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Contributions of three upper leaves of wheat, either healthy or inoculated by *Bipolaris sorokiniana*, to yield and yield components

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Abstract

This work studied contribution of three upper leaves of wheat to yield and estimated grain yield losses due to their defoliation, under healthy and inoculated conditions. We developed a methodology to assess the importance of each leaf to the grain yield. The experimental design was laid out as a split plot with three replications in greenhouse and four replications in the field. Losses and contributions were calculated by dummy regression. Furthermore, defoliating treatment, as a variable, was converted from a categorical to a continuous variable before analyzing its effect by a general linear model. Defoliation effect of two upper leaves on grain yield of 20 wheat varieties was also considered. The results showed that defoliation has similar effect on all varieties. However, durum wheat cultivars were more sensitive than those of bread wheats. In addition, defoliation effect was less than the effect of inoculation. Yield losses were more important when plant lost more than one leaf. Under healthy condition, DF1, DF2 and DF3 reduced similarly grain yield by 17, 19 and 16%, respectively. But, under biotic stress, only DF12 and DF123 induced grain losses of 23 and 36%, respectively. The most important contribution to the grain yield was made by CF1 with 48% under healthy conditions, while it was 39% under biotic stress. Under field conditions, only DF12 reduced grain yield by 20%. Disease severity weighted by the quantified importance of the upper leaves would improve the relationship that may exist between disease severity estimates and grain yield.

Keywords: *Bipolaris sorokiniana*, defoliation, *Triticum aestivum*, flag leaf, grain losses.
Abbreviations: a.i._active ingredient, CV_Cultivar, CF1_contribution of F1, CF2_contribution of F2, CF3_contribution of F3, CF12_contribution of F1 and F2, CF13_contribution of F1 and F3, CF23_contribution of F2 and F3, CF123_contribution of F1, F2 and F3, Def_defoliation, Defc_defoliation treatments, DF_leaf defoliation, DF1_defoliation of flag leaf, DF2_defoliation of F2, DF3_ defoliation of F3, DF12_defoliation of F1 and F2, DF13_ defoliation of F1 and F3, DF23_defoliation of F2 and F3, DF123_defoliation of F1, F2 and F3, F_leaf, F1_flag leaf, F2_penultimate leaf, F3_antepenultimate leaf, Glm_generalized linear model, GS_Growing season TKW_one thousand kernels, HI_harvest index, INRA_National Institute of Agronomical Research, MM_mixed model, SAS_Statistical Analysis System.

Introduction

Cereals grain yield is a complex interaction between its components such as number of plants per unit area, number of spikes per plant, number of grains per spike and grain weight (Birsin, 2005; Karrou et al., 2001). Number of grains per spike or the number of seeds/m^2 are determined within the period from initiation of spikelets to anthesis (Fischer and Stockman, 1986), while average grain weight and final grain yield depend on formation, translocation, partitioning and accumulation of assimilates during the grain filling of post-anthesis period (Zhenlin et al., 1998; Hafsi et al., 2000). All parts of a cereal plant contribute to spike development (Blade and Baker, 1990); however, the upper three leaves are of great importance to grain filling, which determines cereal yield potential (Birsin, 2005; Sen and Prasad, 1996).

Importance of these leaves, especially flag leaf and penultimate leaf, in elaborating grain yield and its components, has been widely discussed (Singh et al., 1983; Seck et al., 1991; Jebbouj and El Yousfi, 2006a). But, wheat flag leaf was found to contribute to grain filling more than 50% (Auiau et al., 1992), while its defoliation generated grain yield losses of 18 to 30% (Youssef and Salem, 1976; Banitaba et al., 2007). For barley, flag leaf contribution to grain yield was 39%, and its defoliation resulted in a yield loss of 21% (Jebbouj and El Yousfi, 2006a, 2009). Other studies pointed out to the role of lower leaves that increases when flag leaf area is affected, either by shading or defoliation (Zhenlin et al., 1998; Ahmadi and Joudi, 2007; Joudi et al., 2006; Binjanzadeh and Emam, 2010).

Contribution of upper leaves to grain yield and its components are estimated with different methodologies such as defoliation, shading or inoculation. Although these techniques are commonly used, the methodologies used are quite different. Indeed, some scientists attributed the magnitude of losses due to defoliation to contribution by comparing yields of treatments lacking specific leaves with a non-defoliated check (Subba et al., 1989; Ali et al., 2010). Jebbouj and El Yousfi (2006a, 2009) have given specific definitions to each of these terms, in which they have defined grain yield losses due to defoliation by comparing a defoliating treatment to a non-defoliated check, while contribution is defined as a comparison of a specific defoliating treatment to the one, where the plants have lost all their upper three leaves. Looking forward to estimate the importance of the three upper leaves of wheat under Moroccan conditions, the present study adopted the same nomenclature defined above, and carried out greenhouse and field experiments to evaluate contribution to yield and estimate grain yield losses using defoliating treatments under healthy and diseased conditions.

Results

Interaction of defoliation and varieties

Analysis of variance indicated a significant effect of both variety and defoliation on grain yield and 1000-kernal weight (TKW) for all tested varieties either durum or bread wheat. However, no interaction between variety and defoliation treatments was noted for grain yield of both wheat species and TKW of durum wheat. However, a significant interaction was only detected for TKW in bread wheat. All defoliating treatments significantly reduced grain yield and TKW for all tested wheat varieties. Losses in grain yield and TKW, due to defoliation DF1 were more important than those induced by DF2. Losses even increased by depriving plants from their two upper leaves F12 (Table 1). We also found that an average yield loss of 42% occurred due to a simultaneous defoliation of the two upper leaves in durum wheat, which was much more important than the one registered for bread wheat cultivars (29%).

Interaction of defoliation and inoculation

Under healthy conditions, defoliation of last three leaves had a significant effect on grain yield, grains number and TKW. Induced losses increased with amplifying defoliation intensity. Simple defoliation DF1, DF2 and DF3 significantly reduced the grain yield, which were 17, 19 and 16%, respectively. This yield reduction increased with defoliation; DF13 and DF12, to reach 21 and 31%, respectively. Losses were at their maximum (40%), when plants had lost all of their three upper leaves (DF123) (Table 2). Similar trend was noted for TKW, and defoliation of flag leaf and penultimate leaf, which similarly induced losses of 10%. These losses increased in the case of defoliation; DF13 and DF23, for which the rates were similar and reached 31%. Losses were even worse; 49% for DF123 (Table 2). For number of grains, treatments; DF1 and DF3 reduced grain number by similar rates of 15 and 17%, respectively. Losses exceeded 20% for DF12 and DF123. However, defoliating treatments DF2, DF13 and DF23 did not have any significant effect on this component (Table 2). Under biotic stress, only defoliating treatments DF12 and DF123 induced significant grain yield loss that reached 23 and 36%, respectively. On the other hand, TKW was only affected when the three upper leaves were defoliated, in which the loss reached 18%. However, none of defoliating treatments caused a significant loss in number of grains (Table 2). Under healthy conditions, flag leaf can contribute to the grain yield up to 48%, while antepenultimate leaf contributed only by 32%. The contribution of these two leaves, when both were present, reached 41%. However, the contribution of CF3 was only significant when it was present with one of the two upper leaves. These later contributions were similar with magnitudes of 36 and 38% for CF13 and CF23, respectively (Table 3). Under biotic stress, contribution of upper leaves to the grain yield became only important once the two leaves (F2 and F3) were present or when one of them is present with the flag leaf (F1). Contribution of F1 was 39% and contributions of CF12 and CF13 were almost similar and reached 48 and 42%, respectively, while the one of CF23 was significantly higher and scored 56% (Table 3).When plants were healthy, all leaves contributed significantly to TKW with almost similar amounts between 19 to 25%, and a maximum contribution was reached by the last two leaves; CF12 which was 35%. However, under biotic stress, leaves contributed to TKW only in a combined state with similar magnitude between 18 to 20% (Table 3). For number of grain, only flag leaf significantly contributed to this variable under healthy state with 22%, but these three leaves did not had a significant contribution under the biotic stress (Table 3).

Losses due to inoculation

Variance analysis of measured variables allowed estimation of a multiple regression model fit, evaluating the effects of inoculation and defoliation on yield and yield component (Table 4). For grain yield, TKW and grain number, regression lines were parallel, indicating the absence of any interaction between defoliation and inoculation. Furthermore, inoculation effect was superior to that of the defoliation. In fact, the inoculation generated an average difference, between inoculated and healthy status of 17.96 g, 0.72 g and 305 for grain yield, TKW and grain number, respectively. The continuous variable, representing intensity of defoliation, had a negative slope for both inoculated and none-inoculated lines of 2.17, 1.56 and 14.78, for grain yield, TKW and grain number, respectively. Therefore, for any unit increase in defoliation intensity, there was a decrease in grain yield of 2.17 g, 1.56 g in TKW and 15 grains in grain number. Mean registered losses in yield and grain number due to inoculation were 31% $[17.96/(40.97 + 17.96) \times 100]$ and $[305/(674.22 + 305) \times 100]$, respectively. However, inoculation effect on TKW was not found to be significant (Table 4).

Defoliation effect under filed conditions

Analysis of variance based on Levene's test and Welch's test suggested to combine data from the two trials because the Levene's test and Welch test for homogeneity of variance were both non-significant. For grain yield, Leven's test showed a F-value of 0.02 and a p-value of 0.956. The Welch's test also showed the F-value of 0.23 and a p-value of 0.6364, while Levene's test for TKW had the F-value of 0.03 with a p-value of 0.8599 and the Welch's test with the F-value of 0.24 and a p-value of 0.627 (for TKW). Analyses of variance of foliar treatment, defoliation and their interaction (Table 5) revealed that only defoliation affected on grain yield and 1000-kernel weight, with probabilities of 0.02 and 0.07, respectively. Foliar and defoliation treatments did not show any significant effect on harvest index (HI). Table 5, also showed absence of any interaction between defoliation and foliar treatment; therefore, the presented data were analysed on average data over foliar treatment. Grain yield as well as TKW were significantly reduced by the effect of

Table 1. Defoliation effect on mean grain yield and 1000-kernal weight (g) over 20 wheat varieties in greenhouse, during 2012-2013 (Mean data) cropping season.

Treatment[a]	Durum wheat		Bread wheat	
	Yield	TKW[b]	Yield	TKW
DF1	3.52	37.06	4.25	27.78
DF2	4.26	38.65	5.01	39.39
DF12	2.64	33.14	3.07	28.41
Control	4.6	42.58	4.31	41.44
S.E.[c]	0.31	1.53	0.42	1.56

[a] DF1, defoliated flag leaf; DF2, defoliated penultimate leaf. [b] TKW, 1000-kernal weigh. [c] S.E., standard error.

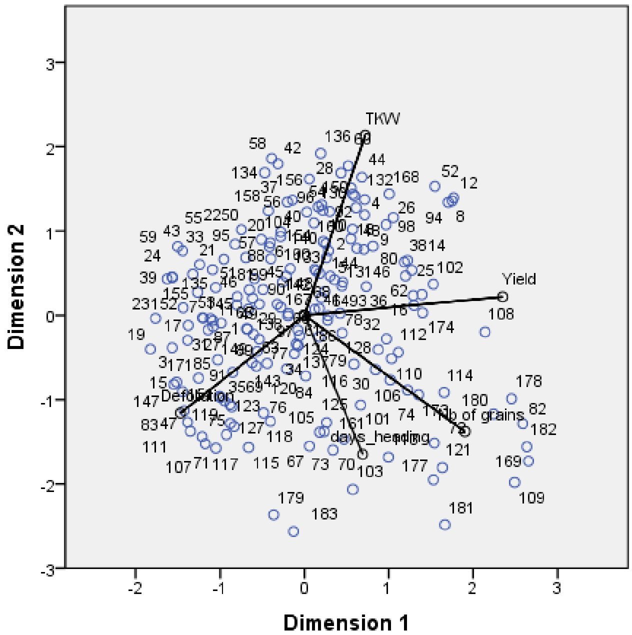

Fig 1. Categorical principal component analysis biplot based on grain yield, grain number, days to heading and TKW as continuous variables, and defoliation as an ordinal variable.

of combined defoliation DF12 (Table 6), and losses were 20 and 8%, respectively. Simple defoliation treatment of flag leaf and penultimate leaf did not significant affect on both grain yield and TKW (Table 6).

The two upper leaves contributed differently to grain yield and 1000-kernel weight. Moreover, only CF2 contributed significantly to the grain yield (by 33%). Flag leaf contribution was only significant when it was combined with penultimate leaf (this contribution reached 25%). However, for TKW, there were the leaves that jointly contributed (CF12) to this yield component by 9% (Table 6).

The quantification of the importance, in absolute values, of defoliation treatments shows that the non-defoliated check and DF2 had the same importance, while the greatest importance was that of DF12 with 1,522 followed by that of DF1 with 0,275 (Table 7). To re-scale this quantification, non-defoliated check, DF2, DF1, and DF12 would be 0, 0.1, 1.22 and 2.5, respectively.

The first two coordinates from Categorical principal component analysis, using data from bread and durum wheat varieties, explained 72% of the total variation. The biplot (Fig. 1) showed the eigen values of 1.92 and 1.66 for the first and the second dimension, respectively. This biplot showed that the more intense defoliation is, the less are the grain yield and TKW. Furthermore, number of grain variable has a positive relationship with grain yield and a negative correlation with TKW. On the other hand, a negative correlation was found between number of days to heading and TKW.

Discussion

Definition of losses and contribution adopted herein are similar to those previously defined by Jebbouj and El Yousfi (2006a, 2009). The magnitude of grain yield and TKW depended on the variety/genotype, but was significantly affected by defoliation. The absence of any interaction between varieties and defoliation on grain yield of two species of wheat and TKW of durum wheat indicated that defoliation had the same effect on all tested varieties. However, the effect of the interaction between variety and defoliation on TKW, of bread wheat, revealed that among bread wheat varieties, there exist genotypes, which tolerate defoliation effects more than others. Furthermore, a difference in grain yield losses of 13% between varieties of durum wheat and those of bread wheat indicated that tested durum wheat varieties were more sensitive to defoliation.

Our results indicated that losses became even more important when plants lost more than one leaf and were even worse with the DF123 defoliation application. Jebbouj and El Yousfi (2006a), Alam et al. (2008) and Bijanzadeh and Emam (2010), also concluded that a combined defoliation of the upper leaves will reduce grain yield and its components more rigorously in comparison to a simple flag leaf removal.

Puckridge (1969) and Subba et al. (1989), suggested that only the three upper leaves are functional photosynthetical units during anthesis and grain filling.

Under healthy conditions, flag leaf had an important contribution to grain yield, 1000-kernel weight and grain number, compared to penultimate and antepenultimate leaves. This result supports the importance of flag leaf as a major photosynthetic organ that contributes to grain filling, and any damage to its green leaf area would generate significant yield losses (Sharma et al., 2003; Buntin et al., 2004; Jebbouj and El Yousfi, 2006a). This importance might be due to its short distance to the sink (spike), longer green period and direct implication in solar radiation interception (Birsin, 2005; Khaliq et al., 2004).

The fact that certain defoliated leaves (especially flag leaf) showed significant contributions and some had no significant or minimal effects on grain yield, and yield components, when defoliated, may be an indication of amplification of the photosynthetic activity of the other remaining parts of the plant. This is in agreement with Zhenlin et al. (1998), Joudi et al. (2006), Ahmadi and Joudi (2007) and Binjanzadeh and Emam (2010), who reported that, in spite of the importance of flag leaf during grain filling, its defoliation could improve the photosynthetic activity of the other leaves. The flag leaves enhance net photosynthesis activity, stomatal conductance and chlorophyll content of most wheat leaves, and generate mobilisation of stored carbohydrates (Schnyder, 1993). However, the range of increase is differed among cultivars (Wang et al., 1997; Joudi et al., 2006; Bijanzadeh and Emam, 2010). This explains the mechanisms employed by plants to overcome any interruption of grain filling from any source of limitations (Richards, 1996).

Grain yield losses due to inoculation were found greater than those due to defoliation. This finding is consistent with barely-net blotch pathosystem (Jebbouj and El Yousfi, 2006b, 2009). In this regard, disease severity is not equivalent to the loss of the same percentage of a green photosynthetic leaf area. Plant seems to boost its use of functional green foliar area to compensate the adverse effect of any disease in order to sustain grain filling for its survival (Jebbouj and El Yousfi, 2006b, 2009). Furthermore, carbohydrate reserves were found to contribute less to grain yield in diseased plant than in healthy ones, especially when yield potential is low (Scott, 1992; Desjardins and Hohn, 1997; Jebbouj and El Yousfi, 2009).

Defoliation effect of upper three leaves (DF123) of healthy plants is greater than that of the same defoliation applied on diseased plants on grain yield and TKW. Similarly, the effect of defoliation on grain number under biotic stress was confounded with disease effect. Therefore, losses in grain number due to defoliation were significant under healthy conditions and non-significant under biotic stress. Therefore, we can conclude that a loss in a healthy leaf area is not equivalent to a diseased one. Therefore, the importance of leaves, depends on their healthy status, either healthy or diseased. Subba et al. (1989) reported that losses due to defoliation under healthy and biotic stress conditions are similar. This difference could be related to periods where applications took place. These authors applied defoliation in pre-anthesis (end of tillering-emergence of flag leaf), whereas in our study, the defoliation took place at heading stage.

Under natural conditions, clipping one of the two upper leaves did not generate significant losses either in grain yield or 1000-kernel weight. Losses only became significant and important after a combined defoliation DF12. Our results supported the importance of both flag and penultimate leaves, in wheat genotypes develop a strong compensation for a loss of one of the upper two leaves (Rosyara et al., 2005). In fact, these two leaves were widely discussed in many studies for their role in grain filling, photosynthetic activity and assimilate translocation (Blum, 1988).

Defoliation did not affect harvest index (HI). The prevailing climatic conditions on 2011-2012 cropping season were not favourable to disease development. Consequently, the effect of fungicide application was not significant. The non-significant effect of defoliation on harvest index showed that the grain yield reduction was proportional to the biomass reduction. An identical study (Rosyara et al., 2005) reported that defoliation at at anthesis stage was significant on grain and biomass yield; however, it was not significant for harvest index.

Categorical principal component analysis seeks a solution to the covariance or correlation matrix of measured variables, and attempts to explain the total variance common to these variables (Tabachnik and Fidell, 1996). It appears that the F2 leaf does not contribute to a reduction of grain yield, when defoliated, while defoliation of flag leaf is important up to 12 times, compared to F2. However, defoliation of both leaves had a score of 2.5, which is 2 times more than that of the flag leaf. Considering these results, in particular those obtained under natural conditions, plants are always exposed to diseases. Therefore, plant pathologists should take the coefficients of leaves importance into consideration, when estimating foliar disease severity specially when they intend to relate estimated disease severity to yield losses. For example, a disease severity of 35% on F2, and 15% on F1, generates an average disease severity of 50 %. However, based on our results, disease severity is 21% [(35%*0.1) + (15%×1.22)], which would over-estimate diseases severity by more than 2 times. Consequently, disease severity is over-estimated when leaves importance is not considered. This discrepancy may induce bias in estimating grain yield losses. In turn, if we use the sum of disease severity on F1 and F2 and divided it by 2.5 score, it will provide an overall 20% disease severity.

Early mature varieties have a short heading period and a longer filling period coupled with a slowing grain filling rate. However, late mature varieties have a longer heading period with a shorter grain filling period with faster grain filling rate (Miege, 1927). Therefore, grain filling period and grain filling rate are negatively correlated (Bahlouli et al., 2008; Erchidi et al., 2000; Triboi, 1990). Furthermore, these two variables were found to explain 97% of the variation in grain weight (Erchidi et al., 2000). Grain filling rate is primarily controlled by number of grains, while grain filling period is controlled by grain filling rate and environmental factors (Triboi, 1990). On the other hand, grain filling period is positively correlated with TKW and negatively correlated with grains number (Bahlouli et al., 2008; Erchidi et al., 2000). These later relationships, explained the negative correlation found here, between TKW and grains number, and heading period.

Table 2. Mean losses (%) in grain yield and yield component induced due to defoliation of the three upper leaves of durum wheat Karim *cv.* under healthy and diseased conditions in greenhouse during 2010-2011 and 2012-2013 (Mean data) cropping seasons.

	Grain yield		1000-kernal weight		Grain number	
Def[a]	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
DF1	17*	-13	10*	3	15*	-11
DF2	19*	0	10*	3	13	-2
DF3	16*	-6	3	1	17*	-5
DF12	31*	23*	9*	11	24*	10
DF13	21*	18	13*	8	10	5
DF23	12*	2	13*	4	-1	-3
DF123	40*	36*	26*	18*	21*	12
S.E.[b]	3.31	3.27	3.31	4.36	64.73	78.24

[a]Def, defoliation; DF1, defoliated flag leaf; DF2, defoliated penultimate leaf; DF3, defoliated antepenultimate leaf.
[b]S.E., standard error. *, values are significantly different from a check with a p-value≤ 0.05 based on a Benferrani test.

Table 3. Contributions (%) to grain yield and yield component of the upper three leaves of durum wheat Karim *cv.* under healthy and diseased conditions, in greenhouse during 2010-2011 and 2012-2013 (Mean data) cropping seasons.

	Grain yield		1000-kernal weight		Grains number	
Trt[a]	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
CF1	48*	39*	20*	16	22*	16
CF2	32*	21	19*	12	11	8
CF3	15	15	25*	8	-3	3
CF12	41*	48*	35*	20*	4	19
CF13	36*	42*	24*	18*	8	16
CF23	38*	56*	23*	18*	6	25
S.E.[b]	3,31	3,42	3,43	4,36	69,12	78,48

[a] Trt, treatment; CF1, flag leaf contribution; CF2, penultimate leaf contribution; CF3, antepenultimate leaf contribution.
[b] S.E., standard error. *, values are significantly different from a check with a p-value≤ 0.05 based on a benferrani test.

Table 4. ANOVA of general linear model for grain yield, 1000-kernal weight and grain number as dependent variables, and defoliation (continuous) and inoculation as independent variables, in greenhouse over the two trials of 2010-2011 and 2012-2013 (Mean data) cropping season data.

		Parameter estimate's	Standard error	DF[a]	T value	Probability
Grain yield	Intercept (β0)	40.97	1.35	2	30.29	0.0011
	Inoculated (β1)	17.96	1.25	187	14.41	<.0001
	Defolmax[b] (β2)	-2.17	0.27	187	-7.97	<.0001
1000-kernal weight	Intercept (β0)	60.45	1.44	2	41.99	0.0006
	Inoculated (β1)	0.72	1.38	187	0.52	0.6035
	Defolmax (β2)	-1.56	0.30	187	-5.16	<.0001
Grain number	Intercept (β0)	674.22	33.06	2	20.40	0.0024
	Inoculated (β1)	305	26	187	12	<.0001
	Defolmax (β2)	-14.78	5.72	187	-2.58	0.0106

[a] DF, degree of freedom.[b] Defolmax, defoliation as a continuous variable.

Table 5. P-values of the analysis of variance of foliar treatment, defoliation and their interaction effect on grain yield, 1000-kernels weight and harvest index in the field, during 2011-2012 cropping season.

	Variables		
	Grain yield	TKW[a]	Harvest index
Foliar treatment	0.13	0.25	**0.10**
Defoliation	0.02	0.07	0.93
Foliar treatment * Defoliation	0.52	0.99	0.45

[a] TKW, 1000-kernal weight.

Table 6. Average grain yield, 1000-kernels weight (g), mean losses and contribution (%) of upper two leaves of durum wheat Karim *cv.* in the field during 2011-2012 cropping season.

	Grain yield			1000-kernal weight		
Leaf[a]	Effect (g)	Losses (%)	Contribution (%)	Effect (g)	Losses (%)	Contribution (%)
F1	90.32	-6	10	45.5	3	5
F2	74.65	12	33*	45.28	3	6
F12	68.08	20*	25**	42.91	8*	9**
Control	85.20			46.86		
S.E.[b]	1.6561			5.9124		

[a] F1, flag leaf; F2, penultimate leaf. When losses are involved, leaves F1 through F12 represent defoliated treatments. Under contribution heading leaves represented treatment were these leaves were kept intact.
[b] S.E., Standard error.
*, values represent the difference between treatment and control and they are significant at 5% of probability; **, values represent the difference between treatment and F123 and they are significant at 5% of probability.

Table 7. Quantification of the importance of different defoliation treatments over 20 wheat varieties in greenhouse, during 2012-2013 cropping season.

Category of defoliation[a]	Quantification	Centroid Coordinates		Vector Coordinates	
		Dimension		Dimension	
		1	2	1	2
Control	-0.947	0.444	0.573	0.552	0.436
DF2	-0.844	0.523	0.350	0.492	0.388
DF1	0.275	0.015	-0.349	-0.161	-0.127
DF12	1.522	-0.970	-0.596	-0.888	-0.700

[a] DF1, defoliated flag leaf; DF2, defoliated penultimate le**af.**

Materials and Methods

Three different experiments were conducted, two of which were carried out in a greenhouse and the third was conducted under natural field conditions.

Defoliation in greenhouse

Plant materiel and experimental design

In the first trial, a widely grown durum wheat cv. "Karim" was chosen for this experiment. Seeding was sown in 20 cm diameter and 30 cm height pots. Each pot contained 10 Kg of natural soil. Five pockets per pot were sown with ten seeds each. After plant emergence, ten plants were kept per pot at two plants per pocket. Plants continued their growth under quasi-natural temperature and luminosity conditions (Jebbouj and El Yousfi, 2009). During plant growth, plants received adequate amount of water and fertilizer as needed, and the time scale adopted to assess growth period was based on the Zadoks decimal codes (Zadoks et al., 1974).

The experiment, repeated once in space and time, was laid out in the first week of November for two growing seasons 2010-2011 and 2012-2013. The experimental design was a split-plot with three replications. The main plots were represented by inoculation with two levels; inoculated and non-inoculated, and the subplot factor represented eight defoliating treatments including a non-defoliated check. Within the same year, this experimental design was repeated once during the first and the second week of November.

Defoliating treatments

At heading stage (GS=55), plants of the main factor under biotic stress were inoculated with *Bipolaris sorokiniana*, while, plants in healthy condition were subjected to defoliation treatments. The inoculated plants were subjected to leaf removal ten days after inoculation. The first defoliation treatment consisted of a single removal of the flag leaf (F1). Similarly, the second and third treatment consisted of a simple defoliation of the penultimate leaf (F2) and of the antepenultimate leaf (F3), respectively. The four other treatments consisted of a removal of combined leaves of (F1 and F2), (F1 and F3), (F2 and F3) and (F1, F2 and F3). These later four treatments were labeled combined defoliations. The last treatment was a controlled non-defoliated check.

Losses in grain yield and yield components due to defoliation were obtained by comparing a defoliating treatment with the check. So, defoliation of F1, F2, F3, F1 and F2, F1 and F3, F2 and F3 and F1, F2 and F3 were denoted by DF1, DF2, DF3, DF12, DF13, DF23 and DF123, respectively. The contributions to grain yield and to its components were obtained by comparing defoliated treatments with the treatment, where plants lost all their three upper leaves. Therefore, contribution of F1 (CF1) was calculated as a difference between yields of DF23 and DF123. In addition, contributions CF2, CF3, CF12, CF13, and CF23 were obtained as a yield difference between DF13, DF12, DF3, DF2, and DF1 yields and DF123 one, respectively.

Inoculation with Bipolaris sorokiniana

Inoculum consisted of *Bipolaris sorokiniana* spores that were previously increased for ten days on a sterilized nutrient medium composed of 20g of tomato, 20g of Agar and 6g of Calcium Carbonate (CaCO3) per liter of distilled water. The spores were collected by scraping culture surface with a brush and sterile distilled water. The resulting suspension was then filtered through two layers of a muslin-cloth and the inoculum was adjusted to a spore concentration of 32×10^3 conidia per mL and 28×10^3 conidia per mL, respectively for the first and the second trial in the first year and to a concentration of 27×10^3 conidia per mL and 22×10^3 conidia per mL, respectively, for the first and the second trial in the second year. Before inoculation, one drop of Tween 20 (Polyoxyéthylène sorbitanmonolaurate) per 100 mL was added to all final solutions. Plants of each pot were sprayed by a hand atomizer with 75 mL of inoculum solution, and each inoculated pot was immediately covered with a transparent plastic bag, for a period of four to five days to maintain high relative humidity and initiate infection.

Disease severity was recorded on inoculated plants. It was estimated as a percentage of infected leaf areas compared to healthy ones. At harvest (GS=90), data for weight and number of kernels as well as the weight of one thousand kernels (TKW) were determined.

Defoliation effect on others wheat varieties

In the second trial, the same procedure was conducted in 2012-2013 to assess yield losses and contribution of upper leaves for additional wheat varieties. Ten varieties of durum wheat and ten of bread wheat were tested. Seeding took place on first week of November, and seeds were put in 20 × 30 cm (upper diameter and hight respectively) as four pockets per pot at five seeds per pocket. These pots were also filled with the same natural soil.

Whenever a variety reached its heading stage (GS = 55), it was immediately subjected to four defoliating treatments of its two upper leaves (F1, F2). Thus, the first, second and third treatments referred to a defoliation of F1, F2 and F1F2, respectively. The last treatment was a non-defoliated control. The trial was also set as a split plot design with three blocks and the trial was repeated once more. Durum wheat varieties were: Irdene 13, Marzak 17, Karim, Anouar 28, Bel Bachir 7, Waha 32, Isli 5, Ourgh 15, Tomouh 3 and Jawhar 26 and those of bread wheat were: Baraka 13, Rajaa 17, Achtar 3, Tilila 15, Merchouch 4, Amal11, Khair 9, Wafia 21, Sais 6 and Rihane 2. Varieties were taken as main plots factor, whereas defoliating treatments as subplot one.

Defoliation under field conditions

Contribution of the upper leaves to wheat yield under natural conditions was studied on the same durum wheat variety Karim, and the trial was installed at Sidi El Aidi experimental station during the first week of November 2011. The experimental design was a split plot with four blocks, where the whole plots represented foliar disease treatment with two levels; treated and untreated, and the sub-plots consisted of defoliating treatments with four levels. At heading stage (GS=55), defoliating treatments included only flag leaf (F1) and penultimate leaf (F2). The first, second and third treatments consisted of a defoliation of F1, F2 and F12, respectively. The last treatment represented a non-defoliated control. Fungicide treatment, Tilt; a. i. propiconazole was applied at spike emergence, and at a recommended dose with a backpack sprayer provided with 1 m long boome with four equidistant nozzles.

A plot of 7 × 2 m^2 of six rows, 30 cm apart represented each treatment. Defoliation treatments were applied on four central rows of 1 m length each. The experiment was repeated once more in the same year within the same station. The second repetition was made around the second week of November. At maturity, each treatment was harvested individually to evaluate biomass production, grain yield and TKW.

Data analysis

The data was analyzed according to split-plot model For losses and contributions of leaves we preferred to use dummy regression for its ease of use in obtaining the requested results. Dummy regression analysis (Hardy, 1993) models a quantitative dependent variable Y (e. i, grain yield), throughout a linear combination of qualitative explanatory dummy variables; here represented by inoculation and defoliation factors. Inoculated treatments took two levels, inoculated and non-inoculated, while the defoliation variables had two levels, 1 and 0 for each defoliated treatments (F1, F2, F3, F12, F13, F23, F123 and the non-defoliated check accordingly). The coding used for the explanatory variable (inoculation) is 0 = healthy treatment and 1 = inoculated treatment. Therefore, the multiple linear regression model for estimating yield losses was as follow:

$$y = \beta0 + \beta1 * \mathrm{F1} + \beta2 * \mathrm{F2} + \beta3 * \mathrm{F3} + \beta4 * \mathrm{F12} + \beta5 * \mathrm{F13} + \beta6 * \mathrm{F23} + \beta7 * \mathrm{F123}$$

While, the multiple linear regression model for estimating leaf contribution was:

$$y = \beta0 + \beta1 * \mathrm{F1} + \beta2 * \mathrm{F2} + \beta3 * \mathrm{F3} + \beta4 * \mathrm{F12} + \beta5 * \mathrm{F13} + \beta6 * \mathrm{F23} + \beta7 * \mathrm{Check}$$

Leaf contribution as CF1, CF2, CF3, CF12, CF13, and CF23 were estimated by a difference between β0 and β's of F23, F13, F12, F3, F2, and F1, respectively, and where β0 is equal to yield of F123. Yield losses and contributions were herein separately estimated for inoculated and non-inoculated treatments.

The dummy regression analysis was corroborated with a mixed model analysis (MM) (Littell et al., 2006) that estimated the main effect of defoliation treatments. These later treatments were represented by one variable that accounted for all levels of the defoliation and had 8 categories that run from 0 to 7. The 0 class level represented a non-defoliated check, and 1 to 7 the defoliation of F1, F2, F3, F12, F13, F23, F123, respectively. This MM had a base line for comparison 0, which represents the non-defoliated check, and modeled the main effect of each category to this based line. In this regard, this comparison informed us about the magnitude of yield loss induced by this particular category of defoliation. Furthermore, when we wanted to estimate contributions, we only had to change the base line to represent the defoliating treatment DF123, and therefore we assigned to this category a 0 code. The mixed model was developed as follow:

$$y = \beta0 + \beta1 * \mathrm{Def}$$

This model treated the Def as a fixed categorical variable, while the blocks were taken as random. This analysis was repeated for each level of inoculation.

Another estimation of the main effects of inoculation and defoliation on yield was performed, and levels of the defoliation treatments were transformed from a discrete character (categorical) to a continuous character (ordinal) given the fact that a defoliation intensity increases with the importance that represent the upper three leaves to a cereal plant. The categories were then ranked accordingly and these categories were represented by one continuous variable, which effect on yields was estimated for inoculated and non-inoculated conditions. According to several studies, flag leaf is more important than penultimate leaf, which is more important than antepenultimate leaf (Rosyara et al., 2005; Jebbouj and El Yousfi, 2006a, 2009; Alam et al., 2008). The defoliation effect on plant performance increases from flag leaf to antepenultimate leaf and it is even worse when the plant had lost more than one leaf (Subba et al., 1989; Jebbouj and El Yousfi, 2006a; Alam et al., 2008; Bijanzadeh and Emam, 2010). Therefore, we classified our defoliating treatments (Defc) as a continuous independent variable according to an increasing effect using a decimal code that ranged from 0 to 7, and where this ranking codes corresponded to a check (non- defoliated), F3, F2, F1, F23, F13, F12 and F123, defoliating treatments, respectively. This proposed mixed model was formulated as follow:

$$y = \beta 0 + \beta 1 * \mathrm{In} + \beta 2 * \mathrm{Defc}$$

Where, β0 represented a non-defoliated and non-inoculated check, β1 was related to an inoculation variable with two inoculated level coded as 0 and non-inoculated as 1. Therefore, it represented the inoculation main effect adjusted for the other explanatory variable (defoliation) in the model. The variable Defc is herein taken as a continuous variable and β2 determines the slope coefficient for the variation in the defoliation intensity running from 0 to 7.

All the analyses (dummy regression and mixed model) were carried out with the software SAS 9.1 (SAS Institute, 1990) and significant treatment effects were judged on their p-value of less than 5% probability. Data of the two field trials were analyzed in combination form because of their homogeneous variance (Levens's and welch test option of the Glm procedure of the SAS system).

Categorical principal component analysis was adopted to investigate the existing relationship between defoliation intensity and yield variables on bread and durum wheat varieties. Before analysis, 184 observations were carried out and defoliation treatments were coded as 1 to 4 following a defoliating intensity. These codes 4, 3, 2 corresponded to defoliation treatments DF12, DF1 and DF2, respectively, while code 1 was given to a non-defoliated control. As result, defoliation treatments were transformed from a classification variable to an ordinal variable. Categorical principal component analysis was undertaken on correlation matrix of number of grains, TKW, yield and the number of days of heading using the proc Factor procedure of the SAS software.

Conclusion

The results confirmed the importance of flag leaf, contributing to cereal yield formation during grain filling. This importance is differentially expressed under healthy and biotic stress conditions. Furthermore, defoliation seems to increase photosynthetic activity of the other leaves to avoid any interruption in grain filling. Importance of all leaves depended on their phytosanitary state, in which healthy leaves are more important than diseased. On the other hand, disease effect is much more important than a simple loss of a similar leaf area. The contribution of leaves to wheat grain yield depends on the interaction of wheat genotype with the environment. In rational estimation of relationship between disease severity and grain yield losses we need to consider the coefficients of importance of every leaf separately. Severity values estimated on these upper leaves, along with their surface and coefficients of importance may be good candidates to improve the efficiency of yield loss models.

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References

Ahmadi A, Joudi M (2007) Effects of timing and defoliation intensity on growth yield and gas exchange rate of wheat grown under well-watered and drought conditions. Pak J Biol Sci. 10:3794-3800.

Alam MS, Rahman AHMM, Nesa MN, Khan SK, Siddquie NA (2008) Effect of source and/or sink restriction on the grain yield in wheat. Eur J Appl Sci Res. 4:258-261.

Ali MA, Hussain M, Khan MI, Ali Z, Zulkiffal M, Anwar J, Sabir W, Zeeshan M (2010) Source-sink relationship between photosynthetic organs and grain yield attributes during grain filling stage in spring wheat (*Triticum aestivum*). Int J Agr Biol. 12:509-515.

Auriau P, Doussinault G, Jahier J, Lecompte C, Pierre J, Pluchard P, Rousset M, Saur L, Trottet M (1992) Le blé tendre. In: Gallais A, Bannerot H (eds) Amélioration des espèces végétales cultivées, INRA Eds. p 22-38.

Bahlouli F, Bouzerzour H, Benmahammed A (2008) Effets de la vitesse et de la durée du remplissage du grain ainsi que de l'accumulation des assimilats de la tige dans l'élaboration du rendement du blé dur (*Triticum durum* Desf.) dans les conditions de culture des hautes plaines orientales d'Algérie. Biotechnol Agron Soc Environ. 12 :31-39.

Banitaba A, Naderi M, Javanmard H, Emami B (2007) Effect of flag leaf and awn removal on vegetative traits, grain yield and yield components of bread wheat (*Triticum aestivum* L.). Res Agr Sci. 2:73-86.

Bijanzadeh E, EmamY (2010) Effect of source-sink manipulation on yield components and photosynthetic characteristic of wheat cultivars (*Triticum aestivum* and T. durum L.). J Appl Sci. 10:564-569.

Birsin MA (2005) Effects of removal of some photosynthetic structures on some yield components in wheat. Tarim Bilim Derg. 11:364-367.

Blade SF, Baker RJ (1990) Kernel weight response to source-sink changes in spring wheat. Crop Sci. 31:1117-1120.

Blum A (1988) Physiological selection criteria for drought resistance. In: Wittmer G. (ed) The future of cereal for human feeding and development of biotechnological research. Int Fair of Agric. 39thed, Foggia, Italy, p 191-199.

Buntin GD, Flanders KL, Slaughter RW, Delamar ZD (2004) Damage loss and control of the cereal leaf beetle (Coleoptera: Chrysomelidae) in winter wheat. J Econ Entomol. 97:374-382.

Desjardins AE, Hohn TM (1997) Mycotoxins in plant pathogenesis. Mol Plant Microbe In. 10:147-152.

Erchidi AE, Talouizte A, Ben bella M (2 000) Croissance du grain chez neuf cultivars de blé dur. In: Royo C, Nachit M, Di Fonzo N, Araus JL (eds). Durum wheat improvement in the Mediterranean region: New challenges. Zaragoza: CIHEAM, p 137-140.

Fischer RA, Stockman MY (1986) Increased kernel number in Norin 10-derived dwarf wheat: Evaluation of the cause. Aust J Plant Physiol. 13:767-784.

Hafsi M, Monneveux P, Merah O, Djekoune A (2000) Carbon isotope discrimination and yield in durum wheat grow in the high plains of Setif (Algeria). Contribution of different organs to grain filling. In: Royo C, Nachit M, Di Fonzo N, Araus JL (eds) Durum wheat improvement in the Mediterranean region: New challenges, Zaragoza, CIHEAM, p 145-147.

Hardy M A (1993) Regression with Dummy Variables. Series: Quantitative Applications in the Social Sciences 93. Sage Publications, Inc. Newbury Park, California, USA.

Jebbouj R, El Yousfi B (2006a) Contribution des trois feuilles supérieures de l'orge défoliées durant tout le cycle de croissance au rendement et comparaison entre la défoliation et les inoculations successives par Pyrenophora teres. Al Awamia 117:34-58.

Jebbouj R, El Yousfi B (2006b) Effet comparé de l'inoculation par Pyrenophora teres f. Maculate et/ou la défoliation simultanée des quatre feuilles supérieures, sur le rendement grain de l'orge. Al Awamia. 120:50-71.
Jebbouj R, El Yousfi B (2009) Barely yield losses due to defoliation of upper three leaves either healthy or infected at boot stage by Pyrenephora teresf.teres. Eur J Plant Pathol.125:303-315.
Joudi M, Ahmadi A, Poustini K, Shrifzadeh F (2006) Effect of leaf elimination on the effectiveness of flag and leaf photosynthesis and seed growth in bread wheat. Iran J Agr Sci. 2:203-211.
Karrou M, El Mourid M, Boulal H, Boutfirass M, El Gharous M (2001) Écophysiologie des céréales en zones semi-aride. INRA, Maroc, p 111.
Khaliq I, Parveen N, Chowdhry MA (2004) Correlation and path co-efficient analyses in bread wheat. Int J Agr Biol. 6:633-635.
Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger L (2006) SAS® for Mixed Models, Second Edition. Cary, NC: SAS Institute Inc.
Miege E (1927) Observations sur la précocité du Blé. In: Revue de botanique appliquée et d'agriculture coloniale. 7e année, bulletin n°72, p 517-526.
Puckridge DW (1969) Photosynthesis of wheat under field conditions. 2. Effects of defoliation on the carbondioxide uptake of the community. Aust J Agr Res. 20:623-624.
Richards RA. 1996. Increasing the yield potential in wheat: Manipulating sources and sinks. In: Reynolds MP, Rajaram S, McNab A (eds.). Increasing yield potential in wheat: Breaking the barriers. CIMMYT, Mexico, DF, p 134-149.
Rosyara UR, Sharma RC, Shrestha SM, Duveiller E (2005) Yield and yield components response to defoliation of spring wheat genotypes with different level of resistance to helminthosporium leaf blight. J Inst Agric Anim Sci. 26:43-50.
SAS Institute (1990) SAS/SAT User's guide, Version 6, Vol. 2, 4th Edition. SAS Institute, Cary, NC.
Schnyder H (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling–a review. New Phytol. 123:233-245.
Scott DB (1992) Assessment of resistance in barely to Pyrenophora teres and Pyrenophora japonica. Crop Prot. 11:240-243.
Seck M, Roelfs AP, Teng PS (1991) Influence of leaf position on yield loss caused by wheat leaf rust in single tillers. Crop Prot. 10:222-228.
Sen A, Prasad M (1996) Critical period of flag leaf duration in wheat (Triticuma estivum L.). Indian J Agr Sci. 66:599-600.
Sharma SN, Sain RS, Sharma RK (2003) Genetic analysis of flag leaf area in durum wheat over environments. Wheat Inf Serv. 96:5-10.
Singh D, Dhaliwal HS, Randhawa AS (1983) Contributions of leaf blades and awns to grain yield and its components at different stages of spike development in wheat. Indian J Agr Sci. 53:212-215.
Subba Rao KV, Yang XB, Berggren GT, Snow JP (1989) A multiple regression model to estimate the contribution of leaves and the effects of leaf rust on yield of winter wheat. Phytopathology. 79:1233-1238.
Tabachnik BG, Fidell LS (1996) Using multivariate statistics. Harper and Row, New York, p 509.
Triboi E (1990) Modèle d'élaboration du poids du grain chez le blé tendre (*Triticum aestivum*) en thell. Agronomie 1:191-200
Wang Z, Fu J, He M, Tian Q, Cao H (1997) Effects of source/sink manipulation on net photosynthetic rate photosynthate partitioning during grain filling in winter wheat. Bio Plant. 39:379-385.
Youssef S, Salem A (1976) Contribution of individual plant parts to grain yield and protein content and quality of two wheat varieties differing in protein content. Alex J Agric Res. 24:55-61.
Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res. 14:415-421.
Zhenlin W, Yin Y, He M, Cao H (1998) Source-sink manipulation effects on post anthesis photosynthesis and grain setting on spike in winter wheat. Photosynthetica. 35:453-459.