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RESEARCH

Estimating grain yield losses caused by septoria leaf blotch on durum wheat in Tunisia

Samia Berraies[1*], Mohamed Salah Gharbi[2], Salah Rezgui[3], and Amor Yahyaoui[4]

Septoria leaf blotch (SLB), caused by *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous, 2011 (teleomorph: *Mycosphaerella graminicola* (Fuckel) J. Schröt.), is an important wheat disease in the Mediterranean region. In Tunisia, SLB has become a major disease of durum wheat (*Triticum turgidum* L. subsp. *durum* [Desf.] Husn.) particularly during favorable growing seasons where significant yield losses and increase of fungicides use were recorded over the last three decades. The objectives of this study were to evaluate the effect of SLB severity on grain yield of new elite durum wheat breeding lines and to measure the relative effect of fungicide control on grain yield. Experiments were conducted during 2007-2008 and 2008-2009 cropping seasons. A set of 800 breeding lines were screened for reaction to SLB under natural infection at Béja research station. To estimate the disease effect, correlation between disease severity at early grain filling stage and grain yield was performed. Results showed that susceptible varieties yield was significantly reduced by SLB. Average yield reduction was as high as 384 and 325 kg ha^{-1} for every increment in disease severity on a 0-9 scale in both seasons, respectively. A negative correlation coefficient varied between -0.61 and -0.66 in both seasons. Treated and untreated trials conducted during 2008-2009 and 2009-2010 showed that yield of treated plots increased by 50% on the commonly cultivated susceptible varieties. The results of this investigation suggested that septoria incidence is related to large grain yield losses particularly on susceptible high yielding cultivars. However, appropriate fungicide application at booting growth stage could be beneficial for farmers. The development and use of more effective fungicide could be sought to alleviate the disease effects and therefore could be considered as a part of the integrated pest management and responsible use strategy on septoria leaf blotch in Tunisia.

Key words: Linear regression, *Zymoseptoria tritici*, *Triticum turgidum*, yields losses, fungicide.

INTRODUCTION

Septoria leaf blotch (SLB) caused by *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous, 2011 (teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt.) (Quaedvlieg et al., 2011), is an important wheat disease in several regions of the world, including Europe, Mediterranean region, South America, USA, and parts of Australia (Eyal et al., 1973; Schluter and Janati, 1976; Eyal et al., 1987; Polley and Thomas, 1991; Loughman and Thomas, 1992; Hardwick et al., 2001; Suffert et al., 2011). High relative humidity, frequent rains, and moderate temperatures are critical for disease development. Substitution of landraces, which have been identified as resistant to SLB (Sebei and Harrabi, 2008), by high yielding varieties resulted in cropping limited numbers of varieties and hence caused greater incidence of SLB over the last three decades. Continuous wheat cropping, high seeding rates, early planting and excessive use of N fertilizers enhanced SLB proliferation (Fernandez et al., 1998; Simón et al., 2003; Ansar et al., 2010).

In many wheat growing areas such as Mexico, Morocco, and USA, durum wheat (*Triticum turgidum* L. subsp. *durum* [Desf.] Husn.) has higher resistance to SLB than bread wheat (*Triticum aestivum* L.) However, in Tunisia bread wheat shows a higher level of resistance to *M. graminicola* compared to durum wheat (Djerbi et al., 1976). The greater incidence of septoria leaf blotch on durum compared to bread wheat in Tunisia suggests a great adaptation of *M. graminicola* pathotypes to durum than bread wheat (Yahyaoui et al., 2000). The virulence and disease incidence are variable and closely related to the frequency of the variety used in a particular area as well as the proportion of durum area as compared to that of bread wheat. Moreover, aggressiveness on durum

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[1] Université de Tunis El Manar, Faculté des Sciences de Tunis, Campus Universitaire 2092, El Manar, Tunis, Tunisie. *Corresponding author (samia_berraiess@yahoo.fr).
[2] Institut National de Recherche Agronomique de Tunis, Laboratoire de Grande Culture, Rue Hédi Karray, 2049, Tunis, Tunisie.
[3] Institut National Agronomique de Tunis, Laboratoire de Génétique et d'Amélioration des Plantes, 43 Avenue Charles Nicolle, 1002 Tunis, Tunisie.
[4] International Maize and Wheat Improvement Center (CIMMYT) km. 45 Carretera México-Veracruz El Batan, Texcoco, Estado de México, CP 56130.
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cultivars in other areas where durum wheat used to be more resistant, particularly in Morocco and South Europe has been observed (Zahri et al., 2008; Maccaferri et al., 2011).

Fungicide applications and the deployment of resistant wheat cultivars are among the most practices aiming to alleviate SLB (Goodwin, 2007; Lehoczki-Krsjak et al., 2010). Fungicides application is effective to control SLB and their benefits have been long acknowledged, but they are not always timely applied, environmentally sound, or economically viable (Paveley et al., 1997). Timing and number of fungicide applications are crucial in crop protection strategy. It has been reported that sprays applied during the period from flag leaf to ear emergence had shown effective control of septoria (Cook et al., 1999). However, the number of applications depends essentially on environmental conditions, disease occurrence, severity, incidence and critical threshold of disease development.

Fungicides have become an integral part of disease-management programs on cereal crops in many countries of the world. The integrated management programs offer appropriate strategies by optimizing the use of fungicides while respecting both the economy and the environment (Burke and Dunne, 2008; Beest et al., 2009; Wiik and Rosenqvist, 2010), in addition to the use of resistant varieties that offer a good insurance of protection and prevent yield losses. Improving quantitative and durable resistance is highly sought by breeders. Durable resistance is highly desirable; however, the selection for quantitative resistance is often difficult to achieve (Chartrain et al., 2004). The development of resistant cultivars to septoria and their use will effectively improve productivity of wheat in areas prone to SLB (Eyal, 1981; Eyal et al., 1987).

In Tunisia currently cultivated durum wheat varieties are very susceptible to SLB and under conducive environmental conditions yield losses can easily reach up to 50% (Gharbi et al., 2008). Yield losses ranging from 25% to 50% have been reported under severe epidemics on durum wheat at other wheat growing areas (Ziv and Eyal, 1978; McKendry et al., 1995).

The impact of foliar diseases on grain yield has been of major interest over long period of time (Madden, 1983). James (1974) and James and Teng (1979) proposed evaluation methods, and several models have been published to determine the relation between incidence and severity of diseases and yield losses (Madden, 1983). These models are based on empirical descriptions of the relationship between disease and yield performance on field crops (Madden et al., 2000). Yield loss is a function of the infection, and one of the common ways to show this relationship is linear regression (Madden, 1983).

Reliable models aiming to predict yield losses and implement alternative strategies for integrated management and application of appropriate fungicides and resistant varieties are crucial in assessing septoria related traits. An estimate of the losses caused by Septoria and understanding of the host-pathogen relationship in a particular environment, can help in selecting the appropriate disease management strategy.

The aims of this study were to estimate yield losses caused by SLB on elite durum wheat lines under the prevailing climatic conditions of northern Tunisia and to define the effects of fungicide application on the cultivar performance.

MATERIALS AND METHODS

The effect of SLB on grain yield was studied on experimental germplasm handled by the National Durum Wheat Breeding Program at the National Institute of Agriculture Research during 2007-2008 and 2008-2009 crop seasons. Reactions to SLB and grain yields were monitored on 800 experimental elite durum wheat breeding lines including commercial cultivars controls. The experiments were conducted at Beja experimental station (36°43'30" N, 9°10'55" E), North West Tunisia, which is characterized by high rainfall with mild winter. For each cropping season, 400 experimental lines were grouped into 16 Durum Wheat (DW) yield trials (DW1 to DW16) containing each 23 experimental lines and two control varieties, 'Nasr' (resistant) and 'Karim' (susceptible). The trials were planted in a randomized complete block design with four replicates. Plots were six rows 5 m long and 20 cm between rows, with a planting density of 300 seeds m^{-2}. Seeding was mechanically performed in mid-November. Fertilizer (ammonium nitrate) and weed control (glyphosate) were those recommended for Beja area. These practices are commonly encountered in the semi-arid cereal areas. Disease development, under natural infection conditions, was assessed at dough stage (Zadoks 70 to 75) (Zadoks et al., 1974) using Saari and Prescott (1975) single digit scale (1-9), which allows to estimate plant response to SLB on the basis of the vertical progression of the pathogen. Grain yield was measured on the four central rows ($5\ m^2$) and expressed in kg ha^{-1}.

Linear regression analysis was used to describe the relationship between grain yield and disease severity. This equation is $Y = a X + b$, where Y is the predicted yield, b is the expected yield in the absence of the disease (both expressed in kg ha^{-1}), X correspond to the disease score, and a is the regression coefficient that expresses the linear yield loss in kg ha^{-1}. Correlation between both parameters was performed using Microsoft excel software.

Yield losses caused by SLB were further estimated in a separate trials conducted at Beja station during 2008-2009 and 2009-2010 where grain yield of the common used susceptible varieties Karim, Razzak, and Khiar and the new released resistant varieties Nasr, Salim, and Maâli were assessed using two applications of fungicide at boot (GS 45) and early grain filling (GS 71) stages. The fungicide was used at the recommended 0.7 L ha^{-1} commercial rate (epoxiconazole 12.5% + kresoxim-methyl 12.5%; Ogam, BASF AGRO, Écully, France) formulation and compared to unsprayed control plots. Seeding date, seeding rate, crop management, and harvested plot size were similar as yield trials described above. The ANOVA was performed on grain yield data to detect year, treatment and variety effects using PROC ANOVA procedure (SAS Institute, Cary, North Carolina, USA). Natural infection in both years was high as recorded on the untreated plots.

RESULTS AND DISCUSSION

Septoria tritici blotch is an important factor limiting durum wheat production in Tunisia. Climatic conditions were favorable for disease development. Early infections in winter were observed almost every year of the study. The vertical progression of infections depends more on rain frequency and overall rain quantity, particularly during March and April. Average temperatures of approximately 20 °C during spring time were optimal for disease development, dissemination, and subsequent infection.

During 2007-2008 cropping season, the incidence of *S. tritici* was high and a wide range of disease severity levels were observed on tested durum accessions. The SLB severity varied from relatively resistant/tolerant (score 2) with small spots on the lower leaves, to very susceptible (score 8 and 9) on flag leaf and spikes with well-developed large lesions and large pycnidia density. During this season, 63% of tested lines had infection levels equal or higher than 8 on the scale 0-9. The susceptible control 'Karim' and the resistant control 'Nasr' were tested within each group in the 16 DW trials. Few lines (9.5%) had infection levels lower or equal to the average infection level of the resistant control 'Nasr'. Moreover none of the tested lines was immune to SLB infection, which confirms the widely reported partial type of resistance to SLB (Eriksen et al., 2003; Chartrain et al., 2004; Arraiano and Brown, 2006). Grain yield of the 400 tested lines ranged from 454 to 5823 kg ha^{-1}. As expected the lowest yields were associated with lines having the highest infection levels.

The results obtained in 2008-2009 showed that infection levels were high on susceptible controls, but the overall infection on the tested material was lower on this set of lines than the previously tested accession in 2007-2008. The differences in disease responses could be attributed to the seasonal conditions, particularly rainfall and temperature that were more conducive to SLB development during winter of 2007-2008 growing season. Although similar severity was noted on control cultivars ('Karim' and 'Nasr') during both cropping seasons; the difference in resistance levels could be due to differential resistance of the components in the second set of accession where 159 (39%) lines had infection levels equal or higher than 8. Whereas 128 (32%) lines had infection levels equal or lower than the average infection level of the resistant control 'Nasr'. Grain yield in 2008-2009 ranged 1623 to 6127 kg ha^{-1} with an average of 5409 kg ha^{-1}.

Regression analysis over all trials evaluated during both cropping seasons indicated a linear relationship between disease severity and grain yield (Figure 1). The estimates of the linear regression coefficient between pathogen severity scores and grain yield was negative indicating that the stronger the disease severity, the greater the grain yield loss. Average correlation coefficients for each four hundred lines were -0.66 and -0.61, respectively (R^2 were 0.44 and 0.39 respectively, $P \leq 0.05$). These results are in agreement with those of Rezgui et al. (2008), who reported a negative correlation coefficient ($r = -0.87$) between grain yield and SLB severity in 48 durum wheat fields.

Average grain yield of each trial were similar to the calculated values Y of the predicted yield. The regression coefficient a, representing the linear yield loss, showed that yield was reduced 384 and 325 kg ha^{-1} during 2007-2008 and 2008-2009, respectively, for each unit increase of disease severity level. Based on this prediction model, grain yield losses can reach up 50% when the flag leaf is infected, implying that flag leaf is the most important contributor to grain filling, hence severe infection may cause high crop reduction (Shaw and Royle, 1989). King et al. (1983) showed that yield-loss caused by septoria can be explained by infection of the flag or second leaf. Grain yield increase due to flag leaf senescence delay using foliar fungicide application on susceptible varieties, can result in important yield increase (Kelley, 2001).

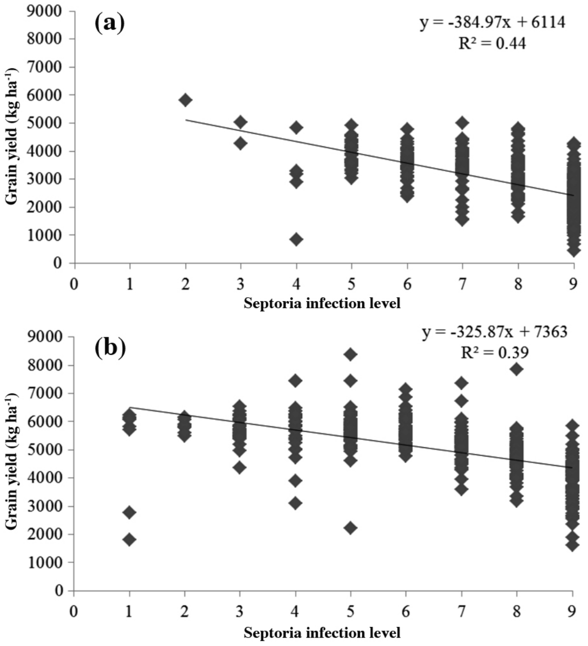

Figure 1. Relationship between septoria infection level and grain yield on durum wheat; 400 lines evaluated during 2007-2008 (a) and 400 lines evaluated during 2008-2009 cropping season (b).

ANOVA of the fungicide control trial indicated a highly significant ($P \leq 0.01$) variety, fungicide treatment, and cropping season effects on grain yield (Table 1). Variability on grain yield was expected due to genetic differences between varieties. The Variety × Treatment interaction significantly affected grain yield suggesting the difference in varietal response to fungicide. Grain yield fluctuates considerably with changes in environmental conditions and number of fungicide applications suggesting that climatic variations have contributed to significant differences in grain yield in both years and that foliar fungicide application could be beneficial in reducing crop losses. The ANOVA for grain yield revealed also a significant mean square due to Variety × Year interaction indicating that the genotype response change considerably with environmental conditions. Those results illustrate the complexity of septoria disease development and hence its management on durum wheat.

The impact of fungicides treatment on grain yield was studied using six durum wheat varieties during 2008-2009 and 2009-2010 cropping seasons. Disease development was adequately high on untreated susceptible varieties. Significant yield losses were recorded in comparison to resistant lines and protected susceptible varieties. Average yields recorded on untreated plots of susceptible and resistant varieties during the crop seasons, was respectively 3969 and 5610 kg ha^{-1} (Table 2). The susceptible 'Khiar' consistently had the lowest grain yield during both cropping seasons, while the resistant 'Maâli' had the highest grain yield in the untreated plots. This result suggests that improvements in SLB resistance could be achieved by the release of resistant varieties. Yield increase attributed to resistant varieties was over 40%. Similar results were reported in France, where 30%-50% of the increase in yield has been attributed to genetic improvements (Brancourt-Hulmel et al., 2003).

Table 1. Mean squares (MS) from ANOVA for grain yield (kg ha^{-1}) assessed during two cropping seasons and using treated and untreated of the most six Tunisian durum wheat cultivars.

Source	DF	MS
Year (Y)	1	117776756**
Block (Year)	6	660923
Treatment (T)	2	28670338**
Variety (V)	5	2759514**
V × Y	5	1139845**
T × Y	2	696252*
V × T	10	1932122**
V × T × Y	10	2432502^{ns}
Error	102	218215

*, **Significant at the 0.05 and 0.01 probability levels; ns: non-significant; DF: degrees of freedom.
CV% = 7.99; $R^2 = 0.91$.

During 2008-2009, a single fungicide treatment controlling septoria applied at booting stage (GS 45), increased yield by 44.34% and 7.31% for the susceptible and resistant varieties, respectively, and an additional treatment at GS 71 resulted and another yield increase by 12.78% and 5.92%, respectively (Table 2). During the next season, the disease pressure was high and a single fungicide application resulted in a 50.06% yield increase for susceptible varieties and 8.6% for resistant varieties. The second treatment did not contribute to a significant yield increase of susceptible varieties. The recorded grain yield increment was 2% for susceptible cultivars and 6.5% for resistant cultivars. These results showed that fungicide application at booting stage gave the best control of SLB. Significant reduction of disease severity on susceptible cultivars and significant increase in grain yield were recorded. The yield increase is attributed to protected flag leaves that contribute and enhance grain filling (Ali et al., 2010; Guo et al., 2010). Various studies showed that

Table 2. Effects of fungicide application on yield increase in six durum wheat cultivars in field experiments conducted in 2008-2009 and 2009-2010 cropping seasons.

	T0		T1		T1-T0		T2		T2-T0	
Varieties	GY	SIL	GY	SIL		Gain (%)	GY	SIL		Gain (%)
2008-2009 Cropping season										
Karim[1]	4728	7	7187	3	2459	52.00	7481	2	2753	58.22
Razzak	4875	6	6481	4	1606	32.94	7190	1	2315	47.48
Khiar	3642	7	5452	3	1810	49.69	6142	1	2500	68.64
Mean	4415		6373		1958	44.34	6937		2522	57.12
Nasr[2]	6433	4	6494	2	61	0.94	7064	1	631	9.80
Salim	6378	3	6734	3	356	5.58	7028	1	650	10.19
Maâli	6458	5	7453	1	995	15.40	7735	1	1277	19.77
Mean	6423		6893		470	7.31	7275		852	13.26
2009-2010 Cropping season										
Karim	3689	9	5277	3	1588	43.04	5326	2	1637	44.37
Razzak	3922	9	5210	3	1288	32.84	5285	2	1364	34.77
Khiar	2958	9	5373	4	2415	81.64	5454	2	2496	84.38
Mean	3523		5286		1763	50.06	5355		1823	52.00
Nasr	4650	5	4987	3	337	7.24	5257	2	607	13.05
Salim	4529	5	4733	3	204	4.50	5166	2	637	14.06
Maâli	5214	6	5912	3	699	13.50	6140	1	926	17.75
Mean	4797		5210		413	8.60	5521		723	15.09

T0: Control trial; T1 and T2: fungicide application at Zadoks growth stage GS 45 and GS 71, respectively; GY: grain yield; SIL: Septoria infection level.
[1] Karim, Razzak, and Khiar are susceptible to septoria leaf blotch.
[2] Nasr, Salim, and Maâli are resistant to septoria leaf blotch.

grain yield increase could result from a single fungicide application between booting and anthesis growth stage (GS 45-61) (Gooding et al., 2000; Wiik, 2009). It has been recognized that fungicides applied during flag leaf emergence (GS 37) to ear emergence (GS 59) offer the best prospect for cost effective foliar disease control (Cook et al., 1999).

The second fungicide application had limited effect on susceptible and resistant varieties during both seasons, suggesting that treatment at booting stage had the main effect of reducing infection and maintain yields to near varietal potential particularly during rainy seasons.

CONCLUSION

This study confirms that septoria leaf botch (SLB) of durum wheat is a major constraint to profitable wheat production in Tunisia. During two consecutive crop seasons data were generated and used to model the relationship between disease severity and yield losses using linear regression analysis. The significance of the breeding efforts to incorporate resistance to SLB in durum wheat in Tunisia was validated since resistant cultivars showed a weak response to fungicide (average gain 441.5 to 787.5 kg ha^{-1}) meanwhile the susceptible response was large (average gain 1860 to 2172 kg ha^{-1}). In addition, results indicate that the infection of the flag or penultimate leaf is a good predictor of yield-loss and also suggest the necessity to treat on time. We conclude that one fungicide application is profitable especially if environmental conditions favor SLB disease development. Timing of fungicide application at GS 45 generally resulted in a higher yield increase on susceptible varieties.

Increasing durum wheat yield at farmer level in SLB prone areas should include proper management of this important disease. Data generated emphasize the importance of continuous breeding to enhance genetic resistance in such an important economic crop to this major biotic constraint.

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