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RESEARCH

Occurrence of Septoria tritici blotch (*Zymoseptoria tritici*) disease on durum wheat, triticale, and bread wheat in Northern Tunisia

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ABSTRACT

Wheat (*Triticum aestivum* L.) is the most important cereal crop in Tunisia, nonetheless production is highly affected by drought and diseases mainly Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* (Roberge ex Desm.) Quaedvl. & Crous anamorph and *Mycosphaerella graminicola* (Fuckel) J. Schröt. 1894 teleomorph; that has become an inherent disease of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) van Slageren) but rarely observed on bread wheat (*Triticum aestivum* L. subsp. *aestivum*) and on triticale (*×Triticosecale* spp.) The main objective of this work was to study the prevalence and geographical distribution of *Z. tritici* on triticale, durum wheat and particularly on bread wheat in different cereal growing regions of North and Northwestern Tunisia to confirm its presence/absence on bread wheat. For this study, 126 wheat fields were surveyed in North and Northwestern Tunisia during 2015-2016 and 2016-2017 cropping seasons. STB on durum wheat was present in the majority of inspected durum wheat fields, where high mean incidence (60%) and severity (40%) were recorded at Jendouba, Bizerte, Beja, and Kef. The survey data revealed low risk on bread wheat with an incidence of 23% and 29% at Bizerte and Beja, respectively. However high incidence of 84% and 52% was recorded at Cap Bon in 2016 and 2017, respectively and mainly at El Haouaria where STB severity was relatively high on bread wheat landrace of unknown origin but called by local farmers as 'Farina arbi'. Sporadic incidence was recorded on Triticale of 100% at Jendouba (in 2016), and 33% at Bizerte (in 2016 and 2017) and absence at all other surveyed sites; likewise for severity at same locations where 13% and 42% were recorded in 2016. The survey data revealed low risk on bread wheat except at El Haouaria where STB severity was relatively high on a bread wheat landrace; while it was considered as high risk at all durum wheat fields in Beja, Bizerte, Jendouba, Zaghouan and Kef regions, such distinct occurrence could lead to clarify host specificity in *Z. tritici*.

Key words: Farina Arbi, survey, *Triticum*, Tunisia, wheat, *Zymoseptoria*.

INTRODUCTION

The cereal sector is of major economic importance in Tunisia. It provides major staple food commodities for most Tunisian households. Cereals are cultivated on almost one third of agricultural land (1.5 million hectares) (Tunisian Ministry of Agriculture, Water Resources and Fishing, 2015), 58% are located in the Northern and Western regions where durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) van Slageren) represents 54%, against 36% for bread wheat (*Triticum aestivum* L. subsp. *aestivum*) and 10% for barley (FAO, 2017). Average production is around 1.05 million tons, which

represent approximately 80% of the country needs (Gharbi et al., 2000). However, cereal production in Tunisia faces many challenges of which drought is the most limiting abiotic stress in semi arid zones (Slama et al., 2005), while biotic stress, mainly leaf rust and Septoria tritici blotch (STB), cause important yield losses particularly on durum wheat in sub-humid regions (Ben Mohamed et al., 2000; Gharbi et al., 2000) of North and Northwestern Tunisia.

STB caused by the ascomycete fungus *Zymoseptoria tritici* (Roberge ex Desm.) Quaedvl. & Crous became more important in Tunisia since the introduction of early maturing, semi dwarf, high yielding varieties. It has become an inherent disease of durum wheat, and thus a significant challenge for breeders to release varieties which combine good resistance and higher yields (Ammar et al., 2011). In contrast to durum wheat, bread wheat varieties grown in Tunisia are almost indemn of Septoria. High incidence of STB on durum compared to bread wheat in Tunisia suggests either an adaptation of *Z. tritici* isolates to durum rather than bread wheat (Yahyaoui et al., 2000) or high levels of resistance in bread wheat. The observed levels of resistance amongst cultivated bread wheat varies from year to year, most likely based on the environmental conditions and the dynamics of STB populations.

Although Septoria was observed on durum wheat annually, up to now not much is known on the occurence of STB on bread wheat. Hence, the main objective of this paper was to study the prevalence and geographical distribution of *Z. tritici* on *Triticum* species and particularly on *T. aestivum* in different cereal growing regions of North and Northwestern Tunisia to eventually confirm its presence/absence on bread wheat.

MATERIALS AND METHODS

Study area description and climatic conditions of the surveyed regions

Surveys were conducted during two cropping seasons (2015-2016; 2016-2017) at seven major wheat-growing areas in North and Northwestern Tunisia (Figure 1). Fifty-seven fields were surveyed in Cap Bon North (El Haouaria), Cap Bon South, Bizerte, Manouba, Beja, and Jendouba during 2016 and sixty-nine fields were surveyed in Cap Bon regions, Bizerte, Manouba, Beja, Jendouba, Zaghouan, and Kef during 2017 (Figure 1). Certain varieties were more predominant than others rendering therefore inter region comparison rather difficult to make.

Meteorological data (temperature and rainfall, Table 1) and geographical coordinates (altitude, longitude and latitude, Table 2) over different climatic regions for each survey areas were recorded. The average rainfall varied from 500 to 800 mm and the temperature ranged between 6 and 33 °C in the sub-humid region (Cap Bon North, Bizerte and Beja). Precipitation and temperature rates varied from 400 to 600 mm and from 5 to 37 °C respectively in the semi-arid regions (Cap Bon south, Manouba, Zaghouan, Jendouba, and Kef).

Figure 1. Map of Tunisia showing the location of survey areas across different climatic regions during 2016 and 2017 cropping seasons. Sub-humid: Cap Bon North (A), Bizerte (B) and Beja (C). Semi-arid region of Northern Tunisia: Cap Bon South (D), Manouba (E), Zaghouan (F), Jendouba (G), and El Kef (H).

Table 1. Climatic conditions of inspected regions during the survey period.

Regions	Weather conditions (range)	
	Rainfall	Temperature (min-max)
	mm	°C
Cap Bon North[1]	500-800	9-31
Cap Bon South[2]	400-500	7-34
Bizerte	600-800	7-33
Beja	500-600	6-32
Manouba	400-600	8-34
Zaghouan	400-600	4-34
Jendouba	400-500	5-37
El Kef	300-400	2-38

[1]Cap Bon North: El Haouaria.
[2]Cap Bon South: Soliman, Beni Khalled, and Grombalia.

Table 2. Geographical coordinates of inspected regions during the survey period.

Treatments	Altitude (range)	Longitude (N) (range)		Latitude (E) (range)	
	m a.s.l.				
Bizerte	16-354	09°03'1"	09°69'83"	32°50'56"	37°14'12"
Cap bon North[1]	11-876	10°02'73"	10°10'95"	36°52'41"	36°67'82"
Cap bon South[2]	24-446	10°45'27"	10°49'10"	36°47'47"	36°92'68"
Manouba	58-244	09°59'1"	09°91'41"	36°30'70"	36°85'04"
Zaghouan	81-320	09°45'43"	10°4'42"	36°21'18"	36°30'53"
Beja	18-290	09°09'01"	10°78'62"	36°22'26"	36°68'31"
Jendouba	21-341	08°32'35"	08°42'45"	36°28'4"	36°32'48"
Kef	100-327	08'39°12"	08°48'50"	36°22'26"	36°11'44"

[1]Cap Bon North: El Haouaria.
[2]Cap Bon South: Soliman, Beni Khalled, and Grombalia.
m a.s.l.: Meters above sea level.

Cereal crops

The survey covered the major cereal growing areas in North and Northwestern Tunisia where commercial durum wheat varieties ('Karim', 'Razzak', 'Maali') occupy over 60% of the area compared to the introduced varieties ('Saragola', 'Carioca', 'Sculpture', 'Soudaine') that cover so far less than 10%. Commercial bread wheat varieties ('Salambo', 'Utique', 'Haidra') and introduced varieties ('Zanzibar') occupy no more than 20% of the area; while triticale ('Bienvenue' and others) covers about 1%-3%; the rest of the area is covered by barley and oats. A unique situation in Cap Bon region where a landrace bread wheat ('Farina arbi') occupies over 60% of the area, the rest is covered by commercial barley, durum and bread wheat varieties. 'Farina arbi', a tall low yielding bread wheat (landrace) of unknown origin is cultivated annually for over a century, according to local farmers, in the same region and exclusively used for pastry known as "Kaak". The seed is maintained by local farmers and not commercialized. Bread wheat landrace ('Farina arbi') and Septoria tritici blotch (STB) differentials (comprised within CIMMYT's ISEPTON) were phenotyped at experimental station of Bou Salem (Tunisia) under artificial inoculation with *Zymoseptoria tritici* ([Roberge ex Desm.)] Quaedvl. & Crous) populations sampled from durum wheat. Inoculation was performed at tillering stage using bulk isolates at a rate of 10^6 spores mL^{-1} according to Ferjaoui et al. (2015) with slight modifications.

Septoria leaf blotch disease assessment

Field surveys were conducted during flowering stage of bread and durum wheat, each field was visited once. STB prevalence was assessed within and between regions based on number of fields surveyed and the presence/absence of Septoria at each location. The incidence was reported on this study based on Saari-Prescott modified "0-9" Cobb-scale (Saari and Prescott, 1975). In this survey, we designed five field classes (Table 3) to assess STB within each region where prevalence, severity and incidence were the main criteria. The relative importance of STB was based on its prevalence at each location where incidence and severity were assessed and averaged at each surveyed field. In this study, prevalence

Table 3. Survey designated Classes for Septoria tritici blotch (STB) prevalence, severity and incidence.

Class	Prevalence[1] %	Severity[2] (Cobb-Scale: 0-9) H	Severity	Incidence[3]
I	0-10	0-3	0-2	Insignificant
II	10-20	3-5	2-3	Low
III	20-40	5-6	3-5	Moderate
IV	40-60	6-8	5-7	High
V	60-100	8-9	7-9	Severe

[1](Number of infected fields (STB present)/total number of fields surveyed) × 100.
[2]H: Level of plant infection.
[3]Percentage of STB within each class level at surveyed field.

indicates how wide spread is STB, whereas incidence conveys information on the risk of the disease within a severity range. In the survey protocol of the CIMMYT septoria phenotyping platform four classes (Class I-V) were adopted. Class I: Low prevalence (%), severity (0-9), and low incidence indicating insignificant risk. Class II: low prevalence and incidence indicating low risk. Class III: moderate prevalence and incidence indicating moderate risk to be monitored. Class IV: fields where STB was wide spread and apparent high severity observed at flag leaf, indicating high risk of the disease. Class V: includes fields heavily infested by STB; situation where the disease is obvious at each field surveyed and where the severity is at its most, i.e. severe symptom on flag leaf and spikes, this is a situation where STB is a high risk. Relevant agronomic data such as variety name, sowing date, fertilization, crop density and spatial pattern and previous crops were recorded. Altitude, longitude and latitude were also recorded using Global Positioning System (GPS).

Data analysis

Linear mixed model was used to analyze disease data (incidence and severity) collected during the survey using ASReml-R software (Gilmour et al., 2002). The years, regions, species, varieties together with their interactions were assumed to be fixed.

RESULTS

Incidence of wheat Septoria tritici blotch

Survey results showed that STB incidence on bread wheat was very limited across surveyed areas in Northern Tunisia *Triticum* species and wheat varieties identified during the survey during the two cropping seasons are presented in Table 4. STB prevalence during the two cropping seasons (2015-2016 and 2016-2017) was insignificant to low on commercial bread wheat varieties in the majority of the surveyed regions (Tables 5 and 6). It was ranked as class I or II except at

Table 4. *Triticum* species and wheat varieties identified during the survey during the two cropping seasons.

Species	Varieties	Surveyed regions
Bread wheat	Zanzibar	Bizerte
	Utique	Bizerte, Beja, Zaghouan
	Haïdra	Bizerte, Beja, Zaghouan
	Vaga	Bizerte, Jendouba
	Salambo	Cap Bon North (El Haouaria), Jendouba
	Bread wheat landrace	Cap Bon North (El Haouaria)
Durum wheat	Karim	Bizerte, Beja, Jendouba, Manouba, Cap Bon North, Cap Bon South, Kef
	Maali	Bizerte, Beja, Cap Bon North, Cap Bon South, Jendouba, Manouba, Kef
	Razzak	Bizerte, Beja, Cap Bon North, Cap Bon South, Jendouba, Zaghouan, Kef
	Monastir	Cap Bon North
	Carioca	Bizerte, Jendouba, Zaghouan
	Saragolla	Bizerte
	Soudaine	Bizerte
	Sculpture	Bizerte, Beja, Jendouba
Triticale	TL4	Bizerte, Cap Bon North,
	Bienvenue	Bizerte, Manouba

Table 5. Prevalence of Septoria tritici blotch in inspected areas during 2015-2016.

Region/District	Number of surveyed fields			Number of infected fields			Prevalence (%)[2]		
	DW	BW	Trit	DW	BW	Trit	DW	BW	Trit
Bizerte	6	7	3	6	2	1	100.00	33.33	33.33
Cap Bon South[1]	8	1	0	8	0	0	100.00	0.00	0.00
Cap bon North (El Haouaria)	3	9	2	2	9	2	66.66	100.00	100.00
Manouba	5	0	1	4	0	1	80.00	0.00	100.00
Beja	4	2	0	4	1	0	100.00	50.00	0.00
Jendouba	3	2	1	3	0	1	100.00	0.00	100.00
Total/mean	29	21	7	27	12	5	93.10	57.14	71.42

[1]Cap Bon South: Soliman, Beni Khalled, and Grombalia.
[2]Prevalence: Number of infected field/number of surveyed field.
DW: Durum wheat; BW: bread wheat, Trit: triticale.

Table 6. Prevalence of Septoria tritici blotch in inspected areas during 2016-2017.

Region/District	Number of surveyed fields			Number of infected fields			Prevalence (%)[2]		
	DW	BW	Trit	DW	BW	Trit	DW	BW	Trit
Bizerte	15	4	3	14	3	1	93.33	75.00	33.33
Cap Bon South[1]	5	0	0	2	0	0	40.00	0.00	0.00
Cap Bon North (El Haouaria)	2	7	1	0	7	0	0.00	100.00	0.00
Manouba	2	0	0	1	0	0	50.00	0.00	0.00
Beja	5	1	0	5	0	0	100.00	0.00	0.00
Jendouba	3	1	0	3	0	0	100.00	0.00	0.00
Zaghouan	7	4	1	6	1	0	85.71	25.00	0.00
El Kef	8	0	0	8	0	0	100.00	0.00	0.00
Total/mean	47	17	5	39	11	1	85.10	66.66	20.00

[1]Cap Bon South: Soliman, Beni Khalled, and Grombalia.
[2]Prevalence: Number of infected field/number of surveyed field.
DW: Durum wheat; BW: bread wheat, Trit: triticale.

El Haouaria (region A, Figure 1) where prevalence and incidence were relatively high (Tables 5 and 6; Figures 2 and 4) putting it as class IV-V level (Table 3). Insignificant prevalence levels were recorded at Zaghouan, Bizerte, and Beja (Tables 5 and 6). Even though relatively high incidence was recorded at Bizerte, the severity was still low; hence it is of low to moderate risk (class II-III). The high incidence observed at Beja was only at 1 out of 2 fields surveyed, hence it is not quite representative and we considered it low. The mean disease incidence and severity on bread wheat landrace reached the maximum levels in 2016 with 84% and 52% respectively at El Haouaria (Figures 2 and 3). Same trend was observed in 2017, where over 77% and 32% disease incidence and severity respectively were recorded in the same region (Figures 4 and 5). Low STB incidence on bread wheat were recorded in 2016 on bread wheat fields at Bizerte 23% and

Figure 2. Incidence of Septoria tritici blotch during 2016 in surveyed areas on three cereal crops: bread wheat (BW), durum wheat (DW), and triticale (TRIT).

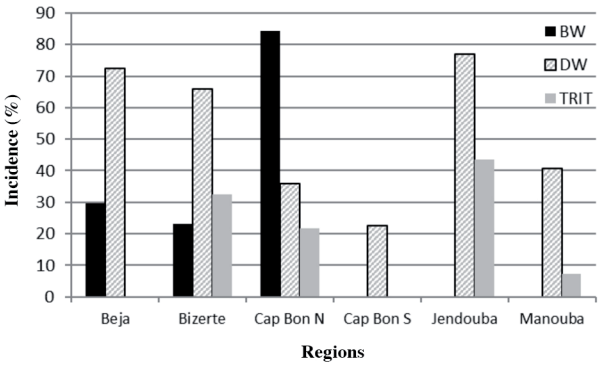


Figure 3. Severity of Septoria tritici blotch during 2016 in surveyed areas on three cereal crops: bread wheat (BW), durum wheat (DW), and triticale (TRIT).

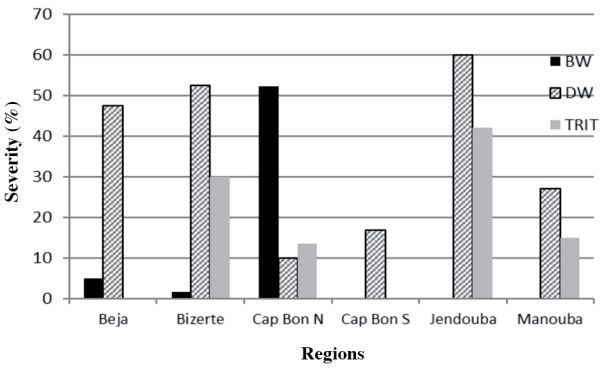
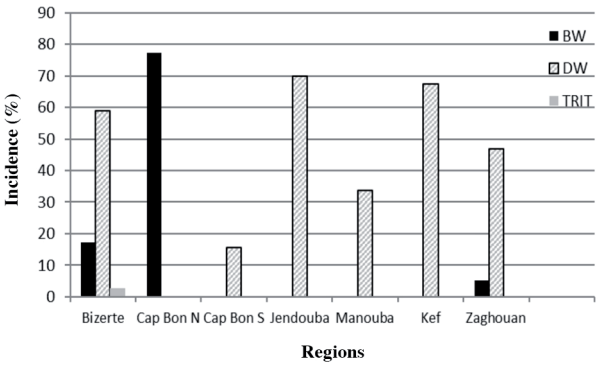


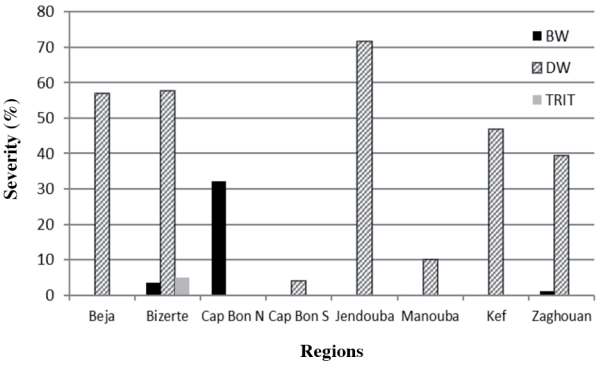
Figure 4. Incidence of Septoria tritici blotch during 2017 in surveyed areas on three cereal crops species: bread wheat (BW), durum wheat (DW), and triticale (TRIT).



Beja 29%, and in 2017 at Bizerte 17% and Zaghouan 5% (Figures 2 and 4). The severity percentages in these regions did not exceed 5% during the two cropping seasons (Figures 3 and 5). These data showed that STB level was at class I and II ratings except at El Haouaria, where it was rated class IV and V.

Unlike the situation on bread wheat, STB was widely distributed on durum wheat and was highly prevalent at Bizerte, Beja, Jendouba, and El Kef where it ranked from class III to V (Table 3). The overall prevalence of the disease was about 50%, 85.71%, 93.3% at Manouba, Zaghouan, and Bizerte, respectively, in 2017 (Table 6). More than 65% and 47% of the disease incidence and severity respectively were recorded in the majority of prospected areas (Beja, Bizerte, Jendouba and

Figure 5. Severity of Septoria tritici blotch during 2017 in surveyed areas on three cereal crops: bread wheat (BW), durum wheat (DW), and triticale (TRIT).



El Kef) compared to 35.77% and 10% in Cap Bon regions during the surveyed period in 2016 (Figures 2 and 3). STB was also found on durum wheat varieties in Southern of Cap Bon area such as Grombalia, Soliman and Beni Khalled (region B, Figure 1) with a prevalence of 100% and 40% during 2016 and 2017 respectively (Tables 5 and 6). In 2017, STB was not observed on durum wheat at El Haouaria.

STB on triticale was observed at only four regions to include Jendouba, Bizerte, Cap bon and Manouba. It was more prevalent (100%) in Jendouba, Cap Bon North and Manouba in 2016 followed by Bizerte 33% during the two survey years (Tables 5 and 6). Greater mean incidence of STB was recorded on triticale at Jendouba (43%, Figure 2) and more than 20% was noted at Bizerte and Cap Bon North (Figure 2). The overall mean severity varied from 13% to 42% in 2016 cropping season (Figure 3). However, STB was very low on triticale at Bizerte region with 3% and 5% disease incidence and severity respectively during 2017 (Figures 4 and 5).

Incidence of Septoria tritici blotch on commercial wheat varieties

Even though the variety distribution between years and surveyed areas varied, general trends show that most durum wheat varieties were highly susceptible to STB at different levels (Figures 6 and 7). The disease incidence reached 100% on the commercial durum wheat varieties 'Saragolla', followed by 'Soudaine' (90%), 'Carioca' (80%) and 'Sculpture' (60%). High incidence was also recorded on the lead commercial durum wheat 'Razzak' (75%), 'Maali' (60%), and 'Karim' (45%) (Figure 6). The lower incidence of the local cultivars was showed by the low STB levels at Cap Bon region, particularly that of 'Karim' that could have been affected by late planting. Despite the high STB disease pressure on durum wheat across the surveyed areas, it was nearly absent at El Haouaria (Cap Bon North) where mainly bread wheat was cultivated. In 2016 high STB incidence (90%) and severity (70%) were observed mainly on the bread wheat landrace ('Farina arbi') at El Haouaria (Figures 6). Mean incidence and severity of 30% and 25%, respectively, were recorded on the bread wheat 'Salambo'. Lower rates (< 10%) were recorded on other commercial bread wheat varieties such as 'Zanzibar', 'Utique' and 'Haïdra', which were below 10%. When tested at experimental station in Northern Tunisia, 'Farina arbi' and the other bread wheat varieties showed no infection of STB despite high levels of infection on most if not all commercial durum wheat varieties. In addition, low levels of susceptibility to STB were recorded on triticale varieties where incidence and severity ranged from 0% to 30%. Out of three triticale varieties, the disease was totally absent on 'Bienvenue' (Figures 6 and 7).

Figure 6. Incidence of Septoria tritici blotch on durum wheat, bread wheat and triticale varieties.

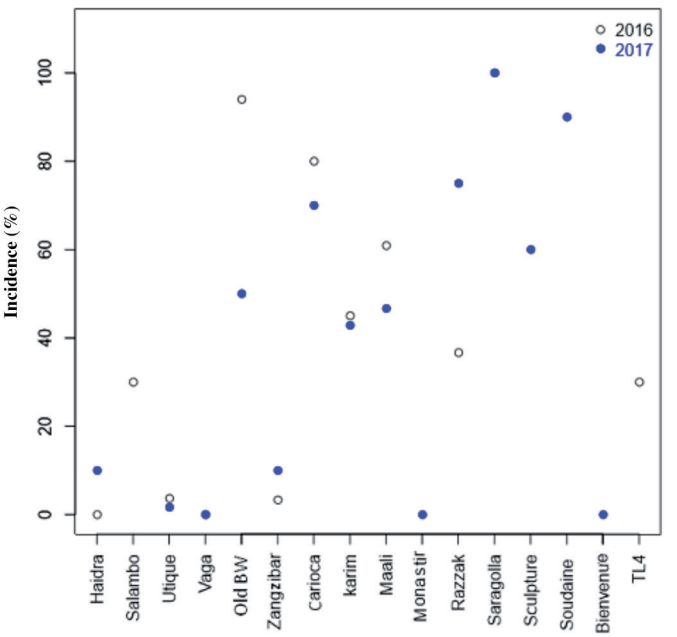
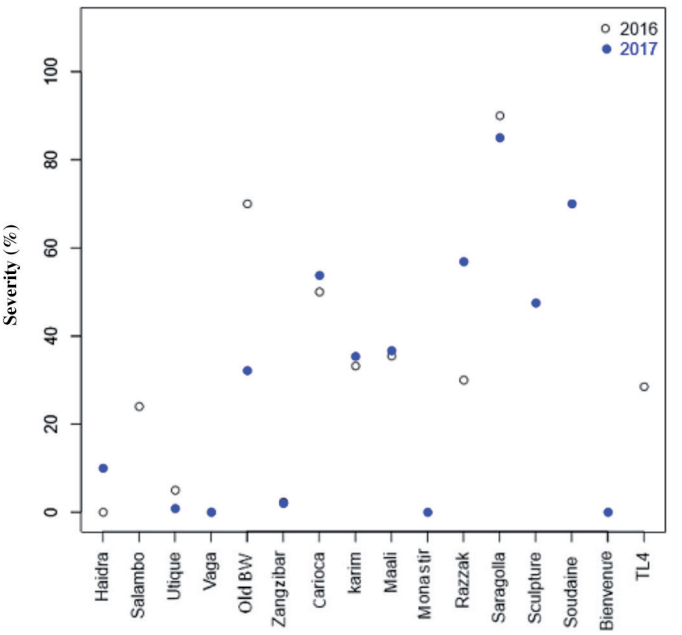


Figure 7. Severity of Septoria tritici blotch on durum wheat, bread wheat, and triticale varieties.



DISCUSSION

The response of durum wheat, bread wheat, and triticale to *Z. tritici* varied according to the crop species. During the surveyed period, *Z. tritici* was more prevalent on durum wheat at the majority of surveyed areas except Cap Bon North (El Haouaria) and confirms the high to moderate risk of STB at Northern and Cap Bon regions of Tunisia, respectively.

This result supports conclusions of previous reports and confirms that Septoria diseases hot spots are prevalent in the sub humid and semi-arid areas at the beginning of winter season (Fakhfakh et al., 2011). The new commercial durum wheat 'Sculpture', 'Saragolla', 'Carioca' and 'Soudaine' were susceptible to Septoria as they were mainly grown at Septoria hot spots where monoculture of durum wheat particularly the susceptible 'Karim' and relatively high rainfall contributed to the development of high infection levels. In particular, high incidence and severity were recorded on 'Karim' and 'Razzak', which confirmed previous findings conducted by Ltifi and Sakkouhi (2008), and Ben Mohamed et al. (2000). In contrast, 'Maali', which was previously characterized by a good level of resistance in Beja (Gharbi et al., 2011), was susceptible to STB in the majority of surveyed areas in this study, which could be explained by a slow decline of host resistance (Kema et al., 2018). The survey data also revealed that triticale was also susceptible to STB across the majority of surveyed areas posing therefore a serious threat to this crop.

On the other hand, the data revealed that STB was very low in the majority of inspected regions on the commercial bread wheat cultivars such as 'Haïdra', 'Vaga', 'Utique', and 'Zanzibar', which could explained by the relative resistance of these varieties to Septoria (Ben Hamouda et al., 2016) while it was higher in 'Salambo' (Saade, 1996). 'Salambo' was released in 1980, period that has known a substantial expansion in bread wheat acreage particularly for varieties with high yield and good level of diseases resistance. It seems that this variety has undergone a slow decline of host resistance over time that is commonly observed in this pathosystem, particularly for bread wheat in Europe (Kema et al., 2018).

Surprisingly from this study, STB on bread wheat poses a great risk only at one region, El Haouaria, where it was rated class IV and V and mainly only on the old bread wheat landrace 'Farina arbi'. The important incidence of Septoria observed on this variety reveals a specific presence of *Z. tritici* population that only develops on this old bread wheat landrace with little or no apparent effect on other bread wheat varieties. This could be mainly associated with the wheat-based mono-cropping system and monoculture of a land race over several decades facilitating thereby the adaptation of the pathogen to this specific variety (Holloway, 2014; McDonald and Mundt, 2016). Similar research reviews on wheat diseases surveys (Teferi and Gerbreslassie, 2015; Takele et al., 2015; Unal et al., 2017) showed that the impact and distribution of diseases varied due to the continuous release and extensive cultivation of susceptible varieties.

Thus, the magnitude of 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 wheat area as compared to that of bread wheat (Yahyaoui et al., 2000).

Testing 'Farina arbi' land race for its resistance/susceptibility to *Z. tritici* at other Northern regions where durum wheat is mostly cultivated showed no STB infection. This unique bread wheat landrace, completely susceptible at El Haouaria (North eastern Tunisia) and completely resistant at Beja Northwestern Tunisia, could be that we are definitely dealing with two distinct *Z. tritici* populations and could give more highlight on STB specificity. Further studies will be conducted to characterize the STB populations from El Haouaria that are mostly specific to the bread wheat land races ('Farina arbi') and have no effect on other bread and durum wheat varieties. Such phenomenon has not been observed before and could lead to further understanding of STB host specificity.

CONCLUSIONS

The survey data revealed low risk of *Zymoseptoria tritici* on bread wheat except at Cap Bon region especially at El Haouaria where Septoria tritici blotch (STB) severity was relatively high on the old bread wheat landrace, while rare occurrence at other sites was observed on some commercial bread wheat varieties. Sporadic incidence and high severity were observed on triticale across the surveyed fields. Although Tunisia is primarily a durum-wheat producing country with *Z. tritici* being mostly prevalent on durum wheat; bread wheat is of great economic importance, even though it occupies small areas. The occurrence of STB on the landrace could lead to development of Septoria population that could become of major importance on bread wheat as is the case in Morocco and other regions. The presence of an STB population at one site and infecting a single cultivar will be further investigated and will possibly lead to better understanding of *Z. tritici* population dynamics that could become an important tool in screening for disease resistance.

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