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Effects of *Septoria* Leaf Blotch Infection on Grain Yield and its Components of Three Bread Wheat Genotypes (*Triticum aestivum* L.)

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ABSTRACT

Septoria tritici (perfect stage, *Mycosphaerella graminicola*) is widely spread all over the world and generates serious crop losses in many wheat-growing regions. This study was carried out *in situ* to determine the effects of infection by *S. tritici* on grain yield (GY) and its components of three bread wheat (*Triticum aestivum* L.) genotypes (Tanit, Tirant and Sérir$_2$). Two treatments were applied. The first one was a control plot treated with a fungicide (Dithane M45) and the second was an artificially infected plot. The development of infection was evaluated by IL and IP, AUDPC and R. Agronomic parameters were used to study the reaction variability of the studied genotypes and to determine the impact of the infection on GY and its components. Results showed that climatic conditions were favourable to the development of infection and that the tested bread wheat genotypes were sensitive to *S. tritici*. 1000-grain weight was the most negatively affected GY component. On the other hand, IL and IP partly explain the loss in 1000-grain weight. On the protected plots, the positive action of the fungicide enhanced plant tolerance and generated a synergy expressed by the improvement of GY and its components.

Keywords: bread wheat, grain yield components, *Septoria tritici*, weight losses
Abbreviations: 1000-GrW, 1000-grain weight; **1000-GrWL**, 1000-grain weight loss; **AB/P**, aerial biomass/plant; **AUDPC**, area under disease progression curve; **DAS**, days after sowing; **DI,** disease severity; **ET/P**, ear tiller number/plant; **GN/E**, grain number/ear; **GN/P**, grain number/plant; **GY**, grain yield; **GYL**, grain yield loss; **HI**, harvest index; **IL**, infection level; **IP**, infection percentage; **LSD**, last significant difference; **ns**, not significant, **OD**, observation date; **R**, apparent infection rate; **SH**, straw height

INTRODUCTION

In Tunisia, annual wheat cultivation covers 927 260 ha, which represents 19% of the arable surface (INS, 2008). Average annual production of the period 2005-2009 was about 1.5 million tons (FAO 2009). For the same period, annual cereal national requirements were 1.2 million tons per year. Deficit, estimated at 25%, is covered by imports (AAC 2000). Despite the deployed efforts to improve the level of production, wheat production remains dependent on climatic conditions, especially precipitation, and is affected by cryptogrammic infections such as by *Septoria tritici* (teleomorph, *Mycosphaerella graminicola*).

Eyal (1981) announced that following *S. tritici* infection, a significant reduction in wheat GY took place. According to climatic conditions and varieties, losses in grain yield varied from 30 to 80% (Eyal *et al*. 1982; Jlibene 1996). Zahri *et al.* (2008) reported that this cryptogrammic foliar disease, met in all areas of wheat production, takes part in the destruction of approximately 2% of the world wheat and causes, each year, million tons of grains and billions dollars of losses. The intensification of wheat production using new farming techniques and the introduction of semi-dwarf and high-yielding varieties has led to an alarming development of *S. tritici* which became epidemic in all wheat cropping areas (Djerbi and Ghodhbane 1975). Perello *et al.* (1991) reported that physiologic specialization has been mentioned in Australia, in Uruguay and in the United States. In Tunisia, an investigation carried out during three cropping years showed that *S. tritici* infects 34% of surfaces, especially in the Northern part of Tunisia, with prevalence on durum wheat (*Triticum durum* L.) (Cherif *et al.* 1994). Whereas other works indicated that this disease affects especially bread wheat (*Triticum aestivum* L.) (Jlibene 1996; Zahri *et al*. 2008). Ezzahiri *et al.* (1996) reported that in Morocco, between 1990 and 1996, 47% of the bread wheat plots and 28% of the durum wheat plots were affected by *S. tritici*. A pathogenic variability of *S. tritici* was shown indicating the presence of specialized physiological races which attack either durum or bread wheat (Weber 1922; Shipton *et al.* 1971; Jlibene 1996). Other works announced that the bread wheat genotypes are more resistant to this fungus than the durum wheat genotypes (Djerbi and Ghodhbane 1975; Ghodhbane *et al.* 1982). Theses authors indicated that the selection for resistance to *S. tritici* and the introduction of resistant genotypes into crossings is relatively older for bread wheat than for durum wheat.

The plant material behavior against this disease can be estimated by two groups of parameters. The first is based on the plant symptoms development. It permits to measure plant resistance level and reflects its aptitude to avoid the disease development in its tissues. The second expresses the GY and its components reduction under the infection. It measures plant tolerance and expresses its predisposition to support a strong disease without a severe loss in GY (Trottet and Merrien 1982). Indeed, GY and its components can be used like indicators of more or less favorable environmental conditions (Meynard and David 1992). GY can be determined as average weight grain production and grain number per unit of area (Jonard and Koller 1951; Meynard and David 1992). Others mentioned that for cereals, GY components are: number of ears/seedling, GN/E and grain weight (Grafius 1964; McNeal *et al.* 1974; Black and Aese 1982).

S. tritici conidia germination occurs at temperatures

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ranging between 2-3°C and 33-37°C with an optimum of 20-25°C. Low temperatures (2-3°C) cause prolongation of spore germination phases, mycelium growth and lesions development. However, a compensation phenomenon between moisture and temperature appears on sensitive cultivars. Indeed, at low temperatures, infection was developed during long wet periods. Conversely, during short wet periods, high temperatures (25°C) accelerated the infection process (Eyal *et al.* 1987). Frequent precipitations and moderate temperatures (15 to 20°C) are favourable of *Septoria tritici* development. It is considered that after a rain, the successful contamination requires a saturation relative humidity from 15 to 20 h. Strong infestations were recorded with 35 h moistening period followed by relative moisture higher than 80% during 48 h (Ezzahiri 2001). The time between infection and pycnidia production depends on the climatic conditions (temperature, moisture and light), cultivar sensitivity and stump virulence. When climatic conditions are optimal, symptoms appear 14 to 21 days after infection (Shipton *et al.* 1971; Eyal *et al.* 1987).

The disease dissemination is due to the projection of irrigation or the rainwater drops on the vegetable debris and the rests of the already infected preceding crops. Infection migrates gradually to attack leaves, sheaths, thatches, glumes and barbs of the ears. It is more severe than sowing is early, used varieties are semi dwarf with abundant vegetation and have a higher yield potential, nitrogen fertilization is massive and precipitations are important, especially, during March and April (Luthra *et al.* 1937; Fellows 1962; Rosielle 1972; Djerbi *et al.* 1974; Ghodhbane *et al.* 1982).

With the development of the pesticide industry, use of chemical against *S. tritici* became undeniable. Nevertheless, pesticides effectiveness is short-term because new virulent stumps appear under the selection pressure. Moreover, chemical pesticides are expensive, environment polluting and their use is delicate for the farmer. Varietal resistance selection to diseases combined with farming techniques represents the best means for the control of *S. tritici* infection development. However, Eyal (1981) reported that the number of resistant genotypes is limited. In Tunisia and Morocco, only 30% of genotypes are resistant to *S. tritici* (Jlibene 1996). Moreover, Ezzahiri (2001) announced that, in Morocco, eight durum wheat and eight bread wheat genotypes were selected for tolerance to *Septoria tritici*. Deghaïs *et al*. (2007) enumerated six durum wheat and eight bread wheat Tunisian genotypes are tolerant to this disease. Although, no *S. tritici* complete resistance was reported on bread wheat genotypes (Jlibene 1996).

The objective of this work was to study the infection impact on the GY and its components and to elucidate the genetic variability and the resistance level of three bread wheat genotypes ('Tanit', 'Tirant' and 'Sérir$_2$') following an artificial infection by a mixture of two local isolates of *S. tritici*, called Fretissa and Utique.

MATERIALS AND METHODS

An experimental trial was conducted *in situ* at Fretissa located in Northeast Tunisia. This area is characterized by continental climatic conditions and clay-silt soil. The precedent crop was flax (*Linum usitatissimum* L.). Before sowing, deep plowing and two surface operations are carried out. Fertilization is composed of 130 kg ha^{-1} of super phosphate (45%) and 150 kg ha^{-1} of ammonium nitrate (33.5%) divided into two contributions with equal shares, one at the tillering beginning and the other at the ear stage formation. Sowing took place at the beginning of November, 2008, with a density of 150 plants m^{-2}. Elementary plots were 3 m^2 (4 lines of 3 × 0.25 m^2) separated by 0.5 m distance. Plant material is composed of three bread wheat genotypes ('Tanit', 'Tirant' and 'Sérir$_2$') characterized by ear formation dates: 123, 135 and 123 days after sowing (DAS), respectively.

Adopted experimental design is a randomized blocks with two treatments isolated by 1.5 m and four repetitions separated by 3 m. There were two treatment types. In the first, four artificial inoculations were realized weekly intervals between tillering and flowering (Eyal *et al.* 1987). The first one took place 30 DAS. Each inoculation was carried out with a mixture of suspensions, containing 10^6 spores.ml^{-1} concentrations of the two local *S. tritici* isolates originating from the two Tunisian regions called Fretissa and Utique. Isolation, culture and artificial inoculation were according to the recommendations of Djerbi *et al.* (1974) and Eyal *et al.* (1987). The second treatment type represents the protected control. It submitted five foliar sprays with the fungicide Dithane M_{45}, (active matter: Mancozeb) at 250 g.hl^{-1} (ATPP 2003). Sprays, repeated every fifteen days, are carried out using dowry pulverizer with flat jet delivering 900 l.ha^{-1} with a maximum pressure of 7 kg.cm^{-2}. The first fungicide application The first fungicide application took place since the appearance of the first *S. tritici* symptoms on the artificially infected treatment.

To ensure infection, inoculations were carried out in the evening when temperature was low and relative humidity was close to saturation. After each artificial inoculation, the seedlings of the two treatments were covered by a polyethylene film (180 µm thickness) over a period of 96 h.

Observations of disease symptom development were carried out every 5 days during a 46-day periods from the beginning April 2009, i.e., 149 DAS. The infection parameters studied were:

- Infection level (IL), which indicates the disease vertical progression is calculated according to the Saari-Prescott scale (Eyal *et al.* 1987) and varies from 0 to 9. At the level 0, the infection is limited to the base of the plant; whereas on the level 9, it has gradually migrated to the ears and has attacked leaves, sheaths, thatches, glumes and barbs.

Infection percentage (IP, %), which indicates the disease severity and expresses the coverage of lesions on a leaf, is expressed as a relative percentage. It varies from 0 to 100% (Peterson *et al.* 1948);

Apparent infection rate (r, in unit/day) (Gregory and Powelson 1971) was calculated according to the formula of Vander Plank (1963):

$$r = \frac{2.3}{t_2 - t_1} \times \log \frac{x_2 \times (1 - x_1)}{x_1 \times (1 - x_2)}$$

where x_1 = portion of a plant infected by the fungus at t_1 and x_2 = portion of a plant infected by the fungus at t_2;

Area under the disease curve (AUDPC) was calculated by trapezoidal integration in accordance with days interval and DS (IP) following the formula (Shaner and Finney 1977):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) * (t_{i+1} + t_i)$$

where Y_i : DS (IP) at the instant t_i ; Y_{i+1} DS at the instant t_{i+1}; n: number of observations.

Harvest took place on July 20th 2009. Twenty seedlings, randomly selected, by treatment, genotype and repetition, were harvested from the two middle elementary plot lines. The agronomic parameters studied were:

Straw height (SH; cm): measured at the end of the vegetative plant cycle and represents the mean straw length from the seedling until the ear bases of each shoot head;

Ear tiller number/plant (ET/P) represents the number of ear tillers per plant. It is counted at the end of the physiological phase of maturity;

Grain number/plant (GN/P): all plant ears are shelled and grains are counted;

Grain number/ear (GN/E): The obtained grain number per plant is divided by the ear number per plant.

Grain yield (GY): represents the grain weight on a plant-by-plant basis;

Aerial biomass/plant (AB/P): after maturity, each plant is cut from the base and weighed. The underground part is neglected;

Harvest index (HI; %): the ratio of the average grain yield to average dry aerial biomass;

1000-grain weight (1000-GrW): determined by cultivar and type of treatment;

1000-grain weight loss (1000-GrWL; %): the ratio of the weight of 1000 grains from unprotected plants to that of the protected control for each cultivar;

Grain yield loss (GYL; %): the ratio of grain yield of unprotected plants to that of the protected control for each cultivar.

The XLSTAT-Pro software was adopted for statistical analyses. One-way variance analysis (ANOVA) was performed and means comparisons were carried out by the Fisher's t-test (LSD) at 5% level. A binary correlation matrix (Pearson's coefficients) was established between agronomic parameters and infection level and percentage of *Septoria tritici*. The path coefficient analysis (Wright 1921; Li 1955) was studied. Infection development curves, R evolution and AUDPC were established on the basis of nine evaluations for each parameter.

RESULTS AND DISCUSSION

Climatic conditions effect on the infection development

Minimum and maximum temperatures and cumulated rainfall recorded during the decade 1996-2005 in the Fretissa region showed that the rainiest months were in February and March. During May and April, climatic conditions were characterized by 14 and 27.7°C, the respectively average minimal and maximal temperatures and by 86 mm of total rainfall (**Fig. 1**). Such conditions favored the development of *S. tritici* infection (Eyal *et al.* 1987) (**Fig. 2**). Indeed, final observations showed that the disease reached average IL of 8 and 3 on the Saari-Prescott scale (Eyal *et al.* 1987) and 70 and 9% average IP respectively in artificially infected and protected control treatments (**Table 1**). During the rainy years, *S. tritici* is placed at the head of the bread wheat parasitic complex (Zahri *et al.* 2008).

Individual analysis of the infection parameters and the agronomic variables

1. Variance analysis

ANOVA showed highly significant ($P < 0.01$) effects of genotype, date of observations (DAS) and their interaction (Genotype × date observations) for IL and IP (**Table 2**). This could indicate that the studied bread wheat genotypes reacted differently toward *S. tritici* infection. At 179, 184 and 189 DAS, 'Tirant' showed a smaller IP than 'Tanit' and '$Serir_2$' (**Fig. 2**). Similarly, 'Tanit' and '$Serir_2$' presented comparable and a higher AUDPC than 'Tirant' (**Fig. 3**). Apparent infection rates, which indicate a variety's partial resistance (Fontem *et al.* 1996), vary according to genotype. At 154 and 184 DAS, 'Tirant' was less sensitive to contamination (**Fig. 4**). For the remainder of the observation dates, all genotypes presented the same degree of sensitivity. This indicates that the climatic conditions were favorable to the infection development, that isolates Fretissa and Utique were virulent and that cultivars 'Tanit', 'Tirant' and '$Serir_2$' were sensitive to the disease (Mathre 1982; Eyal *et al.* 1987). Palmer and Skinner (2002) noticed that no immunity (complete resistance) to *S. tritici* exists, thus necrosis and/or pycnidia are always present, and any restriction or delay in pathogen development is regarded as a form of resistance. Nevertheless, cultivars with good resistance to *S. tritici* are available, but their yield is significantly less than susceptible cultivars treated with fungicides.

ANOVA showed highly significant genotypic differences ($P < 0.01$) for SH, GN/E and 1000-GrW. Also, significant differences were noted for ET/P. It appears that the

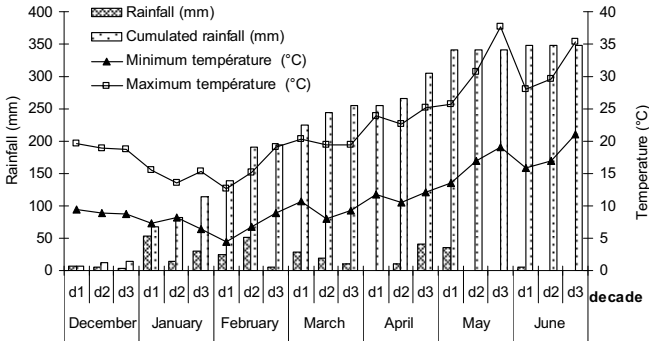

Fig. 1 Rainfall (cumulated monthly) and temperatures (minimum and maximum) means recorded in the Fretissa region during the decade 1996-2005.

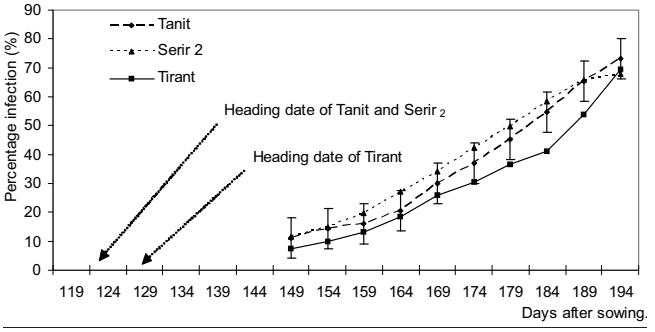

Fig. 2 Progress curve of *Septoria tritici* infection percentage on three bread wheat genotypes in the Fretissa region during the 2009 cropping year.

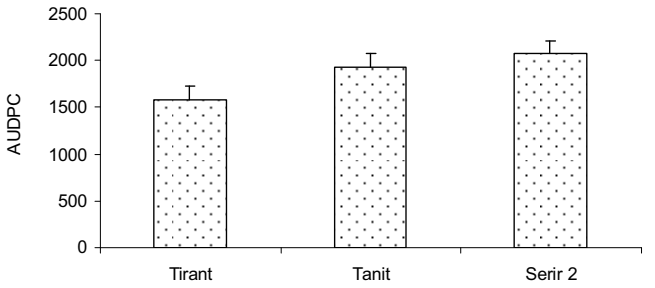

Fig. 3 Areas under disease progress curves of the three bread wheat genotypes.

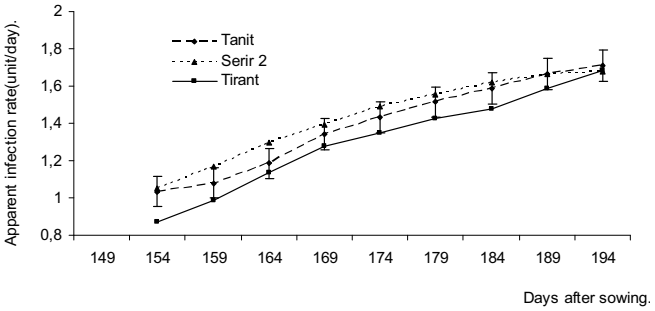

Fig. 4 Apparent infection rates (R) of *Septoria tritici* developed on three bread wheat genotypes.

Table 1 Infection levels (IL) and infection percentages (IP) (final observations) of the three bread wheat genotypes infected by *Septoria tritici*.

Genotypes	Artificially infected		Protected control	
	IL	IP	IL	IP
Tanit	8	73	3	12
Tirant	8	69	3	9
$Serir_2$	8	68	3	7
Averages	8	70	3	9

Table 2 ANOVA results, values and significance of F for the infection levels and percentages of the wheat genotypes infected by *Septoria tritici*.

Variation source	ddl	Infection level	Infection percentage (%)
Genotypes (G)	2	30.474**	10010.798**
Observation Dates (OD)	6	311.812**	105193.87**
Interaction (G × OD)	1659	6.856**	591.764**
Error	1679	0.7	99.275
Total	-	1.914	490.163
Variation coefficient (%)		11.2	29.5
LSD: ($P < 0.05$)	-	1.16	7

**: differences are highly significant ($P < 0.01$) according to the Fisher's *t*-test

bread wheat genotypes used differed according to these agronomic parameters. Treatment effect (i.e., artificially infected or protected control) was highly significant for SH, ET/P, GN/P, GN/E, AB/P, GY, HI and 1000-GrW.

The interaction effect (genotype × treatment) was highly significant for ET/P, GN/E and 1000-GrW and significant at the 5% level for GN/P (**Tables 3, 4**). It seems that, for these bread wheat genotypes, the infection by *S. tritici* affected mainly these last GY components.

2. Correlations and path coefficient analysis

A positive and highly significant correlation between IL and IP (r = 0.82) suggests that these two parameters are closely dependent (**Table 5**). ET/P and GN/P are positively and highly correlated (r = 0.74). Similarly, they are highly and significantly correlated with IL. It seems that the friction of tiller ear provoked by the wind and the microclimate characterized by merciful temperatures and high relative moisture favour the IL development (Djerbi *et al.* 1974; Ghodhbane *et al.* 1982).

GN/P and GN/E were positively and highly correlated with AB/P, IL and IP (**Table 5**). Similar results were noted by Cherif *et al.* (1994). This suggests that vigorous vegetative development contributes to the development of a high GN and creates a favorable microclimate for infection development.

1000-GrW was negatively and highly correlated with IL and IP (r = -0.6 and -0.65, respectively). According to Brönnimann and Neal (1972), the action of disease on GY involves mainly a reduction in the 1000-GrW. It seems that, following infection development on the upper leaves, photosynthetic activity was limited and grain size was reduced (Sebri and Harrabi 1986). Morvan (2006) announced that yield losses are due to the tissue assimilator loss and the nutritive flow source-well moving. The assimilated and nitrogenized components are retained in the leaves what affects finally the 1000-GrW. Similarly, 1000-GrW is negatively and highly correlated ($P < 0.01$) with ET/P, GN/P and GN/E (r = -0.27, -0.18 and -0.34, respectively). The 1000-GrWL were positively and highly correlated with these same parameters. Following a strong tillering, the increase in GN/P involves the reduction of 1000-GrW, which could be accentuated under infection, especially if this takes place during the filling grain phase (Sebri and Harrabi 1986).

SH was not correlated with IL and IP. This reveals that the development of infection is independent from SH and that the latter does not explain the infected leaf area development as indicated by Rapilly *et al.* (1984). However, Ezzahiri (2001) stated that severe attacks of *Septoria tritici* were observed with the introduction of semi-dwarf genotypes.

SH was positively and significantly correlated ($P < 0.05$)

Table 3 ANOVA results, values and significance of F for the studied agronomic parameters.

Variation source	ddl	SH (cm)	ET/P	GN/P	GN/E	AB/P (g/plant)	GY (g/plant)	HI (%)	1000-GrW (g)
Treatments (TRT)	1	986.13**	23.852**	1481.13**	1645.65**	6788.3**	648.91**	293.25**	4624.7**
Genotypes (G)	2	4665.8**	12.881*	17684.8 ns	1411.25**	102.67 ns	14.374 ns	111.89 ns	578.8**
Interaction (TRT x G)	2	2.258 ns	41.602**	22963.06*	535.81**	89.79 ns	3.511 ns	115.3 ns	80.701**
Error	474	27.267	4.301	7000.401	101.49	54.803	7.41	52.364	18.783
Total	479	48.532	4.533	7406.28	111.99	69.207	8.762	53.378	30.995
Variation coefficient (%)	-	7.906	42.379	47.355	27.28	46.543	50.452	21.15	14.327

ns: differences are not significant ($P > 0.05$); *: differences are significant at 5% level ($P < 0.05$); **: differences are highly significant at 1% level ($P < 0.01$); according to the Student's *t*-test

AB/P (g/plant), aerial biomass/plant; ddl, degree of freedom; ET/P, ear tiller number/plant; 1000-GrW (g), 1000-grain weight; GN/E, grain number/ear; GN/P, grain number/plant; GY (g/plant), grain yield; HI (%), harvest index; SH (cm), straw height

Table 4 Comparisons of the average values of the studied agronomic parameters on protected control and infected bread wheat plots.

Treatments	SH(cm)*	ET/P*	GN/P*	GN/E*	AB/P (g/plant)*	GY (g/plant)*	HI (%)*	1000-GrW (g)*
Pprotected control	71 a	6.3 a	215 a	46 a	19.5 a	6.5 a	36.5 a	34.3 a
Artificially Infected	61 b	3.5 b	139 b	28 b	12.3 b	4.3 b	31.9 b	26.2 b

* Different letters within a column indicate significant differences according to the Fisher's *t*-test ($P < 0.05$).

AB/P (g/plant), aerial biomass/plant; ET/P, ear tiller number/plant; 1000-GrW (g), 1000-grain weight; GN/E, grain number/ear; GN/P, grain number/plant; GY (g/plant), grain yield; HI (%), harvest index; SH (cm), straw height

Table 5 Binary correlation matrix (Pearson coefficients) of the agronomic parameters and infection level and percentage of *Septoria tritici*.

	SH (cm)	ET/P	GN/P	GN/E	AB/P (g/plant)	GY (g/plant)	HI (%)	1000-GrW (g)	IL	IP (%)	1000-GrWL (%)	GYL (%)
SH (cm)	1	0.25**	0.26**	0.09	0.41**	0.30**	-0.19**	0.03	0.09	0	0.22*	-0.29**
ET/P		1	0.74**	-0.22**	0.73**	0.71**	0.10	-0.27**	0.17**	0.11	0.32**	-0.72***
GN/P			1	0.40**	0.81**	0.20**	0.31**	-0.34**	0.19**	0.2**	0.37**	-0.90**
GN/E				1	0.23**	0.37**	0.31**	-0.18**	0.07	0.14*	0.16**	-0.36**
AB/P (g/plant)					1	0.87**	-0.07	-0.08	0.08	0.03	0.18**	-0.87**
GY (g/plant)						1	0.39**	-0.01	0.02	-0.02	0.07	-0.99**
HI (%)							1	0.11	-0.12	-0.10	-0.19**	-0.39**
1000-GrW (g)								1	-0.6**	-0.65**	-0.92**	0.02
IL									1	0.82**	0.69**	-0.01
IP (%)										1	0.69**	0.01
1000-GrWL (%)											1	-0.07
GYL (%)												1

*: differences are significant at 5% level ($P < 0.05$); **: differences are highly significant at 1% level ($P < 0.01$); ***: differences are highly at 1‰ level ($P < 1‰$)

AB/P (g/plant), aerial biomass/plant; ET/P, ear tiller number/plant; GN/E, grain number/ear; GN/P, grain number/plant; GY (g/plant), grain yield; GYL (%), grain yield loss; 1000-GrW (g), 1000-grain weight; 1000-GrWL (%), 1000-grain weight loss; HI (%), harvest index; IL, infection level; IP (%), infection percentage; SH (cm), straw height

Table 6 Path coefficient analysis of the agronomic parameters and infection level and percentage of Septoria *tritici* on the grain yield and its components of bread wheat genotypes.

Relation	Direct effects	Indirect effets								Total r
		SH (cm)	ET/P	GN/P	GN/E	AB/P (g/plant)	HI (%)	1000-GrW (g)	IL	IP (%)	
SH (cm)	0.020	-	0.016	0.075	0.004	0.243	-0.06	0.004	0.001	0	0.30**
ET/P	0.064	0.005	-	0.214	-0.009	0.439	0.030	-0.036	0.001	0.001	0.71**
GN/P	0.29**	0.005	0.047	-	0.018	0.486	0.98	-0.044	0.001	0.002	0.90**
GN/E	0.044	0.002	-0.014	0.117	-	0.139	0.099	-0.024	0.001	0.001	0.37**
AB/P (g/plant)	0.599**	0.008	0.047	0.235	0.010	-	-0.022	-0.01	0.001	0	0.87**
HI (%)	0.315**	-0.004	0.006	0.090	0.014	-0.042	-	0.014	-0.001	-0.001	0.39**
1000-GrW (g)	0.130**	0.001	-0.018	-0.098	-0.008	-0.045	0.035	-	-0.004	-0.006	-0.01
IL	0.006	0.002	0.011	0.054	0.003	0.045	-0.036	-0.078	-	0.007	0.02
IP (%)	0.009	0	0.007	0.053	0.006	0.018	-0.031	-0.084	0.005	-	-0.02

**: differences are highly significant at 1% level ($P < 0.01$)

AB/P (g/plant), aerial biomass/plant; ET/P, ear tiller number/plant; GN/E, grain number/ear; GN/P, grain number/plant; GY (g/plant), grain yield; GYL (%), grain yield loss; 1000-GrW (g), 1000-grain weight; 1000-GrWL (%), 1000-grain weight loss; HI (%), harvest index; IL, infection level; IP (%), infection percentage; SH (cm), straw height

with the 1000-GrWL (r = 0.22) and negatively and highly correlated ($P < 0.01$) with GYL (r = -0.29). Trottet and Merrien (1982) indicated that following infection by *S. tritici*, that SH could be a better estimator of the reduction in 1000-GW.

The 1000-GrWL had a positive and highly significant correlation ($P < 0.01$) with IL and with IP with the same coefficients (r = 0.69) (**Table 5**). It can be estimated according to the following model:

$$Y = -21.47 + 3.21x_1 + 0.23x_2;\ R^2 = 0.52$$

with Y = 1000-GrWL, x_1 = IL, x_2 = IP (of the final observations); R^2 = multiple determination coefficient.

R^2 indicates that 52% of the 1000-GrWL are due to infection. IL appears to have a more significant effect than IP on 1000-GrWL. Trottet (1982) found a negative and highly significant correlation (r = - 0.74) between flag leaf attack and 1000-GrW. The most serious yield losses occur when the flag, second and third leaves, which are responsible for providing the photosynthetic products for grain filling, become severely infected with *S. tritici* (Palmer and Skinner 2002; Marroni *et al*. 2006). Indeed, grain formation depends on the effectiveness of the transfer of metabolites from the flag leaf and the highest leaf stages, especially during grain filling phase (Zee and O'Brien 1970; Ledent 1982). Controlling *S. tritici* development is aimed at keeping these leaves free from disease, and is achieved through the development of host resistance and the application of fungicides in the early plant growth stages (Palmer and Skinner 2002; Marroni *et al*. 2006).

In the protected control treatment, relatively reduced 1000-GrWL was due to limited development of infection. Nevertheless, the action of pesticides added to the tolerance of plant material towards infection generated a synergic phenomenon which could be expressed by the improvement of GY and its components. In addition, Sherif *et al.* (1994) noticed a clear improvement of 1000-GW and GY following treatments with fungicides.

GY and GYL were not correlated with IL and IP (**Table 5**). Path coefficient analysis shows that these two infection parameters did not have effects (direct and indirect) on GY (**Table 6**). It appears that GY was not directly affected by infection. These results appear to contradict those given by the multiple regression equation (3). Probably, the determination coefficient 52% is relatively low to assign GYL to infection.

GN/P, AB/P, HI and 1000-GrW had direct effects on the GY. Moreover, GN/P had positive indirect effects on GY through ET/P and AB/P. This parameter had a positive indirect effect on SH, ET/P, GN/P and GN/E. This indicates that compensation phenomena between yield components can appear and attenuate the infection negative impact on GY (Jonard and Koller 1951; Adams 1967; Shipton *et al.* 1971).

CONCLUSIONS

Genetic variability was observed between bread three wheat cultivars: 'Tanit', 'Tirant' and 'Serir$_2$'. Climatic conditions favor their infection and development; two *S. tritici* isolates, Fretissa and Utique, are virulent. Artificially infected plots were severely attacked by the disease whereas plots treated using Dithane M_{45} were partially infected. 'Tirant' was less sensitive to the disease at 154 and 184 DAS. Nevertheless, later, this tolerance decreased and cultivars reached the same degree of sensitivity.

High AB/P and SH contributed to the establishment of a microclimate favorable for infection progression by *S. tritici*. The 1000-GrW yield component was negatively affected by *Septoria* infection and IL seems to determine the importance of 1000-GrWL. However, 1000-GrW contributes to a relatively limited extent to GY development. On the other hand, GN/P, AB/P and HI have the most significant contribution in GY establishment.

Following compensation phenomena between GY components, GY appears not to be directly affected by the infection. Also, it appears that in addition to its protection of the bread wheat cultivars against disease development, fungicide Dithane M_{45} may stimulate plant growth. This fungicide-positive effect, combined with cultivars' tolerance against infection, can generate a synergistic phenomenon expressed by improvement of GY and its components.

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