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Septoria tritici blotch disease progression and physiological traits variation in durum wheat variety mixtures

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Abstract - Variety mixture, a sustainable disease management approach based on functional diversity, is regaining considerable attention by many research groups studying different pathosystems. In this respect, few studies focused on the wheat-*Zymoseptoria tritici* pathosystem. *Z. tritici*, commonly known as the causal agent of Septoria tritici blotch (STB) disease, poses a serious and persistent challenge to durum wheat production where chemical control has become the main control measure available to farmers in Tunisia. In this study, we investigated STB disease progression and the physiological traits behavior during wheat growth stages in two-way mixtures and individual components. Three durum wheat varieties (Monastir, INRAT100 and Karim) with different levels of resistance to STB were assessed in two-way mixtures in various proportions and in four replicates during the cropping season 2018-2019. Disease assessment after heading stage showed that adding 25% or 50% of resistant variety to the susceptible pure stand resulted in a significant disease reduction. However, the efficacy of added 25% of a resistant variety in decreasing the disease seems to be variety dependent. Mixtures with 25% of 'Monastir' showed better performance than those with 25% of 'INRAT100'. Varietal physiological evolution of the canopy showed that STB severity level was negatively correlated to chlorophyll content, normalized difference vegetation index and leaf relative water content. This demonstrates that certain physiological traits can be suitable for screening for resistance to Septoria tritici blotch within-field diversity through the use of variety mixtures.

Keywords: *Zymoseptoria tritici*, durum wheat, variety mixtures, disease progression, physiological traits, sustainable disease management.

1. Introduction

Tunisia is a major durum wheat producer in the Mediterranean region. Durum wheat occupies an average of 800 000 ha of cultivated area and production is around 5.8 million quintals per year (for the last 18 years) (ONAGRI 2018). Durum wheat (*Triticum turgidum* ssp. *durum*) is the basis for the traditional dishes such as couscous and pasta, placing the country as one of the largest cereal per capita consumer of wheat in the world (174.3 kg/ habitat/ year on 2015) (Khaldi 2017). In the Mediterranean and particularly in Tunisia, durum wheat production has been highly affected by fungal diseases as compared to bread wheat. Susceptibility to *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch disease, has been well documented (Yahyaoui et al. 2000; Bel Hadj Chedli et al. 2018) as one of the most important diseases in the Mediterranean basin and it has become a serious inherent problem to Tunisian durum wheat production where the currently cultivated high yielding commercial varieties have become very susceptible to Septoria in addition to other factors such as high seeding rate, early sowing, excessive use of fertilizers and limited control of fungicide applications. The estimated grain yield losses in durum wheat caused by STB disease exceed 40% under conducive conditions (Berraies et al. 2014a).

In general, disease management mainly relies on the use of fungicides and resistant varieties. Yet, chemical control is costly and affects the environment. To-date 21 resistance genes for STB have been identified in bread wheat (Brown et al. 2015) and very few in durum wheat (Aouini 2018). Recent studies of the genetic basis of resistance in durum wheat in Tunisia (Aouini 2018) found that the resistance of 'Agili 39' landrace is due to a natural pyramiding of 3 QTLs; this led to further investigation on durum wheat landraces (Yahyaoui et al. 2019) and intensive crossing of Tunisian varieties (Berraies et al. 2014b) where new sources of resistance are being identified and integrated in breeding programs.

Moreover, the high genetic diversity (Hartman et al. 2018), the genome plasticity of the pathogen (Wittenberg et al. 2009) and the monoculture cropping that reduces host diversity (Wolfe 1985; Rezgui et al. 2008) led to the breakdown of the resistance genes. Hence, there is a need to identify new strategies for identification and deployment of durable resistance as well as introducing alternative disease management strategies to control the disease and reduce the use of chemicals.

A sustainable approach based on the functional diversity principle such as variety mixtures, could be a promising strategy in reducing diseases and increasing yield (Wolfe 1985; Borg et al. 2018; Kristoffersena et al. 2019). Different mechanisms explain mixtures effect on disease progression such as dilution effect, barrier effect, compensation effect (Wolfe 1985), and microclimate modification effect (Vidal et al. 2017). Few studies investigated the effect of durum wheat cultivar mixtures on STB disease; such as the study of Ben M'Barek et al. (2020) that showed its efficacy in reducing disease level based on Automated Image analysis.

Physiological traits are known to sign stress effects indirectly. In fact, during biotic or abiotic stresses, plants decrease all sorts of physiological product losses. The stomata closure is one of the reactions which decreases transpiration rate explaining thereby the high temperature level registered on the infected plant (Lindenthal et al. 2005). The biochemical (chlorophyll and water contents) and the agro-morphological changes during pathogenesis were specifically and highly detectable using the hyperspectral imaging sensing (Mahlein 2016). Hence, early disease detection can be possible with physiological behavior plant analysis (Thomas et al. 2017). Several studies investigated the relationship between disease severity and physiological parameters, using either the hand held instruments or the innovative technology such as the hyperspectral sensors and image analysis, that showed a correlation between several parameters and disease severity (Rosyara et al. 2007; Rosyara et al. 2008; Ashourloo et al. 2014; Yu et al. 2018). However, the relationship between these traits within-field diversity has not been explored so far.

A physiological investigation could thus be a new approach to improve disease detection or assessment, particularly within variety mixtures.

In this study, we investigated the disease progression of STB and the physiological traits in two-way mixtures and their individual components during wheat growth stages in order to identify physiological traits that could be used in screening for resistance to Septoria tritici blotch within-field diversity through the use of variety mixtures.

2. Materials and Methods

2.1. Experimental design

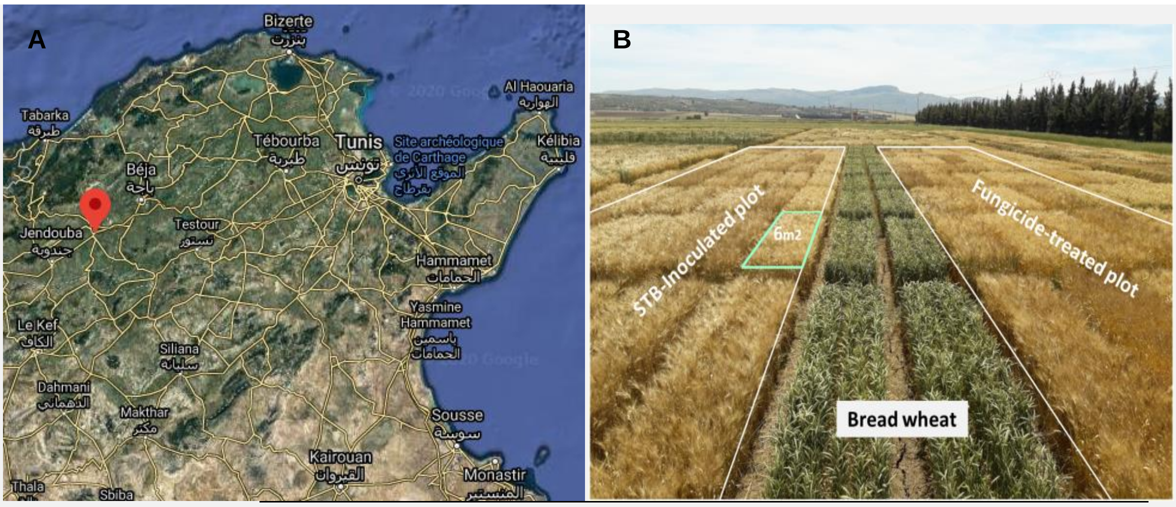
2.1.1. Study area

A field experiment was conducted during 2018-2019 cropping season at the CRP Wheat Septoria Precision Phenotyping Platform-experimental station of Kodia (National Institute of Field Crops), located in the North-West of Tunisia in the semi-arid region, governorate of Jendouba (36°32'51.89 N, 9°0'40.73E) (Figure 1). This region is known for its durum wheat production as well as a major hot spot for STB disease and characterized by a wet weather with annual rainfall means ranging from 400 to 500 mm and a temperature that varies between 9.8 °C to 33 °C.

2.1.2. Wheat varieties and field experiment

Three durum wheat varieties with different resistance level to STB were used in this experiment; Karim, which is highly susceptible to STB and the most cultivated variety occupying more than 60% of durum wheat cultivated areas in Tunisia, INRAT100, moderately resistant and newly released variety, and Monastir, resistant and recently introduced variety that occupies around 5% of durum wheat areas.

In total, there were 10 treatments: three pure stands (Karim, Monastir and INRAT100) and seven two-way mixtures. The mixtures with Karim contained always 50%, 75% or 87.5% of Karim, the rest being resistant varieties. The experimental layout was a complete randomized block design with four replicates. Spatial arrangement of treatments and replicates are shown in Figure 1. A total surface area of 540 m^2 was divided into two equal plots that we named fungicide-treated (protected) and inoculated (infected) plots. The bread wheat varieties were sown in the middle and used as a separation between the two plots. The inoculated plot was subdivided into microplots that were used to inoculate with *Z. tritici*, while the fungicide-treated plot as its name suggests, was devoted to fungicide application (Figure 1).



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	Treatments									
	1	2	3	4	5	6	7	8	9	10
Karim (%)	100	0	0	75	75	50	50	87,5	87,5	0
INRAT100 (%)	0	100	0	25	0	0	50	12 ,5	0	50
Monastir (%)	0	0	100	0	25	50	0	0	12,5	50

Figure 1. (A) Satellite imagery of the study area. **(B)** Spatial arrangement of the ten treatments planted in four replicates. **(C)** Proportions of varieties in pure stands and in two-way mixtures corresponding to ten treatments and planted in four replicates in 2018/2019 growing season at the Septoria Phenotyping Platform (Bou Salem, Tunisia).

The ten different treatments were sown on the same date, November 8, 2018, in an irrigated system. Standard agronomic practices were used to ensure adequate crop development. Plots were maintained and treated the same way except for the inoculation and fungicide treatments. To ensure little or no disease on the control plots, three fungicides composed of different active ingredients: Cherokee (chlorothalonil, propiconazol and cyproconazol), Opus (epoxiconazol) and Ogam (epoxiconazol and kresoxim-methyl), were applied at a dose rate of 1,5 $L.ha^{-1}$, 0.75 $L.ha^{-1}$ and 0.7 $L.ha^{-1}$, respectively during wheat plant growth stages at three dates; on January 22, February 27, March 14, 2019. Two additional treatments were applied using the Ogam fungicide on April 11 and May 3, 2019.

2.1.3. Inoculation

STB inoculum was prepared at the CRP Wheat Septoria Platform laboratory at the National Agronomic Institute of Tunisia (INAT), from five stored *Z. tritici* isolates that were obtained from infected leaves of durum wheat collected in the same region. Approximately 600 ml of the spore suspension was applied per microplot at a concentration of $1x10^6$ spores ml^{-1}. Tween 20 (Merek, UK) (0.1%) was added to the suspension. Wheat plants were inoculated after sunset using a sprayer (Efco AT800, Italy) three times during tillering stage (from GS13 to GS26) (Zadoks et al. 1974); on December 13, December 26, 2018 and on January 10, 2019.

2.2. Data collection

2.2.1. Disease assessment

After heading stage (GS50) (Zadoks et al. 1974), STB progression was evaluated by measuring STB incidence and severity based on the Saari and Prescott double digit scale (00-99) (Eyal et al. 1987). At five different point dates, ten random spikes previously labeled with a blue tape, were assessed following a zigzag path on the inoculated pure and mixed stands. The final disease severity (%) for each microplot is the average of the ten assessed spikes following the formula below:

$$\mathbf{Ds\ (\%) = (STB\ incidence\ /9) \times (STB\ severity\ /9) \times 100}$$

The Area and relative Area Under Disease Progress Curve (AUDPC and rAUDPC) were calculated according to Simko and Piepho (2012):

$$\mathbf{AUDPC} = \sum_{i=1}^{n-1} \frac{(Ds_{i+1}+Ds_i)}{2} \times (t_{i+1}-t_i)$$

Where:
Ds_i (%) = disease severity at time t_i
t_i = date on which the disease was scored
n = numbers of scoring events

$$\mathbf{rAUDPC} = \frac{\textbf{AUDPC genotype}}{\textbf{AUDPC Karim}}$$

Where Karim is the susceptible variety used as a check.

2.2.2. Physiological parameters

In this study four physiological traits were measured on both fungicide treated and inoculated plots in order to evaluate STB and the variety mixture effects on the green biomass, photosynthesis activity and on the water and temperature level variation. Canopy temperature depression (CTD) was measured by estimating the canopy temperature of each plot using a hand-held infrared thermometer Sixth sense LT 300 and applying further the formula below according to Rosyara et al. (2008):

$$\mathbf{CTD = AT - CT}$$

Where:
CTD (°C): Canopy temperature depression
AT (°C): Ambient temperature
CT (°C): Canopy temperature

The normalized difference vegetation index (NDVI) and the leaf chlorophyll content (CC) were determined using the GreenSeeker® hand held crop sensor and a self-calibrating Minolta chlorophyll meter (SPAD-502), respectively (Pask et al. 2012). Ten spikes were selected and labeled with a blue tape to determine the CC on the higher fully emerged leaf during seedling and jointing stages and on their flag leaves when they are fully emerged.

During grain maturity corresponding to GS75, leaf relative water content (RWC) was quantified using Barrs and Weatherley's formula (1962):

$$\mathbf{RWC\ (\%) = (TW - FW) \times 100 / (TW - DW)}$$

Where:
FW (g): Fresh weight (FW = weight of the tubes containing samples – weight of tubes)
DW (g): Dry weight
TW (g): Turgid weight

Based on the protocol described by Mullan and Pietragalla (2011) with slight modifications, ten flag leaves were taken during grain maturity (GS75) from randomly chosen plants for each microplot. These were randomly cut- approximately 5 cm mid-section- and placed immediately into the pre-weighed tubes together and kept cool to avoid leaves dehydration. Using a precision scale, tubes containing 5 cm leaves samples were weighed to obtain the fresh weight (FW) then, 15 ml of distilled water was added to each tube. The tubes were placed in a refrigerator, at 4°C in darkness for 24h to obtain the full turgor. Subsequently, leaves were carefully blotted dry and weighed to obtain the turgid weight (TW). Dried leaves were then placed in the oven at 60°C for 48h and reweighted to measure the dry weight (DW).

2.3. Statistical analysis

All measured variables were subjected to analysis of variance (ANOVA) using procedure MIXED (SAS Institute Inc. 2002). Fisher's protected least significant difference (LSD) test was used for multiple treatment comparisons using the LSMEANS of SAS 9.0 (SAS Institute Inc. 2002) with letter grouping obtained using SAS pdmix800 macro (Saxton 1998). Separations were significant at $p<0.05$. Principal component analysis (PCA) was performed using the JMP®11.0 statistical software (SAS Institute 2014).

3. Results and Discussion

3.1. STB disease progression in pure and mixed stands

The objective of this study was to follow the disease progression in pure and mixed stands to determine the best combination for better resistance to STB disease compared to the susceptible pure stand of Karim and finally to identify physiological traits correlated with STB disease in pure as well as in mixed stands. The choice of the variety Karim is based on the fact that this variety is mostly grown in Tunisia for at last three decades. Although very susceptible to STB, it is considered as the farmers's favourite

due to its large adaptation and productivity (Ammar et al. 2011). The other two varieties, Monastir and INRAT100 are new promising varieties that have shown good resistance level to STB and good yield.
At seedling stage, we evaluated disease progression of the pure stands and difference in disease severity level of the three pure stands on three different dates (GS30, GS33 and GS37-45), that was clearly visible (Figure 2(A)).

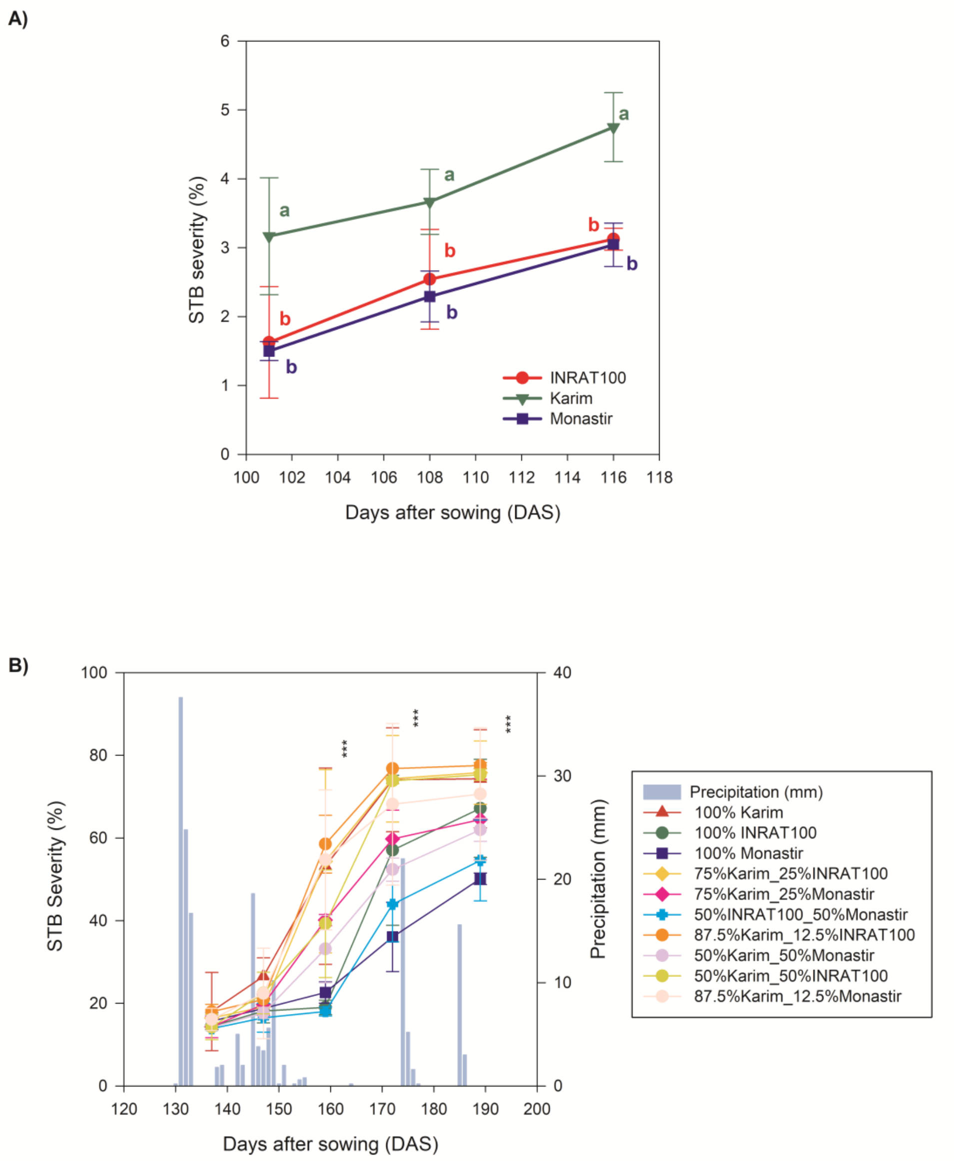

Figure 2. (A) STB severity (%) progression during seedling stage presented by day's number after sowing (DAS): on the inoculated pure stands of the three varieties; Karim (green line), INRAT100 (red line) and Monastir (blue line). STB was assessed using the 0-5 scale. Letters above the lines indicate statistical significance of pairwise differences ($p<0.05$). **(B)** STB disease severity (%) of the ten treatments at five dates presented as day's number after sowing (DAS): 137, 145, 157, 172 and 189 corresponding to growth stages from GS55 to GS87 and precipitation rate (mm) during the growing season. STB disease was visually assessed using the double digit scoring scale.

Karim variety reached the maximum disease level 5 on a 0-5 severity scale compared to INRAT100 and Monastir which showed a lower susceptibility to STB reaching a score of 3; STB severity was 40% higher on the pure stands of the susceptible variety Karim.
From the heading stage (<GS50), disease assessment of the ten treatments of the inoculated plot gave a clear indication of STB severity progress in the mixtures and their pure stands (Figure 2 (B)). For all the treatments and during the first two assessments (137 and 145 days after sowing: DAS), we noticed a slow-disease progression. Subsequently, favorable temperature, humidity and the recorded precipitation that occurred from 140 DAS (GS55) to 155 DAS (GS70) contributed to a significant increase in disease severity at the third assessment, 157 DAS (GS73). From that time point, we could clearly observe a significant difference of the disease level between the ten treatments; the pure stands of Monastir and INRAT100 and their corresponding mixtures (50%INRAT100_50%Monastir) showed the lowest disease level, followed by the two mixtures 3:1 and 1:1 of Karim with Monastir (25% and 50%) and the

1:1 ratio mixture of Karim with INRAT100 in the middle. The variety Karim in pure stand together with, the two mixtures of 87.5% of Karim with either Monastir or INRAT100 (7:1) and the 75% Karim mixture with INRAT100 (3:1) had the highest disease level.
During the fourth assessment (172 DAS, GS75), disease level increased by more than two-fold particularly for the 1:1 ratio mixture of Karim with INRAT100 and the pure stand of INRAT100, that increased from 19% to 50% similar to the pure stand of Karim and unlike that of the pure stand of Monastir (from 22% to 36%). From this point onwards, we noticed a nearly similar trend for all the treatments with continual increase of disease severity though with a slight difference between the treatments. This heterogeneity effect of the seven mixtures reflects the co-existence of different genetic resistance sources reducing the general pathogen fitness and aggressiveness (Borg et al. 2018) which was also clearly observed even with the 1:1 ratio mixture of the two resistant varieties Monastir and INRAT100, that reached more than 50% in disease severity less than that of the pure stand of INRAT100. Another possible explanation could be that the leaf dimension and curvature play an important role on spore dispersal by rain-splash on the canopy (Vidal et al. 2017). The difference of this trait between the three used varieties was not studied in this work but it is an important factor to consider for further mixtures studies. Moreover, even though the ecological benefits of crop diversification are clearly recognized, it is not known which traits plants should have to maximize these benefits. This is because genotypes that have been bred to perform best in monocultures may not be optimal for mixtures and this could be the case for variety INRAT100 when grown in mixtures.

3.2. Mixtures performance on disease severity reduction

In this study, we also calculated the area and the relative area under disease progress curve, AUDPC and rAUDPC respectively, to quantify STB reduction in the different treatments in comparison to the pure stand of Karim. Three S:R (susceptible: resistant) ratios were tested in this trial; 7:1, 3:1, and 1:1 ratios mixtures. Results illustrated that mixing Karim with Monastir showed a better performance in terms of disease severity reduction with lower AUDPC values compared to the mixtures of Karim with INRAT100 (Figure 3). Moreover, rAUDPC values of the three mixtures of Karim with INRAT100 ranged from 0.2 to 0.6 and even higher than 1 (reference value of rAUDPC for the susceptible check Karim, Table 1) on the 7:1 ratio mixture. Although moderately resistant during the early growth stages, INRAT100 mixed with Karim showed a weak performance on STB progression. On the other hand, the three mixtures of Karim with Monastir showed a decrease in the AUDPC values correlating with an increase of the resistant variety proportion in the mixture (Figure 3).

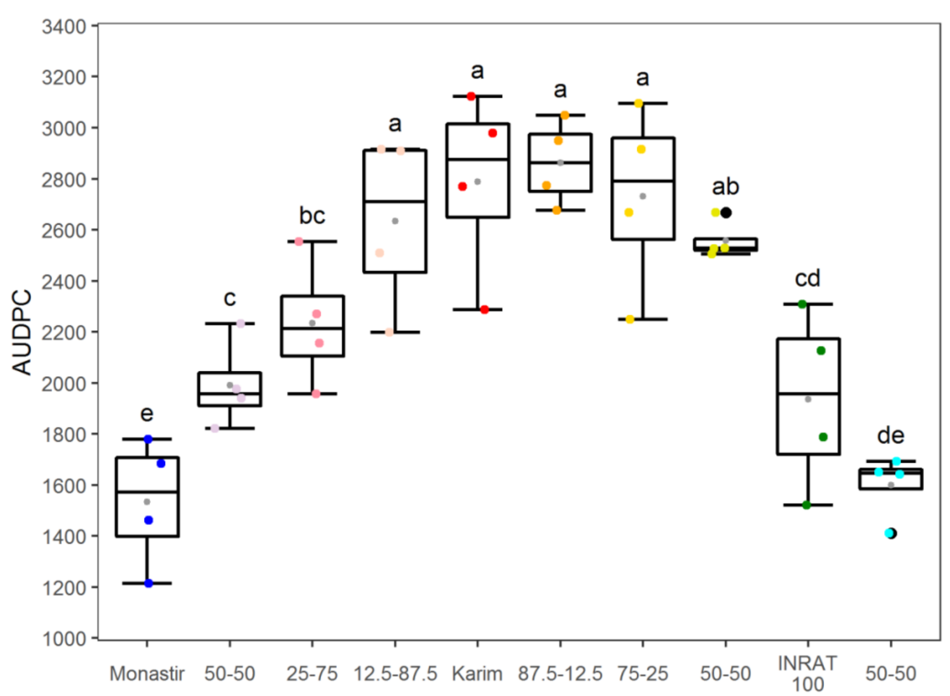

Figure 3. Box plots showing the AUDPC of the ten inoculated treatments; from the middle: Karim in pure stand, to the left: decreasing percentage of Karim in two-way mixtures with Monastir to the pure stands of Monastir; to the right: decreasing percentage of Karim in two-way mixtures with INRAT100 to the pure stands of INRAT100 and finally mixture of INRAT100 with Monastir in equal proportion. Gray and black dots represent the mean values and the outliers, respectively. Letters above the box plots indicate statistical significance difference ($p<0.0001$).

Mixtures including 12.5% of resistant variety either Monastir or INRAT100 did not reduce disease progression; on the contrary they were similar to the susceptible pure stand of Karim (Figure 3). Therefore, the 7:1 ratio mixture (S: R respectively) were not efficient in disease reduction and seem not suited for this specific location. However, this does not preclude that this combination would behave differently under natural epidemics and in another environment. Our results are in agreement with that of Ben M'Barek et al. (2020) that used the Automated Image Analysis (AIA) technique to quantify STB progression and which showed that adding 25% of the resistant variety Monastir to the susceptible Karim substantially reduced STB disease. In our study, the recorded rAUDPC in the mixture (25%Monastir_75%Karim) was around 0.8 (Table 1). However, this was not the case with INRAT100 despite the contrasting levels of resistance between the pure stands of Karim which is susceptible and INRAT100 which is a moderate resistant.
These results support the importance of the choice of the varieties in mixtures which has been discussed by many authors (Borg et al. 2018; Ben M'Barek et al. 2020; Kristoffersen et al. 2019; Vidal et al. 2017).

Table 1. Mean of relative Area Under Disease Progression Curve (rAUDPC) of the ten inoculated treatments; Karim variety was used as the susceptible check

Treatments	Mean ± SE
Monastir	0.56 ± 0.01^{e}
50%INRAT100-50%Monastir	0.57 ± 0.01^{de}
INRAT100	0.69 ± 0.01^{dce}
50%Monastir-50%Karim	0.72 ± 0.01^{dc}
25%Monastir-75%Karim	0.8 ± 0.01^{bc}
12.5%Monastir-87.5%Karim	0.94 ± 0.01^{ba}
50%Karim-50%INRAT100	0.93 ± 0.01^{ba}
75%Karim-25%INRAT100	0.97 ± 0.01^{a}
Karim	$\mathbf{1\pm0.01^{a}}$
87.5%Karim-12.5%INRAT100	1.042 ± 0.01^{a}

a-e: different letters indicate significant statistical differences (α= 0.05, *t* test (LSD))

3.3. Physiological behavior of mixed and pure stands under STB disease pressure

ANOVA analysis was carried out for each physiological parameter namely the chlorophyll content (CC), the normalized difference vegetation index (NDVI), the canopy temperature depression (CTD) and the leaf relative water content (RWC) at each date separately (Table 2). At early growth stages GS55-60, corresponding to time point 119 DAS, we already observed a difference between the inoculated and fungicide–treated plots for the measured CC and CTD. At GS73, CC and NDVI displayed significant differences between the ten treatments and at the beginning of the ripening stage corresponding to 172 DAS (GS75), STB inoculation effect was significant for both CC and NDVI in addition to the treatment effect. Interestingly, at this stage, recorded values of the NDVI and CC were lower in the inoculated plot compared to the fungicide-treated plot and STB severity was at its high level. Generally, under disease pressure, at the plant cell level, *Z. tritici* infects wheat leaves causing cell death and reduction on the photosynthetic activity thereby affecting the canopy green biomass. Simple linear regression showed that CC and NDVI were negatively correlated to AUDPC (R^2 = 0.55 and R^2 = 0.53 for CC and NDVI respectively). Similar results were observed by Yu et al. (2018) using the hyperspectral remote sensing method to measure physiological traits.
Difference in CTD between and within inoculated and fungicide-treated plots varied during the different growth stages and was significant at some time points and not on others (Table 2). This variation could be explained by the climatic conditions in the field during the day of measurement. In this respect, Karimizadah and Mohammadi (2011) suggested that the infrared thermometer was not always suited to measure CTD in field conditions because it is related to temporally variable environmental properties such as irradiance, air temperature, wind speed and vapor pressure deficit unlike to the thermal imager approach, which was more efficient. According to Pask et al. (2012), the best timing to measure CTD is during stem elongation, booting, early grain-filling and at the beginning of late grain-filling stages; the other growth stages were not recommended. However, others found that the difference of CTD between durum wheat genotypes was significant only at the flowering stage GS69 (Bilge et al. 2008; Karimizadah and Mohammadi 2011).
Focusing on the 4th measurement (172 DAS/GS75), for which the data is available for all the traits and the difference between the treatments on STB severity was significant (Figure 2 (B)), we have found that the best four treatments are the pure stands of Monastir and INRAT100 and the two 1:1 ratio

mixtures of Monastir with INRAT100 or with Karim as they showed the highest NDVI and CC and the lowest CTD.

Table 2. ANOVA analysis of the measured physiological parameters; normalized vegetation index, leaf chlorophyll content, canopy temperature depression and leaf relative water content, during the different measurement dates: 119, 137, 151, 172 and 189 days after sowing (DAS) corresponding to 56, 74, 88, 109- and 126-days post-inoculation(dpi).

Source of variation	Df	Physiological parameters				
		119 DAS[a] 56 dpi[b]	**137 DAS 74 dpi**	**151 DAS 88 dpi**	**172 DAS 109 dpi**	**189 DAS 126 dpi**
		Normalized difference vegetation index (NDVI)				
Treatment	9	NS	-	3.56**	6.77***	NS
Inoculation	1	NS	-	NS	180.45***	NS
Treatment x Inoculation	9	NS	-	NS	NS	NS
		Leaf chlorophyll content (CC)				
Treatment	9	NS	NS	0.010**	4.76***	NS
Inoculation	1	9.82**	16.89***	NS	200.82***	31.21***
Treatment x Inoculation	9	NS	NS	NS	NS	NS
		Canopy temperature depression (CTD)				
Treatment	9	NS	2.06*	NS	2.53*	2.91**
Inoculation	1	21.66***	NS	11.43**	NS	59***
Treatment x Inoculation	9	NS	NS	NS	NS	NS
		Leaf relative water content (RWC)				
Treatment	9	-	-	-	3.50**	-
Inoculation	1	-	-	-	92.16***	-
Treatment x Inoculation	9	-	-	-	2.58*	-

*Significant at the 0.05 probability level, ** Significant at the 0.01 probability level, ***Significant at the 0.001probability level, NS; not significant at the 0.05 probability level, a: days after sowing, b: days post-inoculation

In this work, we also measured the leaf Relative Water Content parameter (RWC) at GS75 and the ANOVA analysis showed significant difference between the ten treatments and between the inoculated and fungicide-treated plots in addition to the interaction effect (Table 2). The difference between the RWC rate for each treatment between the inoculated and the fungicide-treated plot was the lowest for the pure stand Monastir and the 50- 50 mixture of INRAT100 with Monastir (Figure 4 A). These two treatments showed only 7% and 10% decrease in RWC (%), respectively while mixtures that contained Karim showed the highest decrease.

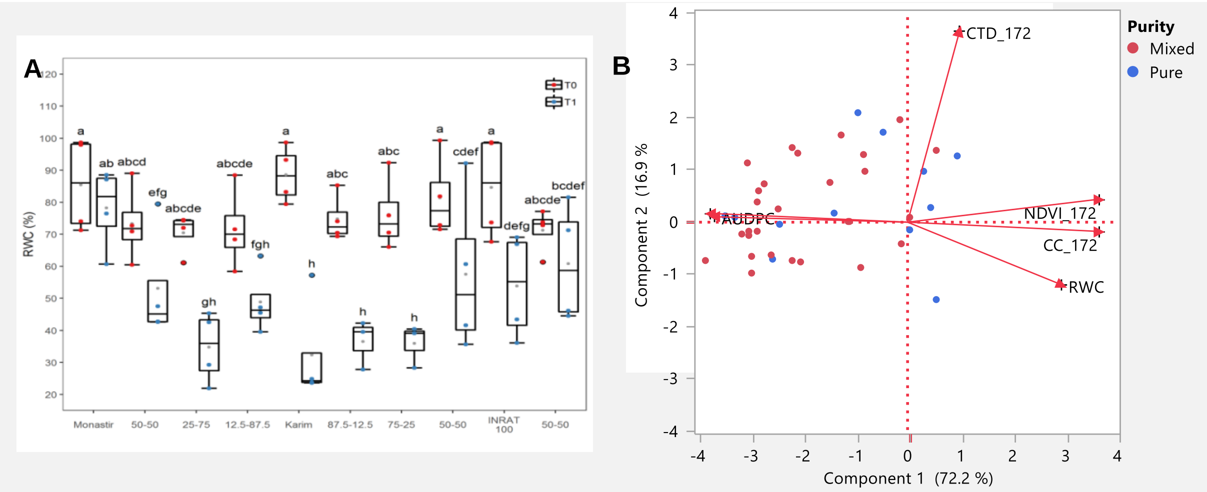

Figure 4. (A) Leaf relative water content (RWC) box plots of the inoculated (T1) and fungicide-treated (T0) treatments. From the middle: Karim pure stand, to the left we have Karim-Monastir two-way mixtures in different proportions and the pure stands of Monastir, to the right we have Karim-INRAT100 two-way mixtures, pure stand of INRAT100 and its mixture with Monastir in equal proportion. Gray and black dots represent the mean values and the outliers, respectively. Letters above the box plots indicate statistical significance of pairwise differences (p<0.05). **(B)** Distribution of the ten inoculated treatments represented in red and blue dots corresponding respectively to mixed and pure treatments on two dimensions Principal Component Analysis (PCA) representing the contribution of seven factors *(RWC: Leaf relative water content, CC_172: leaf chlorophyll content at 172 days after sowing (DAS), NDVI_172: Normalized difference vegetation index at 172 DAS, CTD_172: canopy temperature depression at 172 DAS, AUDPC: area under disease progression curve and FLS: flag leaves severity). A sharp angle between two arrows indicates high correlation between variables; a right angle corresponds to an absence of correlation and a flat angle to a strong negative correlation.*

To investigate the distribution of the inoculated treatments for the physiological parameters and STB severity progression, we applied the principal component analysis (PCA) on the five measured factors, namely NDVI, CC, CTD, RWC at the time point corresponding to 172 DAS/GS75; in addition to FLS which refers to the recorded severity level on the flag leaf samples used for Automated Image Analysis (Ben M'Barek et al. 2020), and the AUDPC (Figure 4(B)). Results showed two dimensions of PCA explaining 89.1% of treatments variance (Figure 4(B)). AUDPC, FLS, NDVI_172, CC_172 and RWC contributed greatly to the first axis of PCA by 72.2%, the higher contribution to the component was measured for FLS and AUDPC (98% and 95% respectively). The second axis was contributed by 94% by the CTD (CTD_172, Figure 4 (B)). With correlation coefficient r = -0.89, AUDPC negatively correlated to both NDVI_172 and CC_172. RWC showed lower coefficient of correlation (r = -0.65) to AUDPC and little higher to FLS (r = -0.72). Similarly, to findings of Yu et al. (2018), the normalized difference water index (NDWI) was used to assess the water content and measured using the hyperspectral canopy remote sensing which showed negative correlation to STB severity at the early and advanced stages with higher correlation coefficient during heading to ripening stages. In comparison with other measured vegetation indices, NWDI showed the best performance and could be a STB indicator at early stages (Yu et al. 2018).
From this work, RWC appears to be a good trait to consider for disease detection even within-field diversification. Although RWC was measured at an advanced growth stage, it will be interesting to determine in future studies, RWC at earlier time points for potential use in early disease detection and efficient management.

4. Conclusion

Based on AUDPC, this study shows that variety mixtures can be an efficient strategy to control STB disease. However, according to previous studies, mixture performance depends on the used varieties, their proportions as well as the environmental conditions. Follow-up studies to investigate whether these mixtures would behave differently or similarly under natural STB infection and under different agro-ecological zones for several years are essential to assess their durability and efficacy. In addition, we also showed a correlation of physiological traits with disease severity, even in mixtures. These traits such as NDVI, CC and RWC can certainly be implemented in disease resistance breeding strategies or early disease detection within variety mixtures.

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