoculation was applied after 25–30 days when all RILs reached approximately the elongation stage (GS37) [85]. The second inoculation was applied to ensure an increase in the disease pressure. Field inoculations were conducted with a spore suspension adjusted to 10⁶ spores/ml of the corresponding Z. tritici isolate using a CO2-pressurized knapsack sprayer with a 1 m hand-held boom till run-off.

**Disease severity scoring in the field tests**

For the field evaluations, we scored pycnidal classes at 28 days post the second inoculation (GS 51) [85] that were later transformed to pycnidia percentages (0 = no pycnidia, 1 = 12%, 2 = 25%, 3 = 50%, 4 = 75% and 5 = 87%) [60, 88].

**Genotyping and construction of linkage map**

Genomic DNA was extracted from fresh leaves using a modified CTAB (cetyltrimethylammonium bromide) method and quantified using NanoDrop 8000 spectrophotometer V 2.1.0. Whole-genome profiling was performed using DA'T-Seq™ technology by Diversity Arrays Technology Pty Ltd, Australia, as described by Kilian et al. [89]. In brief, the DA'T-Seq™ technology was optimized by selecting the most appropriate complexity reduction method for wheat (PstI-Msel restriction enzymes). DNA fragments digested with restriction enzymes were ligated with PstI adaptors and unique barcodes, and then amplified following PCR. Amplicons were pooled and sequenced in a 96-multiplex on a HiSeq2000 (Illumina, USA) resulting in a total of 5,891 raw DA'T-Seq SNP markers.

The SNP markers were first filtered according to their polymorphism between the parents ‘Agili39’ and ‘Khiar’. A total of 3,459 filtered SNPs was subsequently tested for the segregation distortion that was determined by the calculation of the Chi-Square (X²). Hence, SNPs with a major distortion of p < 0.001 were removed. Moreover, identical SNPs, which should be mapped to the same position on the linkage group, were identified (loci similarity = 1). Hence, only one marker of ‘similar loci’ was retained on the linkage map to reduce the calculation burden. The final set of filtered SNPs was then used to generate a genetic linkage map using JoinMap® 4 software [90]. The regression mapping algorithm was used to construct the genetic map. Linkage between markers, recombination rate (Θ), and map distances was calculated using the Kosambi mapping function in JoinMap [91]. Markers were grouped using a minimum independence LOD (logarithm of the odds) score of 10.0 and linkage groups were established at a minimum LOD score of 3.0. Markers were linearly aligned in each linkage group, converting the recombination rates into the Kosambi’s map distance in centimorgans. A sequential map builds up strategy was followed to determine the best marker position [92]. The best fitting position of markers was examined based on the goodness-of-fit test (chi-square) for the resulting map. The final map included bin markers that excluded similar SNP markers.

Linkage groups were subsequently assigned to chromosomes by aligning the SNP marker sequences of the linkage groups to the reference genome of the *Triticum turgidum subsp. durum Svevo.v1* and using the BLASTn function in the publicly available sequence database ‘Ensemble Plants’ (https://plants.ensembl.org/Triticum_turgidum/Tools/Blast?db=core) [93].
Statistical analyses

Seedling data statistical analyses

Pycnidia AUDPC scores (P-AUDPC) of the pre-screening experiment (experiment 1) were analysed for their variance using the `aov()` function in the R environment [42] to test for the effect of ‘line’, the effect of ‘isolate’ and the effect of any ‘line x isolate’ interactions. The following linear model was fitted to the observed P-AUDPC scores:

\[
Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}
\]

where \(Y_{ijk}\) is the P-AUDPC score in the \(Kth\) replicate with isolate \(i\) and line \(j\). \(\mu\) is the overall mean. \(\alpha\) is the isolate \(i\) main effect. \(\beta\) is the line \(j\) main effect. \((\alpha\beta)ij\) is the interaction effect between isolate \(i\) and line \(j\) and \(\epsilon\) is the unexplained error.

For the split-plot seedling experiment performed on the RIL population (experiment 2), an analysis of variance for the necrosis and the pycnidia AUDPC scores (N-AUDPC and P-AUDPC) was performed using the `sp.plot` function available in the `Agricolae` package in the R environment [42, 94]. Isolates were the whole-plot factors and RILs were the sub-plot factors. We fitted the observed N-AUDPC and P-AUDPC scores to the following linear model:

\[
Y_{ijk} = \mu + \alpha_i + \eta_k(i) + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk},
\]

where \(Y_{ijk}\) is the N-AUDPC or P-AUDPC score in the \(Kth\) replicate of a plot with isolate \(i\) and RIL \(j\). \(\mu\) is the overall mean. \(\alpha\) is the fixed effect of isolate \(i\). \(\eta\) is the whole-plot error. \(\beta\) is the fixed effect of RIL \(j\). \((\alpha\beta)ij\) is the interaction effect between isolate \(i\) and RIL \(j\) and \(\epsilon\) is the sub-plot error.

Significant differences between isolates were determined using the least significant difference (LSD) of N-AUDPC and P-AUDPC scores and using the `Agricolae` package in the R environment. ‘RIL x isolate’ grouping of necrosis and pycnidia AUDPC scores was defined based on the Bonferroni test at \(p<0.05\). Homogeneity of the seedling replicates was checked and homogeneous data across replications were subsequently averaged and used for the seedling QTL analysis [95].

Repeatability was estimated for the necrosis and pycnidia AUDPC generated at the seedling stage as follow:

\[
\text{Repeatability} = \frac{\sigma^2G}{\sigma^2G + \frac{\sigma^2GE}{r} + \frac{\sigma^2\epsilon}{r}}
\]

where \(\sigma^2G\) is the variance component due to genotypes, \(\sigma^2GE\) is the variance component due to the interaction between the genotype and the isolate, \(E\) is the number of isolates which is eight in the seedling experiment, \(\sigma^2\epsilon\) is the variance component due to the unexplained error and \(r\) is the number of replicates which is three per isolate for all seedling assays.

Field data statistical analyses

Field pycnidia scores generated by the inoculation of adult plants with Tun1 and Tun6 Z. tritici isolates were tested for their variance to check for the effect of ‘genotype’, ‘block’, ‘year’, ‘isolate’ and for any two-way and three-way interactions between the independent variable ‘genotype’ and the independent factors ‘block’, ‘year’ and ‘isolate’. A linear model was fitted to the observed pycnidia at the adult plant stage as follow:

\[
Y_{ijkt} = \mu + \alpha_i + \beta_j + \gamma_k + \eta_t + (\alpha\beta)_{ij} + (\gamma\beta)_{kj} + (\eta\beta)_{tj} + (\alpha\eta\beta)_{ijt} + \epsilon_{ijkt}
\]

where \(Y_{ijkt}\) is the pycnidia coverage score of RIL \(j\) inoculated with isolate \(i\) in the \(Kth\) block at year \(t\). \(\mu\) is the overall mean. \(\alpha\) is the main effect of the isolate \(i\). \(\beta\) is the main effect of the RIL \(j\). \(\gamma\) is the main effect of the block \(k\). \(\eta\) is the main effect of the year \(t\). \((\alpha\beta)ij\), \((\gamma\beta)kj\), \((\eta\beta)tj\) are the two-way interaction effects of the RIL \(j\) with the isolate \(i\), the RIL \(j\) with the block \(k\) and the RIL \(j\) with the year \(t\), respectively. \((\alpha\eta\beta)ijt\) is the three-way interaction effect of RIL \(j\) with isolate \(i\) and with year \(t\) and finally, \(\epsilon\) is the residual.

For the Tun1 and the Tun6 isolates tested on multiple consecutive years, a broad sense heritability (\(H^2\)) was estimated as follow:

\[
H^2 = \frac{\sigma^2G}{\sigma^2G + \frac{\sigma^2GE}{r} + \frac{\sigma^2\epsilon}{r}}
\]

where \(\sigma^2G\) is the variance component due to genotypes. \(\sigma^2GE\) is the variance component due to the interaction between the genotype and the isolate. \(E\) is the number of years which is 2 and 3 for Tun1 and Tun6 isolates, respectively. \(\sigma^2\epsilon\) is the variance component due to the unexplained error and \(r\) is the number of blocks which is five for all adult assays.

The least-square means (Lsmeans) of pycnidia coverages were derived from the individual year trials using the SAS software [96]. Transformed field data were subsequently considered for the field QTL analysis.

Correlation between seedling and adult plant assays and frequency distribution

A heatmap correlation matrix was calculated for the generated phenotypes at the seedling and the adult plant stages using the “Corr” function and visualized using the “corrplot” package in the R environment [43]. The “Corr” function was set to calculate the Pearson correlation. Positive and negative correlations were displayed in the heatmap matrix by a color gradient from red, indicating a positive correlation, to blue, indicating a negative
correlation between the phenotypic traits. Frequency distribution figures were generated using the “hist” function in the R environment.

**QTL analysis procedure**

An inclusive composite interval mapping of additive (ICIM-ADD) functionality in QTL IciMapping v4.1 [97] was used. Additive QTL were detected using a walk speed of 1.0 cM and the probability used in stepwise regression for additive QTLs was 0.001. The logarithm of odds (LOD) value of 3.0 was chosen to declare significant QTL, and the LOD value was calculated from 1000 permutations with type I error of 0.01. The phenotypic variance explained (PVE) and additive effect of individual QTL at the LOD peaks were also obtained. Identified QTL were plotted against their corresponding linkage groups using the MapChart © software version 2.3 [98]. Subsequently and for the identified QTL contributing to resistance, we aligned the linked SNP markers to the reference genome of the *Triticum turgidum* subsp. durum Svevo.v1 and using the BLASTn function (https://plantsensembl.org/Triticum_turgidum/Tools/Blast?db=core) in the publicly available sequence database ‘Ensemble Plants’ [93].

Epistatic interactions between QTL were identified by the inclusive composite interval mapping of digenic epistatic QTL (ICIM-EPI) method implemented in QTL IciMapping software v4.1 [97]. The LOD threshold was set at 5.00 to declare significant epistatic QTL and LOD value was calculated from 1000 permutations at the significance level of 0.05.

The highest epistasis interactions detected between the QTL (highest LOD) were subsequently selected for a two-way interaction test to examine pycnidia AUDPC means of the variant allele combinations linked to the epistatic QTL. Alleles linked to the detected QTL were named by ‘R’ and ‘S’ denoting the resistant and susceptible alleles, respectively. Hence, pycnidia AUDPC means of the four allele combinations ‘RR’, ‘RS’, ‘SR’ and ‘SS’ were examined and Boxplots were generated using the `ggplot2` package in the R environment [99].

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08560-2.

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**Authors’ contributions**

GHJK and SH designed the study. SF produced the ‘Agili39’/Khiar RIL population, provided the plant materials, and generated the field phenotypic data. LA generated the seedling phenotypic data. KA and SD genotyped the ‘Agili39’/Khiar mapping population. HJS, LA and RBS generated the linkage genetic map. LA performed the statistical analysis. BB and SS performed the QTL and the epistasis analyses. LA, SH and SF wrote the manuscript. All authors read and reviewed the manuscript.

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**Availability of data and materials**

The used plant materials are available at the CIMMYT gene bank upon a reasonable request to the co-author Karim Ammar (<k.ammar@cgiar.org>). The sequencing raw data files generated during this study are uploaded to the CIMMYT data repository (https://hdl.handle.net/11529/10548618). The analyzed data are available as additional files to this article.

**Declarations**

**Ethics approval and consent to participate**

The used plant material comprises either registered *Triticum durum* cultivars and landrace accessions or experimental lines developed by the corresponding author Professor Sonia Hamza at the National Institute of Agronomy (INAT) and by Dr. Sahbi Ferjaoui at the Regional Field Crops Research Center of Beja (CRRGC). Experimental research and field studies, including the collection of plant material complies with relevant institutional, national and international guidelines and legislation.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Page 19 of 20
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