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Enhancing Resistance to *Fusarium graminearum* in Danish and Iraqi Local Winter Wheat Varieties under Greenhouse Conditions

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Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum*, has become one of the most destructive diseases in the world's wheat growing areas, especially in the humid and semi humid regions. *F. graminearum* results in yield and quality reduction and accumulation of mycotoxins. Nine winter wheat cultivars widely grown in Denmark or Iraq have been evaluated for FHB resistance during 2014/2015. The evaluation was made by means of artificial inoculations with *F. graminearum* under greenhouse conditions. Two inoculation methods were used Injection method, detect type II resistance by injecting fusarium conidia into individual florets and detect resistance to initial infection type I resistance by Spraying *Fusarium* conidia on the spikes. More precise data relating the effects of FHB on yield have been obtained using inoculated trials. Based on these data, there was no significant difference between syringe inoculation and spray inoculation (p<0.0001), whereas a significant differences between cultivars and method of inoculation on cultivars, Eiba99 and Rabiea which were assessed as resistant, and the cultivars, Skalmeje, Ritmo, Sham6, Aras, Mariboss, Eiba95 and Hereford as susceptible to FHB. Response to FHB was assessed by the percentage of visually infected spikelets and head weight relative to the non-inoculated (control). In the resistant cultivars, both the total grain yield and some of the yield elements (spike weight, grain /spike and 1000 kernel weight) were significantly less affected by FHB than in susceptible cultivars. Furthermore, the yield components (spike weight, grain number /spike, grain weight/spike, TKW) were significantly and negatively affected by artificial inoculation with FHB in all nine tested wheat cultivars. On the basis of experimental results it is concluded that artificial inoculation with *F. graminearum* could provide additional information about selection of both resistant and susceptible wheat cultivars.

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Introduction

Fusarium head blight (FHB), caused by *Fusarium* spp., has become one of the most destructive diseases in the world's wheat growing areas, especially in humid and semi humid regions (Paillard et al., 2004; Mesterhazy, 1978; Stack and McMullen, 1985; Kiecana et al., 1987; Kiecana et al., 1988). FHB has been linked with up to 17 causal organisms, which occur in most cereal-growing areas of the world. The most common species are

Fusarium graminearum (*Gibberella zeae*), *F. culmorum*, *F.avenaceum* (*G. avenacea*), *F. poae* and *Microdochium nivale* (*Monographella nivalis*) (Browne and Cooke, 2004). Among *Fusarium* species, *F. graminearum* Schw., the conidial stage of *Gibberella zeae* (Schwabe) Petch (Schroeder and Christensen, 1963) is the most aggressive species for wheat ears according to pathogenicity tests (Mesterhazy, 1978; Stack and McMullen, 1985).

The disease reduces kernel set and grain weight, thereby causing significant yield losses, which range from 30% to 70% under epidemic conditions (Miedaner, 1997; Mesterhazy, 1978). Consequently, grains are contaminated with fungal metabolites, mainly deoxynivalenol (Sutton, 1982; Jenkinson and Parry, 1994; Scott, 1990), which are toxic for human beings and animals (Sutton, 1982; Mirocha et al., 1989; Jenkinson and Parry, 1994; Miedaner, 1997; Dill-Macky and Jones, 1997). The occurrence and type of mycotoxins may depend on several factors, including environment, species of fungus present, severity of infection and the variety or kind of crop (Tanaka et al., 1988; Tan et al., 2004; Nakamura et al., 1994). Besides the toxicological aspect, the germination percentage of infected grains is substantially reduced (Bechtel et al., 1985). The baking quality of flour from infested crops was also detracted from the *Fusarium infected* grains (Tanaka et al., 1988; Mirocha et al., 1989; Scott, 1990).

Fusarium spp. can survive on crop residues in the soil for more than six years as mycelium, ascospores, conidia or chlamydospores (McMullen et al., 1997). Stack (1999) reported that the chlamydospores, formed either in soil or in host tissues are the main survival structures of many spp. Infection of wheat spikes by *Fusarium* spp. may occur from spike emergence, growth stage 50, through late milk stage. However, infection occurs most frequently during anthesis. Infection occurs primarily when ascospores or conidia are deposited on or within the flowers. Flower infection may also occur via ascospores, mycelia, conidia, chlamydospores or hyphal fragments deposited directly on or into glume, rachis or palea (McMullen et al., 1997).

Early infections can lead to aborted kernel development, tombstone kernels or reduced size of immature kernels, whereas later infections often lead to symptoms that can only be distinguished by toxicological screening (Stack and McMullen, 1985). Under favorable environmental conditions following infection, *F. graminearum* will colonize through the rachis and adjacent florets, growing systemically or saprophytically (Bai et al., 2000). In the case of saprophytic colonization, the fungus may spread to adjacent heads via physical contact (conidia or mycelia), splash dispersal (conidia) or wind dispersal (ascospores). Mycelia are the primary means of saprophytic growth and appear as orange-red lesions on the rachis or glume. Systemic colonization often appears as premature senescence of the spike from the infection point to the top of the spike and downward to the culum (Bai et al., 2000), or degradation of cell wall materials through toxins produced by the fungus (McMullen et al., 1997).

Resistance mechanisms to FHB in wheat are classified as either passive or active (Mesterhazy, 1995). Passive mechanisms are associated with phenotypic traits such as plant height, presence of awns, spikelet density and time to flowering. Active mechanisms include five different components: (1) resistance to initial infection also called Type I resistance; (2) resistance to spread of infection or Type II; (3) resistance to kernel infection or Type III; (4) tolerance or Type IV; and (5) resistance to mycotoxin accumulation or Type V (Schroeder and Christensen 1963; Wang and Miller 1988; Mesterhazy 1995). The biochemical and molecular bases for resistance have not been established; however, putative mechanisms operating in Type V resistance have been proposed (Miller et al., 1998).

Schroeder and Christensen (1963) postulated that there were two mechanisms of FHB resistance (type I and type II) that varied independently among cultivars. Type I resistance operates against initial infection and type II against the spread of the pathogen within the host. Resistance to infection (type I) may be due to morphological characters or physical barriers. Similarly, resistance to colonization (type II) may be of a chemical or physical nature (Mesterhazy, 1995).

Host resistance has long been considered the most practical and effective means of controlling FHB (Sutton, 1982; Liu and Andersen, 2003). Several studies have reported differences between susceptible and moderately resistant cultivars in both infection severity and mode of resistance (Miedaner, 1997). There is a consensus that resistant cultivars will provide the most stable and durable solution to the problem (Gilbert and Tekauz, 2000). However, no cultivars are yet available with complete resistance (Sutton, 1982). Even with resistant cultivars, the growing environment may influence the occurrence of the disease. Hall and van

Sanford (2003) reported a highly significant genotype by – environmental interaction for FHB resistance.

Until cultivars with high levels of resistance to FHB are developed, integrated crop management practices including foliar fungicide application, crop rotation and tillage practices may be the best means to keep the disease under control. Parry et al. (1995) found FHB in small cereals and concluded that there were surprisingly few reports of successful fungicidal or biological control treatments for FHB in the field. Dion et al. (2001) reported some benefits of fungicide spray application on controlling FHB, but did not detect significant differences in DON content. Similarly, Martin (2001) concluded that foliar fungicides were inconsistent in their effectiveness to control FHB in cereals. Control of the disease is, at best, inconsistent in the field. The subject of FHB control is dominated by work on selection of resistant cultivars and it is not surprising that fungicidal and biological control has received so little attention (Khan et al., 2006).

The main objective of this study was to identify sources of resistance to FHB in selected winter wheat cultivars from Denmark and Iraq under artificial inoculation with *Fusarium graminearum* under greenhouse conditions

Materials and methods

Factorial experiments were conducted in the greenhouse of Department of Horticulture, Faculty of Agriculture Science, University of Sulaimani during the 2014-2015 period.

Plant material and design of experiments

Four winter wheat varieties which were widely grown in Denmark and five local winter wheat varieties form Iraq were used in this study. All cultivars were planted on October 25, 2015 (Table 1).

Table 1. Winter wheat varieties used in this experiment.

Cultivars	Obtained from
Aras	Iraq
Rabie	Iraq
Eiba99	Iraq
Eiba95	Iraq
Sham6	Iraq
Mariboss	Denmark
Ritmo	Denmark
Hereford	Denmark
Skalmeje	Germany

Experimental plots were arranged in a randomized complete block design (CRBD) with three replications. Each replication consisted of three parallel plot rows with 25 cm between rows. Plots were 100cm long and 100 cm wide. A split-plot design was used, with main plots representing the two inoculation methods, syringe, spray and non-inoculated (control). Each treatment was replicated three times.

Fungal material (inoculum)

Fungal isolate used in this study was *F. graminearum* (R-7775), provided by (L. Nistrup Jørgensen), Institute of Integrated Pest Management, The Faculty of Agricultural Sciences, Århus University, Denmark have been used in this experiment. The isolate was chosen because they are known to be aggressive and has been used resistance tests in resin Germany and Sweden (L. Nistrup Jørgensen Personal communication).

In 2015, macroconidia of isolates were prepared using solid medium methods (Naito et al., 1984). The isolate was were cultured for four to five days on oat meal agar (oat meal 50g, sucrose 20g, agar powder 15g water 1L). The aerial hyphae on the media were removed by brushing and then the cultures were illuminated for three days with fluorescent light. Numerous macroconidia were produced on the surface of the cultures. Subsequently, the macroconidia were suspended in distilled water and adjusted to concentration 5×10^5 spores /ml.

Methodology of artificial inoculation

The evaluation was made by means of artificial inoculations with *F. graminearum* and assessment of symptom intensity 20 day after inoculation. For syringe inoculation, a 10-μl droplet of conidial suspension of *F. graminearum* was injected with a hypodermic syringe directly through the glumes in a central floret on each side of 10 chosen wheat heads (cultivar) per plot, that were marked by a colored clip (Fig. 1).

Since wheat is most susceptible to infection at anthesis (Schroeder and Christensen, 1963), experimental inoculations were, as far as possible, carried out at that time (when approximately 50% of the plants within the plot were in the flowering stage). In 2015 due to differences between cultivars in time of anthesis the inoculation period lasted, roughly from April 25 to May 25, inoculation has been performed using Danish inoculum.

Fig. 1: Injection method used in inoculation of wheat heads with *F. graminearum* by syringe directly through the glumes in a central floret on each side of the chosen heads (original).

For spray inoculation, ten heads per plot (cultivar) were chosen at anthesis and 0.5 ml of inoculum (conidia suspension) was sprayed by a portable sprayer directly through the glumes into a central floret of spikes (one spray on each side). The inoculated spikes were marked with colored clips. Both methods of inoculation were always performed on the same date for a given cultivars.

Assessment of FBH after artificial inoculation

Disease severity was assessed in the injection-inoculation treatment (marked heads) by counting the number of visually diseased spikelets, 10 and 20 days after inoculation, in relation to the total number of spikelets of the respective head. The result was percent infected spikelets (Fig. 2).

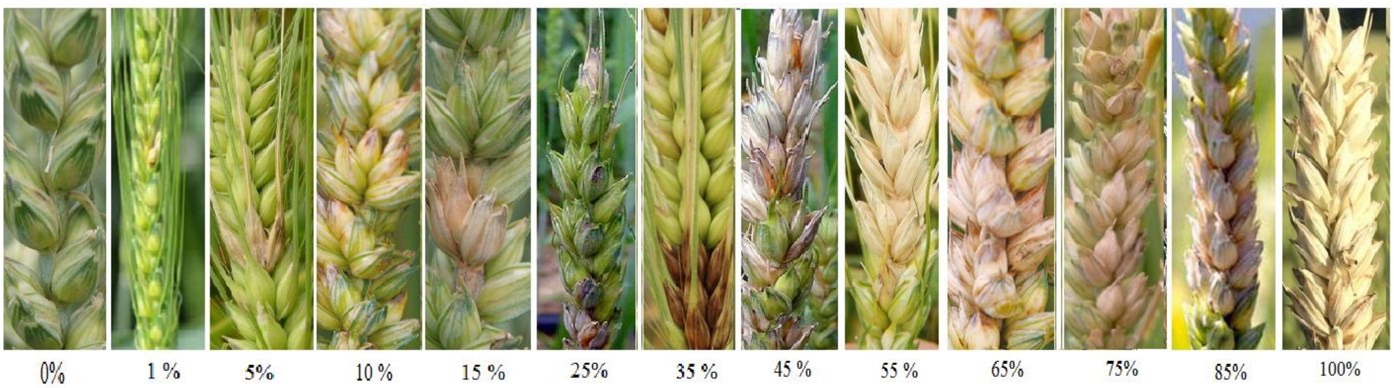

Fig. 2: *Fusarium* head blight severity on spikes in order to evaluate the response to FHB caused by *F. graminearum* (original).

Description: 0% no disease symptoms; 1% and 5% a single floret is diseased; 10% and 15% a single spikelet is diseased; 25%, 35%, 45% and 55% each degree of spikelets and rachis internodes are diseased, respectively; 65%, 75% and 85% each degree of spikelets and excess number of rachis intemodes are diseased ; 100% spikes are completely diseased. The first evaluation of disease (10 days after inoculation) was made on the 9th of May and the second 20 days after inoculation, on 12th of June, in 2015.

Upon maturity, the infected spikes were harvested separately, as they remained marked from the moment of infection. Also, the same number of non-inoculated spikes was harvested from each plot in order to determine post harvesting indices for disease manifestation. As such, determinations were conducted regarding spike productivity indices under.

***Fusarium* attack:** spike weight, number of grains in a spike, weight of grains in a spike and thousand kernel weight (TKW). These biometrics were conducted individually on 30-40 plants, for artificially-infected spikes, as well as non-infected, control ones. The ranges of values obtained for each productivity character under analysis were the basis for subsequent statistical processing towards obtaining inoculated and non-inoculated statistical parameters.

Statistical analyses

All analyses were based on plot means. Plot means in each treatment was calculated for comparison and analysis. For all data sets, we make two analyses. The first was by two factors both inoculation methods and varieties. There was a statistical interaction between these two factors, meaning that cultivars react differently to inoculation. Therefore, data for each variety was analysed separately. Single plant observations of the syringe-inoculated treatment were also averaged plot-wise to reduce the within-plot error. Number of decimals on spike weight should also be reduced to one decimal place. Variances were stabilized by appropriate transformation of data if necessary. The differences between inoculation methods were not significant different (p>0.05). To see the specific differences to the control, one has to look in Analysis of Maximum Likelihood Parameter Estimates. Consequently, these data were analysed by logistic regression, assuming a Poisson distribution (corrected for over-dispersion when present). Hypotheses were rejected at p<0.05. All statistical analyses were performed with the program PC-SAS (release 9.4; SAS Institute, Cary, NC).

Results

First of all, the data showed that all analyzed characteristics were affected by inoculation with FHB. There are some differences between cultivars in their reaction to FHB, which were expressed by post-harvest indices. Furthermore, tables below shows that generally, FHB ratings were highly correlated with yield reduction as well as with significant reductions of yield elements (spike weight, grain no/spike, grain weight/spike and TKW. Disease progresses up and down from the central infected spikelets and may infect up to all spikelets in a spike when weather conditions are favorable and the genotype is susceptible to FHB (Fig. 3).

Fig. 3: Injection method used in inoculation of wheat heads with *F. graminearum* by syringe directly through the glumes in a central floret on each side of the chosen heads (original).

Effect of *Fusarium* head blight on the main quantitative characters in the different cultivars

Spike weight

There were a main effect of cultivars and treatments, meaning that there were significant (p<0.0001) differences between cultivars and inoculation methods. It can be observed that in all situations the loss in weight of spike, as a result of the disease effect, is important when the comparison is made to the mean of the experiment in inoculated and control individuals.

Ritmo and Sham6 which had been rated as resistant cultivars, lost less in spike weight by inoculation, while for Rabia there was no significant (p=0.1601) difference in effect of spike by artificially inoculation with *Fusarium*. In the case of Rabia the loss of weight of spike by inoculation with *Fusarium* is not so severe as compared to the mean of the experiment. Rabia seems to be the most tolerant to *Fusarium* attack. In the same way, Sham6 showed a good tolerance to *Fusarium* as expressed by weight of spike.

Despite the fact that no highly resistant cultivars were found obvious differences have been noted in the reaction to FHB of the nine studied cultivars. Four of the analyzed cultivars (Aras, Ritmo and Sham6) reached the highest level of weight spike, while scored lower than the mean FHB severity (Table 2).

For cultivar Eiba99 the differences were highly significant for the values of weight of spike in both and syringe inoculation compared to control. When the cultivars were compared with the mean of experiment, we observed that they have significant differences. Thus, Rabiea shows no significant differences as compared with the mean of the inoculated control. Sham6, Ritmo, Maribo, Eiba95 and Aras have positive differences, while Eiba99, Hereford and Skalmeje have negative significant differences for both inoculated and control.

Table 2. Weight of spike (g) in nine winter wheat cultivars tested in Bakrajo, 2014/2015.

Cultivar	Eiba99	Aras	Eiba95	Hereford	Mariboss	Rabiea	Ritmov	Sham6	Skalmeje
Spray	1.752B	1.877B	1.886B	0.916B	1.488B	2.682A	1.316B	2.322B	1.612C
Syringe	1.515B	2.578AB	1.898B	2.526A	1.855AB	3.077A	2.094A	2.181B	2.093B
Control	4.077A	3.126A	2.960A	2.461A	2.257A	3.224A	2.089A	2.900A	2.978A
p-value	<0.0001	0.0147	0.0004	<0.0001	0.0042	0.1601NS	0.0120	0.0117	<0.0001

Means with the same letter are not significantly different at p<0.05

Spike number

There was a main effect of cultivars and treatments, meaning that there were significant (p<0.05) differences between cultivars and different inoculation methods, meaning that cultivars react differently to inoculation. For the cultivar Rabiea, Ritmo, Sham6 and Skalmeje the control were significantly different (p<0.05) to both syringe and spray inoculation. Cultivars Skalmeje and Hereford were significantly more susceptible when spray inoculated, as assessed by infected spikelets. In contrast, Aras had a lower relative head weight when treated by syringe inoculation. The Iraqian cultivar, Sham6 was among the most resistant cultivar for both methods and treatments. Furthermore, there were no significant (p<0.05) difference between syringe and spray inoculation for following cultivars, Rabiea, Eiba99, Ritmo, Sham6 and Maribo.

For syringe and spray inoculation, cultivars Aras, Eiba95, Hereford and Skalmeje differed significantly (p<0.05) for spike number. The Danish cultivars Hereford and Skalmeje were significantly more susceptible when spray and syringe inoculated, as assessed by infected spikelets. In contrast, Eiba95 had a lower relative head weight when treated by spray inoculation. Cultivars Eiba99, Ritmo. Sham6, Maribo and Rabiea were among the most resistant genotypes for both methods and traits (Table 3).

Table 3. Spike number in nine winter wheat cultivars tested in Bakrajo, 2014/2015.

Cultivars	Inoculation methods (*p*-value)		Control (*p*-value)
	Spray	Syringe	
Eiba99	*p*=0.0051A	*p*=0.0016A	*p*=0.0021A
Aras	*p*=0.2220B	*p*=0.9146B	*p*=0.0001A
Eiba95	*p*=0.0007B	*p*=0.0175B	*p*=0.0347A
Hereford	*p*<0.0001A	*p*=0.2680B	*p*=0.0018A
Ritmo	*p*=0.0719A	*p*=0.6348A	*p*=0.1759A
Sham6	*p*=0.7463A	*p*=0.9566A	*p*=0.9197A
Skalmeje	*p*=0.0025B	*p*<0.0001A	*p*=0.0000A
Maribo	*p*=0.896B	*p*=0.1681B	*p*=0.0037A
Rabiea	*p*=0.5843A	*p*=0.7004A	*p*<0.8541A

Means with the same letter are not significantly different p<0.05.

Thousand kernel weight (TKW)

There were a main effect of cultivars and treatments, meaning that cultivars react differently to inoculation, there were significant differences between cultivars and between inoculation methods. However, there was a significant difference between the inoculation methods. TKW is an important character that is mainly under genetic control. TKW was affected in different ways by *Fusarium* attack. So Eiba99, which is a cultivar with the highest grain weight, lost more than half in TKW (Table 4).

Table 4. Thousand kernel weights in nine winter wheat cultivars tested under greenhouse condition in Bakrajo.

Cultivar	Eiba99	Aras	Eiba95	Hereford	Mariboss	Rabiea	Ritmov	Sham6	Skalmeje
Spray	1.2B	1.3B	1.5B	0.7B	1.1B	1.9A	1.0B	1.6B	1.2C
Syringe	1.1B	1.9AB	1.3B	2.0A	1.5A	2.0A	1.9A	1.6B	1.6B
Control	2.9A	2.2A	2.1A	1.8A	1.6A	2.2A	2.0A	2.1A	2.0A
p-value	<0.0001	0.0101	0.0066	<0.0001	0.0078	0.2038	<0.0001	0.0169	<0.0001

Means with the same letter are not significantly different p<0.05.

In the case of the Hereford cultivar, which is very sensitive to *Fusarium* inoculation, in what concerns the disease evaluation index, as well as the postharvest index (ear weight, number of kernels, and weight of kernels/ear), it can be observed that Thousand Kernel Weight (TKW) was less affected. For Syringe and spray inoculation, three cultivars differed significantly (p<0.05) for thousand kernel weights percent infected spikelets and relative head weight. Cultivars, Hereford, Maribos, Ritmov and Skalmeje were significantly more susceptible when syringe inoculated, as assessed by infected spikelets. In contrast, Eiba 99, Rabiea and Sham6 had a lower relative head weight when treated by spray inoculation. The Iraqian cultivar Rabia was among the most resistant cultivar for both methods of inoculation. For Rabiea cultivar no significant difference has been recognized.

Number of grain/spike

Cultivars react differently to inoculation; there were significant differences between cultivars and between inoculation methods. Furthermore, there were significant differences between both inoculation methods compared to control. For spray and syringe inoculation, cultivars Eiba99, Eiba95, Hereford, Ritmo, Skalmeje and Mribo differed significantly ($p<0.05$) for number of grain/spike. The greatest loss in kernel number/spike was observed in the Eiba99, Eiba95, Hereford Maribo and Skalmej cultivars which means they were susceptible to *Fusarium*. In contrast, Rabiea, Aras, Sham6 and Ritmo seem to lose the less number of grain/spike as compared to the control (Table 5).

Table 5. Number of grain/spike in nine winter wheat cultivars tested in Bakrajo, 2014/2015.

Cultivars	Inoculation methods (*p*-value)		Control (*p*-Value)
	Spray	Syringe	
Eiba99	p<0.0001B	p<0.0001B	p=0.2209A
Aras	p=0.0027B	p=0.0051 B	p=0.8328A
Eiba95	p <0.0001B	p<0.0001B	p=0.0149A
Hereford	p <0.0001B	p<0.0001B	p=0.0053A
Ritmo	p <0.0001B	p=0.0004B	p=0.0708A
Sham6	p <0.0001A	p=0.2186B	p=0.0051A
Skalmeje	p <0.0001B	p<0.0000B	p<0.1163A
Maribo	p <0.0001B	p=0.0001B	p=0.0775A
Rabiea	p=0.0137B	p=0.3485A	p=0.1258A

Means with the same letter are not significantly different $p<0.05$.

Cultivar Sham6, Maribo, Eiba95, Ritmo were significantly more susceptible when spray inoculated, as assessed by infected spikelets. Therefore, there were a lower relative number of grain /spike when treated by both syringe and spray inoculation. Cultivar Rabiea was among the most resistant cultivar for both inoculation methods. For cultivar Sham6, and Ritmpo there were a significant ($p<0.05$) difference between syringe and spray inoculation whereas, for Eiba99, Eiba95, Hereford, Skalmeje and Maribo there no significant difference between spray and injection compared to the control.

Discussion

Cultivars have reacted differently to inoculation methods. It means that there was a significant difference between the inoculation methods. Weight of spike is an important characteristic in the evaluation of the effect of FHB damage, and provides a good explanation of the behavior under *Fusarium* attack, noted for each variety (Fig. 4).

This means that previous data were confirmed the results obtained by (Sardar et al., 2009; Rozalia and Sardar, 2012). The number of grain /spike evaluated for the nine cultivars that were used in our study expressed the differences between cultivars. In all situations, the number of grain/spike was affected by inoculation in different degrees, depending on the cultivars potential and resistance to *Fusarium* (Sardar et al., 2009; Rozalia and Sardar, 2012; Dill-Macky and Jones, 1997).

The characteristic number of grains/spike does not express so well the relation to *Fusarium* attack because the effect on the number of grains was strong for all cultivars. As we know, the character number of grains/spike is negatively correlated with the thousand kernel weight (Sardar et al., 2009). Although the character kernel/ear is genetically conditioned, its decrease due to *Fusarium* infection may be a consequence of unmolding of kernels in inoculated ears, the inoculation being performed in the period of flowering (Miedaner et al., 2003). Furthermore, grain weight/spike, were significantly and negatively affected by artificial inoculation with FHB in some tested wheat cultivars (Fig. 5).

However, sources of resistance to FHB are distributed worldwide (Mesterhazy, 1995) and resistance tests across wide ranges of environments are necessary to analyze the environmental stability of possible donors. Spore flow arrives most likely by splash dispersal, reaching the head from outside (Parry et al., 1995). Cultivars, that are resistant to spray but susceptible to syringe inoculation should have type I resistance, whereas cultivars susceptible to spray but resistant to point inoculation should possess type II resistance.

However, spray inoculation covers both types I and II resistances and this classification is, therefore, not as clear (Schroeder and Christensen, 1963).

Type I resistance can reduce infection efficiency. Because it is not fully efficient, some *Fusarium* propagules will be successful in infecting the head and then type II resistance should be activated to limit disease progress, and consequently, limit yield losses and mycotoxin content in the grain (Miedaner et al., 2003).

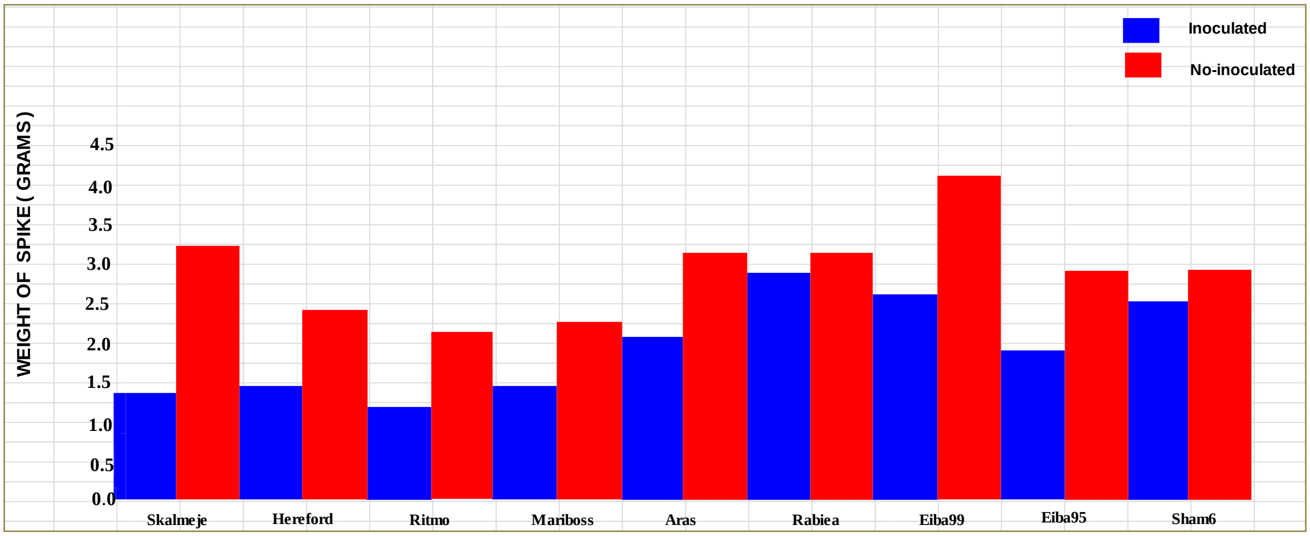

Fig. 4: The effect of *Fusarium* Head Blight on weight of spike (9 winter wheat varieties, Bakrajo 2015).

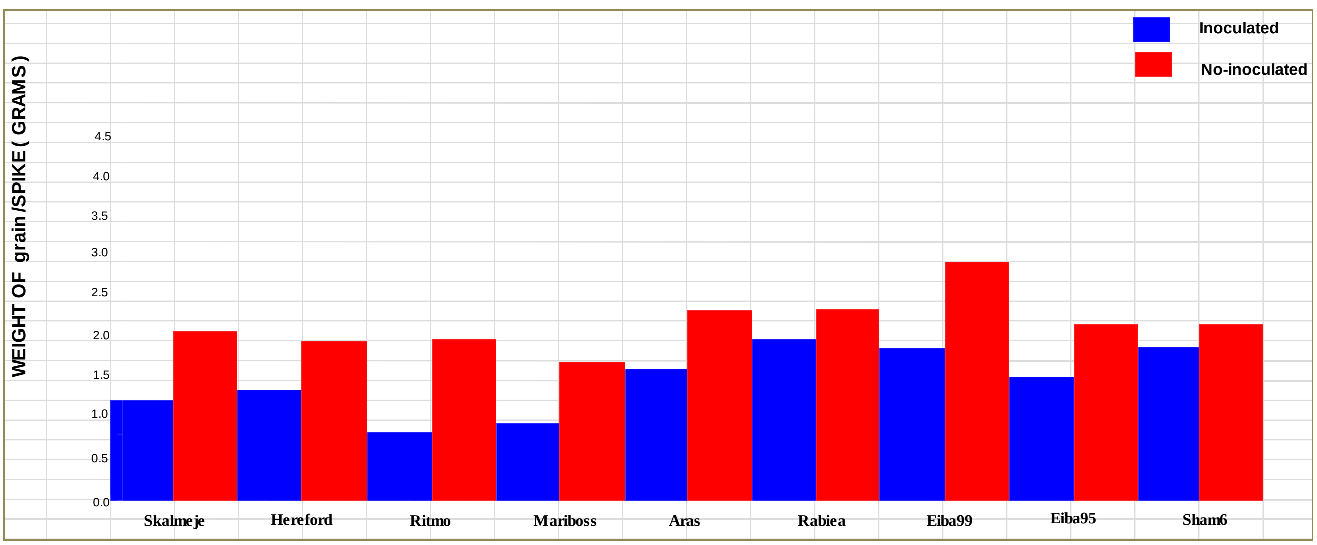

Fig. 5: The effect of *Fusarium* Head Blight on weight of grain/spike (9 winter wheat varieties, Bakrajo 2015).

Conclusion

On the basis of experimental results presented, the following conclusions have been formulated concerning the main research objectives which the thesis aimed at:

Yield components (spike weight, grain no. /spike, grain weight/spike, TKW) were significantly and negatively affected by artificial inoculation with FHB in all nine tested wheat cultivars. Weight of grain / spike and Thousand Kernel Weight are the yield components that react to *Fusarium*, by diminishing their values. So for all the varieties in the situation of inoculation was reduced as compare with control.

In spite of the fact that among the nine winter wheat varieties tested none was immune or highly resistant, obvious differences were noted in the reaction to artificial inoculation with FHB of these six cultivars. The most severe losses in the quantitative traits mentioned were noted in Eiba99, Eiba95, Hereford, Skalmeje and Maribo while in Rabiea, Aras, Ritmo and Sham6 the level of these losses were less.

In spite of the fact that among the nine winter wheat varieties tested none was immune or highly resistant, obvious differences were noted in the reaction to artificial inoculation with FHB of these six cultivars. Finally, on the basis of our results, we have found that artificial inoculation with *F. graminearum* could provide additional techniques about selection of both resistant and susceptible wheat cultivars.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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