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RESEARCH ARTICLE

Measuring resistance in durum wheat-*Zymoseptoria tritici* interaction using aggressiveness quantitative traits

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ABSTRACT

Phenotyping for quantitative resistance to Septoria tritici blotch (STB), one of the most economically devastating diseases of durum wheat (*Triticum durum* Desf.) in North African region, is a limiting factor for breeding progress. Here we tested main phenotypic markers of aggressiveness affecting host damage and pathogen reproduction reported in earlier studies for measuring differences in quantitative host resistance. A collection of 11 Tunisian durum wheat genotypes varying in resistance to the pathogen was inoculated by a highly aggressive and virulent isolate of *Zymoseptoria tritici* in a replicated field and controlled experiments, conducted in 2017-2018 at the experimental station of the Regional Field Crops Research Center of Beja in Tunisia. The severity of infection caused by *Z. tritici* isolate, including percentage of leaf area covered by chlorotic lesions (PLACCL), percentage of leaf area covered by necrosis (PLACN), and percentage of leaf area covered by pycnidia (PLACP) as well as latent period (LP) and pycnidia density (PYC_{dens}) were assessed visually and quantified. The results showed that there were significant differences in all assessed traits on the tested genotypes and significant positive correlations were obtained between PLACN, PLACP and PYC_{dens}. Similarly, there was a significant negative correlation between LP and these last traits. The PLACP was found as the more reliable trait to discriminate genotypes to different classes of resistance, consistent with the results obtained for the remaining traits. These data suggest that using both necrotic leaf area (PLACN) bearing pycnidia and PYC_{dens} can improve selection for quantitative STB resistance.

Key words: Aggressiveness, durum wheat, quantitative resistance, selection, *Triticum durum*, *Zymoseptoria tritici*.

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is one of the most important cereal crops in Tunisia. Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* (formerly known as *Mycosphaerella graminicola*, anamorph *Septoria tritici*; Quaedvlieg et al., 2011) is the most recurrent and important wheat disease in North African region (especially in Tunisia) where STB is often more severe on durum wheat than in other regions. The STB can cause yield losses that typically range 10%-25%, but under conducive weather conditions, losses can easily exceed 50% particularly in humid zones where disease management is frequently suboptimal. Plant breeders and phytopathologists in Tunisia have shown an increasing interest in breeding for resistance to STB (Ferjaoui et al., 2015; Ben M'Barek et al., 2020).

Inheritance studies have suggested that STB resistance is controlled either qualitatively or quantitatively; however, much of attention has been focused on qualitative *Z. tritici*-wheat interactions, i.e., isolate-specific resistance that follows a gene-for-gene relationship (Brading et al., 2002). Specific resistance is manifested by near-immune response to certain isolates and is usually conferred by a single gene. At present, 21 major *Stb* genes conferring qualitative resistance to septoria have been identified and mapped mainly in bread wheat (Brown et al., 2015). Each of these genes is effective against much more than only one or a few known isolates of *Z. tritici*. The fairly high frequency of sexual reproduction in populations of *M. graminicola* (Boukef et al.,

2012) increases the risk of adaptation of the pathogen population to resistance genes deployed in the host population, and the loss of the effectiveness of resistance in new resistant cultivars (Goodwin, 2007). The breakdown of STB resistances has been reported in many wheat growing areas throughout the world (Krenz et al., 2008; Kildea et al., 2021). Partial or quantitative resistance to *Z. tritici* is incomplete, controlled by genes with small-to-moderate effects and assumed to provide more durable than specific resistance due to its reduced intensity of selective pressure exerted on the pathogen population. These genes have generally weaker specificity than qualitative genes and effective against all genotypes of a pathogen (Brown et al., 2015; Arraiano and Brown, 2017). Therefore, this resistance seems more durable and appears to dominate in this pathosystem, but also complex to work and may be harder to select. Indeed, the description of quantitative aspects of wheat-*Z. tritici* variations is still incomplete and largely understudied despite being the subject of several discussion over the last two decades (Zhan et al., 2016).

Zymoseptoria tritici is considered a hemibiotrophic fungus, as it undergoes two distinct phases of plant colonization. The first infection stage consists of a latent phase, frequently referred to as biotrophic, asymptomatically lasting at least 1 wk, which is subsequently followed by a necrotrophic phase that begins with the appearance of chlorotic lesions 14-21 d after infection associated with the formation of leaf lesions that eventually coalesce into necrotic blotches bearing pycnidia (Steinberg, 2015).

Aggressiveness is defined as quantitative variation of pathogenicity induced by a pathogen on susceptible host (Lannou, 2012) and was suggested as being conditioned by minor gene-for-minor gene interactions with additive effects to host defense (Niks et al., 2015). It is an important parameter whose assessment has become relevant research and applied topic in plant pathology (Suffert et al., 2013; 2018; Bock et al., 2021). This parameter can be divided into several components, such as infection latent period, sporulation rate, lesion size, and pycnidia density (Caffier et al., 2010). These components are highly environment-dependent and can be affected by environmental conditions (Zhao et al., 2020), host genetics, and the physiological status of both pathogen and host (Lannou, 2012; Pariaud et al., 2012). Their assessment can be either in the field or in controlled conditions (greenhouse or glasshouse) but the disadvantage of the field tests is that environmental factors cannot be controlled (Suffert et al., 2013; Zhao et al., 2020). Both percentage of leaf area covered by chlorotic (PLACCL) and necrotic lesions (PLACN) and percentage of leaf area covered by pycnidia (PLACP) were the most commonly used to quantify the aggressiveness of *Z. tritici* and also to evaluate the level of wheat cultivars resistance (Morais et al., 2016; Zhan et al., 2016). The rating of such traits requires methodological approaches that correspond to the pathosystem under study (Lannou, 2012). Moreover, some aggressiveness traits are directly reflected in a single observation (e.g., disease severity), whilst others need to be estimated by aggregation of temporal data (e.g., latent period) (Suffert et al., 2013).

This study undertakes an investigation in the aggressiveness quantitative traits of an aggressive and virulent *Z. tritici* isolate on a collection of 11 durum wheat genotypes differing in their level of resistance. Based on this, we aim to develop and validate a method to assess interaction in the durum wheat-*Z. tritici* system, in order to conclude on both the "resistance" traits in wheat genotype and the "aggressiveness" traits in *Z. tritici*.

MATERIALS AND METHODS

Plant material

Nine old durum wheat (*Triticum durum* Desf.) accessions and two Tunisian improved durum wheat cultivars were used in this study (Table 1). They showed different levels of resistance to *Zymoseptoria tritici*: a highly susceptible 'Karim' (Ferjaoui et al., 2011), a highly resistant accession 'Agili39' (Ferjaoui et al., 2015), and moderately resistant 'Salim' and eight old accessions (Berraies et al., 2011; Ferjaoui et al., 2015). Currently, 'Karim' is the most cultivated improved variety in Tunisia which raised in popularity and accounted for more 60% of the area under wheat since 2000 (Berraies et al., 2014). 'Salim' is a source of partial resistance to Septoria tritici blotch (STB) in the field, contains one quantitative trait loci (QTL) for resistance, identified and located on chromosome 3B (Berraies et al., 2011). 'Agili39' is the most STB-resistant source in Tunisia. Indeed, major genetic loci for resistance have been identified in this accession (Ferjaoui et al., 2011; 2016).

Table 1. Source, origin and disease behavior of durum wheat accessions and improved genotypes. INRAT: National Institute of Agronomical Research of Tunisia; INAT: National Institute of Agronomy of Tunisia; BNG: National Genebank of Tunisia; S: susceptible; R: resistant; MR: moderately resistant.

Genotype	Source	Origin, selection or release history (Ammar et al., 2011)	Disease behavior
Karim	INRAT	Introduced from CIMMYT in 1973 (F_4), released and grown since 1980	S
Agili39	INAT	Local landrace with some types introduced from Morocco, pure line selection started in 1908, spread commercially in 1915, abandoned in 1920	R
Salim	INRAT	Recent variety, derived from Tunisian cross and released in 2009	MR
Jenah khottifa (JK14)	INAT	Local landrace from the north, pure line selection started in 1908, spread commercially in 1905, abandoned in 1930	MR
Taganrog120	INAT	Introduced from Italy and selected from Taganrog landrace	MR
Mahmoudi114	INAT	Local landrace first introduced from Algeria, present pre-1893, pure line selection started in 1908, no longer grown	MR
Souri92	INAT	Local landrace, pure line selection started in 1908, abandoned in 1930	MR
Sbei83	INAT	Local landrace, pure line selection started in 1908, abandoned in 1930	MR
15607	BNG	Local accession selected in 2008 from Mahmoudi landrace	MR
15975	BNG	Local accession selected in 2008 from Azizi landrace	MR
15974	BNG	Local accession selected in 2008 from Mahmoudi landrace	MR

Fungal isolate and inoculum preparation

One isolate of *Z. tritici* named TunBz-1 belonging to the most virulent and aggressive Tunisian pathotype P1 was used in this experiment, based on a previous population study (Ferjaoui et al., 2015). Inoculum was produced by culturing the isolate on yeast-glucose liquid medium (yeast extract 10 g L^{-1}, glucose 30 g L^{-1}), as described by Ferjaoui et al. (2015). The spore suspension was adjusted to 10^7 spore mL^{-1} and supplemented with Tween 20 (1 drop 100 mL^{-1}) for inoculation.

Field experiment

The experiments were conducted during two consecutive wheat growing seasons 2016-2017 and 2017-2018 at the experimental station of the Regional Field Crops Research Center of Beja (Oued Beja, Tunisia; 36°44′05″ N, 9°13′35″ E), located in the most important Tunisian wheat-producing area. This region is in the sub-humid bioclimatic zone with an average annual rainfall ranging from 600 to 1000 mm and a daily mean temperature ranging between 10 and 28 °C. The area is well-known as a hot spot for STB (Ferjaoui et al., 2015). The experiments were set up as a complete randomized block design with three replicates per treatment, spaced 1.5 m apart. Plots were three rows 1 m long and 20 cm spacing, with sowing density of 40 seeds per row. The spacing between plots was 0.5 m. Sowing was performed on 20 November 2016 and 27 November 2017 by hand. Fertilization and weed control were conducted according to standard experimental practices in the station.

The spray inoculation was done at growth stage (GS) 31 (Zadocks et al., 1974). 'Karim' and 'Agili39' were used as controls. Ten plants within the middle row for each plot were randomly selected and tagged to be used consistently to assess STB traits. At 28 d post inoculation (dpi) time point that corresponds to the maximum expression of the symptoms on the susceptible control, the percentage of leaf area covered by chlorotic lesions (PLACCL), percentage of leaf area covered by necrosis (PLACN), and percentage of leaf area covered by pycnidia (PLACP) of the whole inoculated leaf layer of each plant were estimated visually.

Glasshouse experiment

The glasshouse trial set up in 2017-2018 was conducted in a completely randomized design (CRD) with three replicates. In each replicate, five clean and healthy seeds of each genotype from the collection mentioned above were sown in 30 cm diameter pots containing field soil (Vertisol):sand (2:1). Three seedlings having similar maturities were selected for inoculation and treated as an experimental unit. Plants were watered, fertilized as needed and maintained in glasshouse fitted with an air conditioning unit to keep a temperature at approximately 20/12 °C day/night with natural light intensities and relative humidity (RH) around 70%. At stem elongation stage (GS 31), plants in each pot were inoculated with the same spore inoculum concentration as for the field experiments until run-off using hand-operated sprayer. After inoculation, the pots were arranged according to CRD and covered with transparent plastic sheet to provide a 100% humidity environment and kept at 18-22 °C/16-20 °C light/darkness on glasshouse benches to promote infection for 72 h before being returned to the first conditions.

Development of STB symptoms (chlorosis and necrosis) was regularly observed. The latent period (LP) was recorded for each plant as the number of days from inoculation to the appearance of the first pycnidia in the lesions. At 28 dpi, one infected leaf of each plant was excised and fixed to sheet of plain paper, adaxial side up, with labels identifying the accession name and plant number as well as replicate number to aid in later analysis. Leaves were then pressed by placing the stacks of paper under a 5 kg weight and stored at 4 °C for 2 d to facilitate the assessment of aggressiveness traits. In total, 99 leaves were collected and the PLACN and the PLACP were visually estimated. The sporulating area (SPO_{area}) on rectangular infected leaf sections of length 70 mm and various width was estimated as a percentage and then transformed into square centimeter (cm^2) on each of the 99 scored leaves. Subsequently, the pycnidial number (PYC_{num}) on each SPO_{area} was counted visually using a hand lens (magnification 10X). The pycnidial density (PYC_{dens}) was calculated as the surface density of pycnidia of the sporulating area (PYC_{num}/SPO_{area}) (Suffert et al., 2013).

Data analysis

The average scores of different aggressiveness traits over all plants in each plot were calculated. The ANOVA was performed for all data recorded from the field and glasshouse experiments, using general linear model (GLM) procedure in the SAS software (SAS Institute, Cary, North Carolina, USA). The main scores of all traits and effects of genotype, replicates, and the year, as well as Genotype × Year interaction were determined by GLM. Duncan's multiple range test (DMRT) was used to determine the significance among mean values of assessed traits at 5% level. Correlations between recorded traits in all experiments were done to evaluate their association based on Person's correlation coefficient (r).

RESULTS

***Zymoseptoria tritici* symptoms development and assessment of aggressiveness traits**

Under field conditions, the first typical symptoms of the disease appeared on average 19 dpi on 'Karim' and consisted of chlorotic spots on the leaf surface with small necrosis lesions bearing few pycnidia. Few days later, necrosis lesions enlarged with expression of abundant pycnidia. The symptoms on the remaining genotypes were observed from 23 dpi excepted the accession 'Agili39' where only small and rarely chlorotic lesions were recorded, but no further disease symptoms developed.

The ANOVA of the generated data showed that the genotype effect was highly significant ($P < 0.001$). This result indicates that the observed variation in the symptom expression mainly accounted for genotypic differences. The aggressiveness traits mean values revealed a significant difference between years (2017 and 2018). Similarly, Genotype × Year interaction was significant in analyzed traits, except for PLACCL. In fact, a disease pressure was observed in 2017 for the tested germplasm. However, a nonsignificant effect was observed between replicates (Table 2).

Therefore, tested genotypes exposed an average between 4% to 41.3% of PLACCL, from 0% to 74% of PLACN ad from 0% to 65% of PLACP (Table 3). 'Agli39' was the most resistant genotype, no typical symptoms of the disease were observed; only a few small chlorotic spots (PLACCL< 5%) without necrosis and pycnidia (Table 3; class a). On the leaves of the susceptible genotype 'Karim', the aggressiveness of the isolate measured using PLACCL values ranged from 5% to 15%, with an average around 10%-11%; whereas typical necrosis of *Z. tritici* developed displaying as large merging necrosis areas containing abundant pycnidia (average of PLACN and PLACP up to 40%; Table 3; class e). On the moderately resistant wheat genotypes, the

average PLACCL ranged from 11% to 41.3%. However, a larger necrotic area (up to 20% leaf surface) with an average of PLACP up to 10% were recorded on '15607' and '15974' (Table 3; class c) and up to 20% on 'Salim' and '15975' (Table 3; class d), while the five other genotypes ('JK14', 'Mahmoudi114', 'Taganrog120', 'Souri92' and 'Sbei83') had an average of PLACN and PLACP less than 20% and 10%, respectively (Table 3; classes b, bc).

Table 2. ANOVA for aggressiveness traits on the 11 durum wheat genotypes inoculated with the virulent isolate TunBz-1 at the adult plant stage. PLACCL: Percentage of leaf area covered by chlorotic lesions; PLACN: percentage of leaf area covered by necrosis; PLACP: percentage of leaf area covered by pycnidia; LP: latent period; PYC_{dens}: pycnidial density; DF: degree of freedom. *Significant at $P < 0.05$.

Experiment	Traits	Source of variation	DF	Mean square	F value	Pr > F
Field	PLACCL	Year	1	6.182	5.527	0.023*
		Genotype	10	620.933	555.109	< 0.001
		Genotype × Year	10	1.644	1.470	0.185
		Replicate	2	2.267	2.026	0.145
		Residual	42	1.119		
	PLACN	Year	1	13.636	43.473	< 0.001
		Genotype	10	2553.244	8139.842	< 0.001
		Genotype × Year	10	12.186	38.851	< 0.001
		Replicate	2	0.163	0.519	0.599
		Residual	42	0.314		
	PLACP	Year	1	0.970	6.054	0.018*
		Genotype	10	2001.657	12496.830	< 0.001
		Genotype × Year	10	4.678	29.206	< 0.001
		Replicate	2	0.470	2.932	0.064
		Residual	42	0.160		
Glasshouse	PLACN	Genotype	10	4718.533	526.053	< 0.001
		Replicate	2	8.859	0.988	0.377
		Residual	86	8.970		
	PLACP	Genotype	10	3735.691	1003.587	< 0.001
		Replicate	2	2.939	0.790	0.457
		Residual	86	3.722		
	LP	Genotype	10	414.719	1410.112	< 0.001
		Replicate	2	0.576	1.958	0.147
		Residual	86	0.294		
	PYC_{dens}	Genotype	10	4578.473	1229.999	< 0.001
		Replicate	2	2.939	0.790	0.457
		Residual	86	3.722		

On the other hand, and under controlled growing conditions, ANOVA performed on assessed traits revealed that the genotype effect contributed significantly ($P < 0.001$) to their variation; however, no difference was found between replicates, indicating homogeneity of the inoculation (Table 2). The first pycnidia was detected on the susceptible genotype 'Karim' 16-17 dpi (Figure 1). At 28 dpi, the visual assessment of the PLACN became easy and reached 85% with an average of 80%, and the PLACP ranged from 70% to 80% with an average of 73% (Figure 2). The PYC_{dens} was 80 to 90 pycnidia cm^{-2} with an average of 83 pycnidia cm^{-2} leaf area (Figure 1). For the resistant genotype 'Agili39', up to 28 dpi no visible signs or symptoms of STB were observed. Only small light-green spots covering 1%-2% were noted in some leaves.

For the moderately resistant genotypes, typical symptoms of the disease were well established a few days after their expression on the susceptible control 'Karim'. Indeed, the first pycnidia was detected between 19

and 25 dpi (Figure 1). Based on DMRT for comparing mean values of aggressiveness traits (PLACN and PLACP) in an ANOVA, these genotypes (moderately resistant in the a priori assessment) were classified into three phenotypic groups. The first group (classes b to c for PLACN and b-bc for PLACP) contains resistant genotypes ('JK14', 'Mahmoudi114', 'Taganrog120', 'Souri92' and 'Sbei83'), which showed an average of PLACP less than 10%, an average of PLACN ranged from 6% to 22%, a LP ranged between 22 and 25 d and an average of PYC_{dens} ranged from 8 to 15 pycnidia cm^{-2} (Figures 1, 2). Whereas the second group (classes cd-d for PLACN and c for PLACP), encompass the genotypes '15607' and '15974', considered as moderately resistant by displaying an average of, PLACP less than 20% (16% and 17%, respectively), PLACN 34 and 27%, LP 21 and 19 d, respectively and an average of PYC_{den} around 21 pycnidia cm^{-2} leaf area (Figures 1, 2). The third group (classes e for PLACN, and d for PLACP) encloses the two remaining genotypes, 'Salim' and '15975' which are considered as moderately susceptible by exhibiting an average of PLACP 24% and 27% (higher than 20% and less than 40%), an average of PLACN 44% and 39%, a LP 20 and 19 d, and an average of PYC_{dens} 28 and 32 pycnidia cm^{-2}, respectively (Figures 1 and 2). This classification is consistent with the results obtained in the field experiments (Table 3).

Table 3. Mean values of the aggressiveness traits of the isolate TunBz-1 assessed on 11 durum wheat genotypes under field conditions on 2017 and 2018. PLACCL: Percentage of leaf area covered by chlorotic lesions; PLACN: percentage of leaf area covered by necrosis; PLACP: percentage of leaf area covered by pycnidia. Different letters among means of aggressiveness traits in every year and every genotype indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

Genotypes	Aggressiveness traits					
	PLACCL		PLACN		PLACP	
	2017	2018	2017	2018	2017	2018
	——— % ———		——— % ———		——— % ———	
Agili39	4.0a	4.7a	0.0a	0.0a	0.0a	0.0a
JK14	11.0b	12.2b	5.0b	7.2b	4.0b	5.1b
Mahmoudi 114	11.7b	12.5b	5.0b	8.3b	4.0b	6.2b
Taganrog 120	22.3c	25.2c	12.5bc	8.0b	5.0b	4.5b
Souri92	27.3c	28.1c	12.2bc	11.4b	8.4bc	6.0b
Sebei83	25.7c	26.5c	17.8bc	14.5bc	9.4bc	6.5b
15607	41.3d	40.5d	29.7c	31.3c	12.0c	11.5c
15974	23.0c	24.2c	25.0c	27.2c	13.0c	14.6c
Salim	21.7c	24.6c	39.3d	35.4d	25.0d	23.3d
15975	20.3c	22.2c	36.3d	32.1d	27.2d	25.6d
Karaim	10.4b	10.8b	74.0e	70.1e	65.0e	63.7e

Correlation between aggressiveness traits

'Agili39' was specifically resistant (immune) to TunBz-1. Thus, due to the absence of disease symptoms, this genotype was removed from the correlation analysis. The correlations between different aggressiveness traits assessed on the 10 remaining genotypes in each experiment are listed in the Table 4. According to field data, the correlation between PLACN and PLACCL was negative and insignificant ($r = -0.059$ in 2017 and -0.045 in 2018, with $P > 0.01$). Correlation between PLACP and PLACCL was negative but significant ($r = -0.281$ in 2017 and -0.270 in 2018, with $P < 0.001$). While a positive and clearly significant correlation ($r = 0.948$ in 2017 and -0.925 in 2018, with $P < 0.001$) was detected between PLACP and PLACN. Under glasshouse conditions, highly significant correlation ($r = 0.956$, $P < 0.001$) was obtained between PLACP and PLACN. Latent period (LP) exhibited highly significant negative correlation ($P < 0.001$) with PLACN ($r = -0.900$), PLACP ($r = -0.831$) and PYC_{dens} ($r = -0.834$). Whereas perfect significant positive correlations were obtained between PYC_{dens} and the traits PLACN ($r = 0.961$, $P < 0.001$) and PLACP ($r = 0.999$, $P < 0.001$).

DISCUSSION

The most studies treating the interaction wheat-*Z. tritici*, have been skewed towards bread wheat (Brown et al., 2015). The analysis presented here is based on visual estimates of the most informative traits of aggressiveness described in previous *T. aestivum-Z. tritici* interaction studies (Stewart and McDonald, 2014; Zhan et al., 2016) and aimed to measure the reaction of set of 11 Tunisian durum wheat genotypes known by their contrasting levels of STB-resistance to an aggressive and virulent isolate TunBz-1 (Ferjaoui et al., 2015). The same set of genotypes was inoculated at the same growth stage in field and glasshouse trials. This was intended to compare and validate the reaction of tested genotypes to the isolate TunBz-1 through the measured traits in both environments and to exclude the probable effect of additional inoculum in the field experiment from either endogenous (local) or exogenous (distant) sources. In addition, some traits (LP and PYC_{dens}) were assessed only in controlled environment since they can be influenced by the field conditions, including climatic parameters and pathogen maintenance (Mundt et al., 2002).

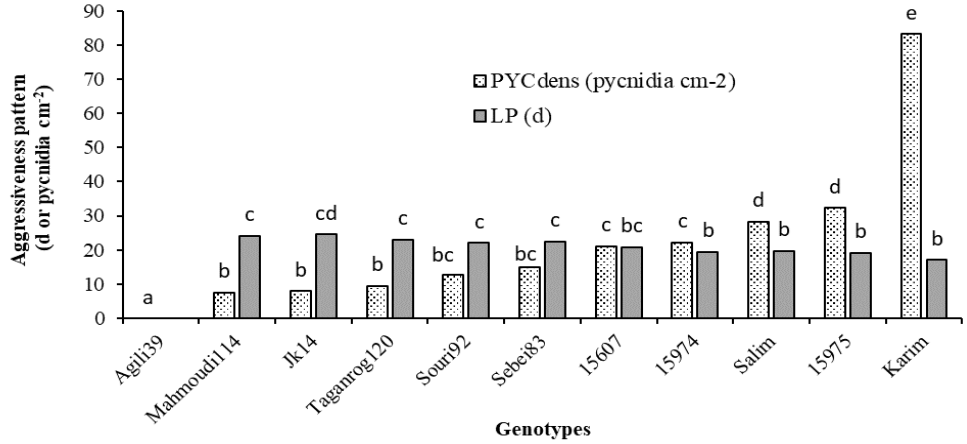

Figure 1. Aggressiveness pattern of TunBz-1 isolate on 11 durum wheat genotypes under glasshouse conditions, measured using the traits pycnidial density (PYC_{dens}) and latent period (LP). Different letters above the PYC_{dens} and LP columns indicate significant differences ($P < 0.05$) according to Duncan's multiple range test after performing ANOVA.

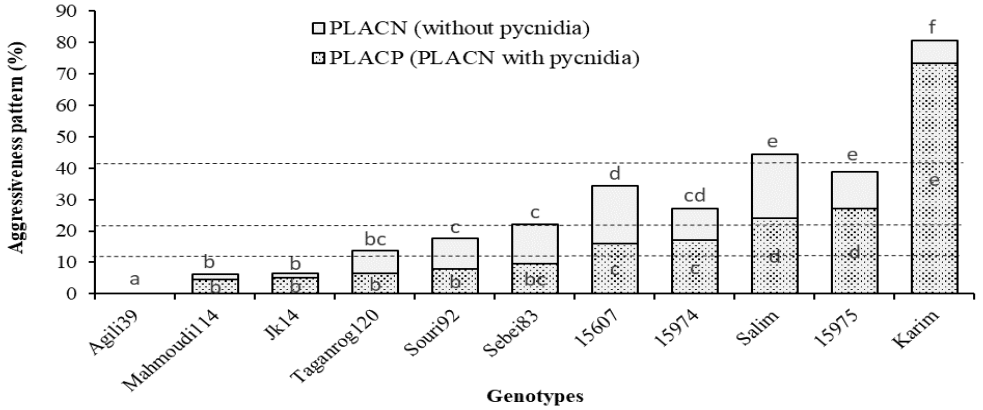

Figure 2. Aggressiveness pattern of TunBz-1 isolate on 11 durum wheat genotypes under glasshouse conditions, measured using the traits percentage of leaf area covered by necrosis (PLACN), with and without pycnidia, and percentage of necrotic leaf area covered by pycnidia (PLACP). Different letters indicate significant differences ($P < 0.05$) between genotypes according to Duncan's multiple range test after performing ANOVA.

At 28 dpi, the most chlorotic lesions observed early, especially on the susceptible and moderately susceptible genotypes become necrotic. This observation is in agreement with those of Duncan and Howard (2000), who reported that chlorotic lesions rapidly evolve to necrotic lesions on the susceptible hosts. Therefore, PLACCL was not correlated with PLACN while, a significant but weak negative correlation was obtained with PLACP. This result shows that chlorosis, which could be caused by multiple concomitant infection events, cannot be proved as indicator of host resistance or pathogen aggressivity, therefore, this trait was not assessed in the glasshouse experiment. The presence of small, black pycnidia in the necrotic lesions is the most reliable in-field character for identifying the disease (Suffert et al., 2013) as observed early in this study on the leaves of the susceptible genotypes. This character helps to separate STB lesions from lesions caused by other leaf spotting diseases (e.g., tan spot).

Table 4. Pearson correlations coefficients between aggressiveness traits recorded on the tested durum wheat genotypes expected 'Agili39' under field (2017/2018) and glasshouse conditions. ***Correlation is significant at $P < 0.001$. PLACCL: Percentage of leaf area covered by chlorotic lesions; PLACN: percentage of leaf area covered by necrosis; PLACP: percentage of leaf area covered by pycnidia; LP: latent period; PYC_{dens}: pycnidia density.

Aggressiveness traits		Coefficient of correlation (2017/2018)				
		PLACCL	PLACN	PLACP	LP	PYC_{dens}
Field	PLACCL	1				
	PLACN	-0.059/-0.045	1			
	PLACP	$-0.281^{***}/-0.270^{***}$	$0.948^{***}/0.925^{***}$	1		
Glasshouse	PLACN		1			
	PLACP		0.956^{***}	1		
	LP		-0.900^{***}	-0.831^{***}	1	
	PYC_{dens}		0.958^{***}	0.999^{***}	-0.834^{***}	1

The absence of pycnidia as observed here on 'Agili39' was interpreted by Karisto et al. (2018) as an absence of STB, even if necrotic or chlorosis lesions were visible. PLACCL and PLACN provide a measure of the damage of host leaf tissue caused by *Z. tritici* or other pathogen during infection process or by host defense reactions, whereas PLACP provides a rough estimate of the potential degree of *Z. tritici* reproduction (Stewart and McDonald, 2014; Karisto et al., 2018). Therefore, PLACP seems to be a preferable measure of aggressiveness for *Z. tritici* and more reliable than necrosis as a guide to a plant's susceptibility. However, PLACN is easier and faster to score. The high positive correlation coefficient (r) found between PLACN and PLACP in this study (Table 4) as observed by Radecka-Janusik and Czembor (2014), suggests that, as necrosis size increases, the leaf area covered by pycnidia also increases (Zhan et al., 2016). These results indicate that necrosis may be used to evaluate the reaction of wheat genotypes to *Z. tritici* and the aggressiveness of the isolate provided that no other factors, such as senescence or other diseases, causes extensive necrosis.

The high significant differences observed among the tested genotypes for all studied aggressiveness traits are reflected by the important number of significantly different groups of genotypes. Indeed, the expression of disease severity measured using different traits depends on the host and the symptoms were ranged from complete immunity with total absence of the disease symptoms, observed on 'Agili39' to total susceptibility expressed by high percentage of the leaf necrotic area with abundant production of pycnidia recorded on the susceptible 'Karim'. These two genotypes ('Agili39' and 'Karim') were used as references of resistance and susceptibility and allowed to control the level of virulence and aggressiveness of the isolate. In most previous studies, aimed to identify and characterize new sources of resistance to *Z. tritici*, the PLACP was the most widely used quantitative trait (Ferjaoui et al., 2015; Ouaja et al., 2020). In the present study, the PLACP was used to classify the reaction of tested genotypes to TunBz-1 isolate, taking into consideration the other traits (particularly PLACN and PYC_{dens}). In fact, according to DMRT analysis and levels 10%, 20% and 40% of PLACP, the genotypes were classified into different classes and phenotypic groups. Same classification was obtained in both experiments showing the response stability of wheat genotypes to the disease across the two environments.

In addition to the aforementioned aggressiveness traits, latent period (LP) has been used to characterize the interaction wheat genotypes-TunBz-1. As STB epidemic is polycyclic and result from the integration of many overlapping infection cycles (Suffert and Thompson, 2018), therefore the LP is a crucial component of aggressiveness of the pathogen isolate and could be used to characterize host susceptibility (Lannou, 2012). Nevertheless, it is challenging to assess this trait in field conditions because there was the possibility that primary inoculum originated within the field (Morais et al., 2016), and therefore we cannot estimate the date of infection (Suffert et al., 2013). Here we have shown that this trait was negatively associated with all other aggressiveness traits and can impact on their expression. It varies significantly between genotypes and on average, has been shown shorter on the susceptible and moderately susceptible than on the resistant and moderately resistant genotypes (Figure 1). Hence, it could be considered that the time between the start of the infection process by a unit of inoculum and the start of pycnidia production decrease with the degree of host susceptibility, while the host damage and pathogen reproduction (PLACN, PLACP and PYC_{dens}) increase. This observation is supported by the strong negative correlation identified between LP and the other assessed traits (Table 4), extending the reported correlations in previous studies (Suffert et al., 2013).

The PYC_{dens} reflects the ability of the isolate to convert the damaged host tissue into reproductive structures or the host's ability to suppress pathogen reproduction (Karisto et al., 2018). This trait was significantly different between genotypes and increases with increasing PLACN and PLACP. Kelm et al. (2012) noted that necrotic lesions are needed for pycnidia formation for many fungal pathogens such as *Z. tritici*. Therefore, strong positive correlation was found between these traits (Table 4). Although there was a general trend for association between these traits, there were any cases where isolates produced a high PLACN but few pycnidia and vice versa as mentioned by Stewart and McDonald (2014) and Karisto et al. (2018). The isolate TunBz-1 induced smaller necrotic lesions and fewer pycnidia on the resistant and moderately resistant genotypes (e.g., 'Mahmoudi114' and '15607') than on the moderately susceptible or susceptible genotypes (e.g., 'Salim' and 'Karim'). These results are in agreement with results from previous studies indicating that number of pycnidia increases with degree of susceptibility (Stewart and McDonald, 2014; Zhan et al., 2016). The variation in the number of pycnidia between genotypes suggests that pycnidia production is also a trait of interest when seeking to measure and characterize the level of quantitative resistance of wheat genotypes to *Z. tritici*.

The low level of pycnidia production has an impact on reducing damaging STB epidemics because it will reduce the rate of epidemic and enhancing the resistance to host damage (Karisto et al., 2018). In this way, we believe that the PLACP and the density of pycnidia can better characterize overall host resistance or susceptibility and pathogen aggressiveness as mentioned by Stewart and McDonald (2014). In that respect, breeders may select for genotypes that combine both resistance to host damage and resistance to pathogen reproduction as suggested by Karisto et al. (2018) and supported by our results.

CONCLUSIONS

The genotypes 'Salim', 'JK14', 'Taganrog120', 'Mahmoudi114', 'Souri92', 'Sbei83', '15607', '15975', and '15974' known as moderately resistant to *Zymoseptoria tritici* responded differently to the virulent isolate TunBz-1 using different aggressiveness traits. Highly significant positive and negative correlations were observed between traits, i.e., percentage of leaf area covered by necrosis (PLACN), percentage of leaf area covered by pycnidia (PLACP), latent period (LP), and pycnidia density (PYC_{dens}) scored on infected leaves. Conventional phenotyping for *Z. tritici* resistance under conducive weather conditions based on visual assessment of PLACN, PLACP and PYC_{dens} could be a relatively convenient method of assessing *Z. tritici*-wheat interaction and making decision for resistance selection. However, to screen large number of wheat populations, in different sites within a short period of time and with greater precision and efficiency, PLACN bearing pycnidia taking into consideration their density appears more fast, easy and reliable toll. In addition, including genotypes known by their high level of resistance and susceptibility in the screening for resistance to *Z. tritici* as references sources allowed us to evaluate the virulence and the aggressiveness level of the pathogen, and correctly classify the reaction of tested durum wheat genotypes based on the aforementioned aggressiveness traits.

Author contributions
Conceptualization: S.F., A.S. Methodology: S.F., A.S. Formal analysis: S.F. Investigation: S.F., K.H. Resources: S.F., A.S. Data curation: S.F. Writing-original draft: S.F. Writing-review & editing: S.F., A.S., K.H. All co-authors reviewed the final version and approved the manuscript before submission.

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