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Identification of Wheat Sources Resistant to Hessian Fly, *Mayetiola destructor* (*Diptera: Cecidomyiidae*) in Tunisia

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ABSTRACT

Hessian fly, *Mayetiola destructor* Say, is one of the most destructive pests of wheat (*Triticum* species) in Tunisia. Genes in wheat that confer resistance to this insect provide the most efficient and economical means of crop protection. We investigated six wheat sources of resistance to Hessian fly from the United States, for response to this midge in one of the wheat growing regions in North Tunisia. Experimental results revealed that cultivars harbouring H11 and H13 genes expressed highly significant resistance levels. Among thirteen Tunisian durum and bread wheat varieties tested against this insect, four of them expressed resistance in different infestation circumstances, indicating that these varieties hold great promise in breeding wheat for resistance against Hessian fly in Tunisia.

Key Words: Evaluation; Hessian fly; Resistance genes; Sources; Wheat

INTRODUCTION

The Hessian fly, *Mayetiola destructor* Say, is the most important pest of bread wheat, *Triticum aestivum* L. and durum wheat *Triticum durum* Desf., throughout most productive areas in the world, including the USA, Asia, New Zealand, Southern and Western Europe and North Africa (Ratcliffe & Hatchett, 1997). In Tunisia, numerous outbreaks of this midge occurred since 1930 (Pagliano, 1935; Miller *et al.*, 1989; Makni *et al.*, 2000) and damage was estimated to 40% for durum and 26% for bread wheat (Makni, 1993). Surveying Hessian fly infestation showed that severe damage on wheat was caused by *Mayetiola destructor* in the North of Tunisia, namely, in the regions of Jedeida, Mateur, Goubellat, Fahs and Mejez El Beb (Ben Salah *et al.*, 1993; Makni, 1993). These regions represent a great part of arable land and meet more than one half of the nation's wheat requirement.

In order to reduce the damage caused to wheat by the Hessian fly, many strategies were adopted, but no control method was effective. In fact, cultural methods such as burning or ploughing under wheat stubble are not compatible with present farming practices. Chemical control has been expensive, toxic and not always gave adequate control (Bennani & Riany, 1978; Lhaloui *et al.*, 1987). Of all management practices, only the use of genetically resistant wheat varieties appeared to be a practical and economical method for crop protection. Sixty Hessian fly –resistant wheat cultivars were released in the USA, during the period from 1950 to 1983 and losses by this were reduced to less than 1% in areas where resistant varieties were grown (Ratcliffe & Hatchett, 1997).

Thirty–one Hessian fly resistance genes, designated H1 to H31, have been identified in wheat (Williams *et al.*, 2003). Most of these genes are dominant or incompletely dominant, and they condition antibiosis to larvae.

Although a resistance gene could be effective against the Hessian fly in a considered region, there is no evidence that it would confer resistance to this insect in a different region, due to genetic variation among the fly populations. In fact, 16 Hessian fly biotypes, classified as "GP" (Great Plains) and "A" through "O", have been identified on the basis of their differential response to the resistance genes H3, H5, H6 and H7H8 in wheat (Gallun, 1977). The "GP" biotype is not virulent to any resistance gene; whereas, Biotype "L" is the most virulent as it can attack wheat varieties with any resistant gene.

In Morocco, efforts to find wheat varieties resistant to the Hessian fly were unsuccessful and all the Moroccan varieties tested were susceptible to Hessian fly damage (Tegyey, 1965; El–Bouhssini, 1981). In contrast, varieties from Tunisia expressed high resistance levels, when they were subjected to infestation by Hessian fly. In fact, Amri *et al.* (1990) reported that 191 varieties were resistant to biotype GP, 186 resistant to biotype D and 129 resistant to biotype L, out of 217 Tunisian durum wheat varieties tested for response to infestation by selected biotypes of the Hessian fly. Makni (1993) reported that 11 Tunisian wheat varieties were resistant to the Hessian fly in field tests carried out in Mateur.

Because of the major shifts in biotype composition in Hessian fly populations by mutation or by selection pressure (Foster *et al.*, 1991), resistance tests must be conducted regularly in different regions. Objectives of the present study were to (i) evaluate, in Tunisia, different sources of Hessian fly resistance from United States wheats, in order to identify genes that condition resistance to the Hessian fly in Tunisia and (ii) assess the level of resistance in the Tunisian wheat varieties against this pest.

MATERIALS AND METHODS

Plant material. The tested plant material consisted of six cultivars of the "Uniform Hessian fly Nursery" (UHFN), each carrying one or more Hessian fly resistance genes. These cultivars and their resistance genes were "Monon" (H3), "Ribeiro" (H5), "Seneca" (H7H8), "IN657C1" (H11), "KSH8998" (H13) and "R.J.91" (H16). "Newton" cultivar, carrying no Hessian fly resistance gene, was used as a susceptible check. All of these cultivars originated from USA and were grown in the « Centre National de Recherche Scientifique (CNRS), Gif / Ivette, France". Thirteen Tunisian bread and durum wheat varieties used were "Ajili", "Hamira", "Jenah Khottifa", "Mahmoudi", "Médéa", "Sébéi", and "Souri" (local durum wheats, *Triticum durum*), "Karim" and "Razak" (durum wheats, *Triticum durum*, improved by the "Institut National de Recherches Agronomiques de Tunisie", INRAT) and "Byrsa", "Salambo", "Tébica" and "Utique" (bread wheats, *Triticum aestivum*, improved by the "Institut National de Recherches Agronomiques de Tunisie", INRAT).

Evaluation of known sources of resistance in U.S. wheats, to the Hessian fly in Jedeida. Because of shortage of seed, the test was conducted only in greenhouse condition, at the "Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie". Wheat entries were subjected to Hessian fly infestations in the greenhouse. A Hessian fly culture (Cartwright & Lahue, 1944) from Jedeida was used to infest the plants. Thirty seeds of each variety carrying a resistance gene, and 30 seeds of the susceptible check, "Newton" were planted, separately, in two opposite sides of a pot. At 2 –to –3 –tillers stage, plants in each pot were infested by 1 male and 1 female of Hessian fly under a cheesecloth tent. Mating and oviposition proceeded for 24 h before all adult flies were removed. Four weeks after infestation, the plants were classified as resistant or susceptible. Susceptible plants were stunted with dark green leaves and carried live larvae; whereas, resistant plants had normal appearance and harboured dead first – instar larvae.

Evaluation of Tunisian varieties of durum and bread wheat, for resistance to the Hessian fly in Jedeida.

Field test. Entries of wheat including the thirteen Tunisian durum and bread wheat varieties, stated above and the susceptible check, "Newton", were planted in Jedeida, 30 km to the North West of Tunis. The experimental design used was a randomised block with three replications. Fifty seeds of each variety were sown in a 1 m long row. Successive rows were spaced 50 cm apart. Because of low germination rates, Mahmoudi, Jenah Khottifa and Médéa could not be included in the study. Thirty plants of each variety were randomly sampled at 3-5 tillers stage. These plants were taken to the laboratory and each plant was recorded as either susceptible or resistant, as described above.

Greenhouse test. The test was conducted at the "Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie". Methods of infestation and determining resistance were similar to those used for US cultivars testing.

Data analysis. Data from both field and greenhouse tests were analysed using the same approach. Each wheat entry from a given cultivar or variety was treated as either susceptible or resistant. Data from all entries belonging to the same cultivar or variety were combined. The one–way between subjects ANOVA test" (Maxwell & Delaney, 1989; Armitage & Berry, 1994) was carried out with cultivar/variety as "factor" and infestation as "variable". Two contrast procedures i.e. (i) the "many against control" method and (ii) the "all –pairwise" method were used. The Least Significant Difference (LSD) method ($P<0.05$) between varieties or between cultivars was used for each contrast procedure. In addition, a Spearman correlation coefficient (rs) (Siegel & Castellan, 1988) was calculated, to compare the data obtained from field tests with those obtained from greenhouse tests. The entire data analysis was performed using the "Analyse –it for Microsoft Excel software version 1.71" (Analyse –it for Microsoft Excel. Leeds, UK).

RESULTS

Evaluation of known sources of resistance in U.S. wheats, to the Hessian fly in Jedeida. U.S. cultivars carrying different Hessian fly resistance genes and the susceptible check, "Newton", were characterized by variable percentages of susceptible plants. The one–way ANOVA test (many against control) showed significant differences for the percentage of plants susceptible between each cultivar carrying a resistance gene and the susceptible check "Newton" carrying no resistance gene (Table I). This meant that, in greenhouse condition, all resistance genes, H3, H5, H7H8, H11, H13 and H16, conferred resistance to the Hessian fly from Jedeida.

The difference between resistances conferred by these genes was estimated by an additional one–way ANOVA test (all –pairwise) using LSD ($P<0.05$). Cultivars with H5, H7H8 and H3 demonstrated weak –to –moderate levels of resistance; whereas, those carrying the H16, H11 and H13 genes were effective in preventing high infestation. Less than 7% of the plants with H11 or H13 were susceptible (Table II).

Evaluation of Tunisian varieties of durum and bread wheat, for resistance to the Hessian fly in Jedeida. The tested Tunisian varieties showed variable reactions to infestation in the field as well as in the greenhouse. In the field test, "Newton" checks were infested at 50% only. There was no significant difference in reaction to infestation

Table I. Contrast for the percentage of plants susceptible, between cultivars carrying resistance genes and the susceptible check, "Newton".

Cultivar	*Percent plants susceptible	All-against-control contrasts LSD (P<0.05)
"Newton" (No gene)	100.00±0.00	*H3* versus No gene Significant
"Ribeiro" (*H5*)	76.66±6.66	
"Seneca" (*H7H8*)	43.33±11.54	*H5* versus No gene Significant
"Monon" (*H3*)	23.33±8.81	*H7H8* versus No gene Significant
"R.J.91" (*H16*)	13.33±5.77	*H11* versus No gene Significant
"IN657C1" (*H11*)	6.66±0.00	*H13* versus No gene Significant
"KSH8998" (*H13*)	0.00±0.00	*H16* versus No gene Significant

Percentages followed by the same letter are not significantly different. *mean ± standard deviation (n=30)

Table II. Effect of resistance genes in US cultivars, on greenhouse infestation by the Hessian fly from the region of Jedeida.

Cultivar	*Percent plants susceptible & All-pairwise contrasts, LSD (P<0.05)
"Newton" (No gene)	100.00±0.00a
"Ribeiro" (*H5*)	76.66±6.66b
"Seneca" (*H7H8*)	43.33±11.54c
"Monon" (*H3*)	23.33±8.81d
"R.J.91" (*H16*)	13.33±5.77de
"IN657C1" (*H11*)	6.66±0.00de
"KSH8998" (*H13*)	0.00±0.00e

Percentages followed by the same letter are not significantly different. *mean ± standard deviation (n=30)

Table III. Evaluation of Tunisian varieties reactions to infestation by the Hessian fly in field, in the region of Jedeida.

Variety	*Percent plants susceptible & All-pairwise contrasts, LSD (P<0.05)
Susceptible check "Newton"	50.00±6.66a
Souri	40.00±8.82ab
Tébica	30.00±10.00abc
Karim	26.66±5.77bc
Byrsa	23.33±5.77bcd
Utique	23.33±5.77bcd
Ajili	23.33±0.00bcd
Razak	13.33±11.54bcd
Salambo	13.33±3.33bcd
Sébéi	10.00±8.81cd
Hamira	3.33±3.33d

Percentages followed by the same letter are not significantly different. *mean ± standard deviation (n=30)

between "Souri" and "Tébica" varieties and "Newton" cultivar. This meant that a first group including "Souri" and "Tébica" was susceptible to infestation by the Hessian fly. A second group was "Karim", "Byrsa" "Utique" "Ajili", "Razak" and "Salambo", which were resistant as they discarded significantly from "Newton" and the percentage of susceptible plants within these varieties was comprised between 27 and 13%. The most resistant group included "Sébéi" and "Hamira" with less than 10% susceptible plants (Table III).

In greenhouse conditions, the susceptible check "Newton" was infested at 76.66%. The one–way ANOVA test with the LSD procedure (P<0.05) showed the grouping of the tested varieties into three major groups. One group included "Byrsa", "Jénah Khottifa", "Souri" and "Mahmoudi" which did not differ significantly from "Newton". "Ajili", "Karim", "Salambo", "Razak" and "Sébéi" constituted a second group moderately resistant, and "Hamira" represented a third group highly resistant as all plants from this variety were resistant in the greenhouse test (Table IV).

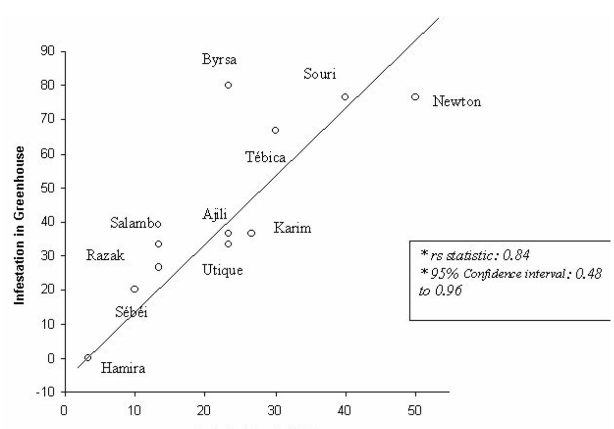
In order to investigate the extent of relatedness between results obtained in field and those in greenhouse, a Spearman rank correlation analysis was performed, which equaled 0.84. This implied that results of both tests were positively correlated (Fig. 1). "Hamira", "Sébéi", "Razak" and "Salambo" varieties demonstrated the highest levels of resistance in field test as well as in that achieved in greenhouse.

Table IV. Reactions of Tunisian varieties to greenhouse infestation, by the Hessian fly from Jedeida.

Variety	*Percent plants susceptible& All-pairwise contrasts, LSD (P<0.05)
Byrsa	80.00±12.02a
Jénah Khottifa	80.00±5.77a
Susceptible check "Newton"	76.66±8.82a
Souri	76.66±3.33a
Mahmoudi	70.00±12.02a
Ajili	36.66±8.82bc
Karim	36.66±3.33bc
Salambo	33.33±6.66bc
Razak	26.66±5.77c
Sébéi	20.00±8.82cd
Hamira	0.00±0.00d

Percentages followed by the same letter are not significantly different. *mean ± standard deviation (n=30)

Fig. 1. Comparison of infestation results obtained in field and those obtained in greenhouse, by the Spearman rank correlation analysis.



DISCUSSION

We screened a series of cultivars from the "North American Uniform Hessian Fly Nursery" (UHFN), carrying different resistance genes, for reaction to the Hessian fly from the region of Jedeida in greenhouse conditions. Results showed that all genes tested, namely, H3, H5, H7H8, H11, H13 and H16 expressed resistance. Two genes of this nursery gave a high level of resistance to the Jedeida population of this pest. These were H11 and H13. Indeed, plants carrying one of these genes were stunted at low percentages less than 7%.

Similar tests conducted, previously, in the region of Mateur (Makni, 1993) indicated a high level of resistance conferred by three genes, H5, H11 and H13, in both field and greenhouse conditions. El–Bouhssini *et al.* (1988) reported that H5, H11 and H13 genes were resistant to the Moroccan Hessian fly, in four different locations where tests were conducted. Later, 10 genes, H5, H7H8, H11, H13, H14H15, H21, H22, H23, H25 and H26 were selected as conferring resistance to Hessian fly in the Moroccan fields (El–Bouhssini *et al.*, 1996). However, in this study only a restricted level of resistance was expressed by "Ribeiro" cultivar carrying the H5 gene. This fact is probably a consequence of the H5 susceptibility at high temperatures (Sosa, 1979). In contrast to H5, the H11 and H13 resistance genes are highly resistant and allow for little larval survival across different temperatures (El–Bouhssini *et al.*, 1999). Therefore, our results provide support to previous studies showing that H5, H11 and H13 genes exhibit sufficient resistance to be of value in breeding for Hessian fly resistance, in Tunisia. Moreover, a limited number of Hessian fly biotypes would be present in North Africa with a presumed predominance of biotype D, against which H5, H11 and H13 genes are efficient. Although H5, H11 and H13 appeared to be effective over the geographical regions where tests were conducted, it is possible that biotypes capable of overcoming resistance conferred by these genes would be present in other regions.

Resistant sources in Tunisian germplasm are a valuable reservoir in breeding for Hessian fly resistance. However, due to variation in the insect biotypes leading to new gene–for–gene interaction profiles, there was a need to broaden and renew the genetic base for resistance screening among Tunisian wheat varieties.

Thirteen Tunisian varieties, of which nine of durum wheat and four of bread wheat were tested for Hessian fly response under controlled and field conditions. Results, inferred from the one way ANOVA showed that "Hamira", "Sébéi", "Razak" and "Salambo" varieties expressed the highest level of resistance in field test as well as in that achieved in greenhouse. The Spearman rank correlation analysis indicated a 0.84 significant correlation between results obtained from both tests. Results indicated that these varieties should be included in breeding programs aiming to transfer Hessian fly resistance genes into high yield varieties used by farmers in Tunisia.

Molecular markers are, now–a–days, becoming essential tools in plant breeding (Dubcovsky, 2004). Availability of tightly linked molecular markers can now be used in marker–assisted selection (MAS) programs, especially for disease resistance genes where it is possible to infer the gene by the marker without depending on the natural pest or pathogen occurrence or waiting for its phenotypic expression (Najimi *et al.*, 2002). A number of Hessian fly resistance genes–linked markers were identified by molecular techniques such as RFLP (Ma *et al.*, 1993), RAPD (Dweikat *et al.*, 1997) and AFLP (Najimi *et al.*, 2002; Williams *et al.*, 2003). Our further research focus will be search for the existence of DNA markers incorporated in the resistant Tunisian wheat varieties that are linked to genes conditioning resistance to the Hessian fly.

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