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Monogenic Inheritance of Resistance to Septoria Tritici Blotch in Durum Wheat 'Agili'

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ABSTRACT

Full resistance to Septoria tritici blotch caused by the fungus *Mycosphaerella graminicola* and its genetic inheritance has rarely been described in durum wheat. A high level resistance to a virulent Tunisian isolate 'Tun6' has been detected in an old local durum wheat cultivar 'Agili'. High yielding but susceptible durum wheat cultivars. 'Karim' and 'Khiar' were crossed with the resistant 'Agili'. In both F_2 populations, a 3: 1 (resistant: susceptible) segregation was observed after inoculation in the field with the isolate 'Tun6' at the seedling and adult stages, indicating that resistance is controlled by a single dominant gene. This genetic analysis was confirmed by F_2-derived F_3 families segregation of 1: 2: 1 (homozygous resistant: segregating: homozygous susceptible) ratio. Genetic analysis results are consistent with a single gene segregation indicating that there is a gene-for-gene interaction in the wheat-*M. graminicola* pathosystem and provides evidence that a qualitative resistance to Septoria tritici blotch exists in durum wheat.

Keywords: *Mycosphaerella graminicola,* Septoria tritici blotch, *Triticum turgidum* subsp. *durum*

INTRODUCTION

Septoria tritici blotch (STB), caused by the ascomycete fungus *Mycosphaerella graminicola* (Fu¨ckl) J. Schrot. in Cohn (anamorph *Septoria tritici*), is currently the most important foliar disease of wheat (*Triticum aestivum* and *T. turgidum* subsp. *durum*) in many regions of the world (Eyal and Levy 1997; Van Gin keel and Rajaram 1993; Cowger *et al.* 2000) it is particularly a major problem in regions characterized by frequent rains and moderate temperatures, such as the Mediterranean Basin, Eastern and Central Africa (Magboul *et al.* 1992; van Ginkel and Rajaram 1993). Yield losses ranging from 25 to 50% have been reported (Ziv and Eyal 1978; McKendry *et al.* 1995). Fungicides are used to control STB (Cook *et al.* 1999) but are expensive and not entirely reliable. Additionally, the recent discovery of resistance to fungicides has further enhanced interest in breeding and growing resistant cultivars. Incorporating genetic resistance into wheat cultivars is an economically and environmentally sound method of controlling this disease.

Resistance to STB may be either quantitatively or qualitatively inherited. Quantitative resistance is partial or polygenic (Jlibene *et al.* 1994; Simon and Cordo 1998; Zhang *et al.* 2001) and isolate non-specific (Chartrain *et al.* 2005) with additive and dominant gene effects and effective against all *M. graminicola* isolates (Zhang *et al.* 2001; Chartrain *et al.* 2004; Simon *et al.* 2004). Specific resistance is near-complete, isolate-specific and oligogenic (Somasco *et al.* 1996; Arraino *et al.* 2001; McCartney *et al.* 2002) and follows a gene-for-gene relationship (Brading *et al.* 2002). A simply inheritance controlled by one or two dominant or partially dominant genes (Wilson 1979; Somasco *et al.* 1996; Arraino *et al.* 2001; Brading *et al.* 2002; McCartney *et al.* 2002), or by two or three recessive genes has been reported (Rosielle and Brown 1979; Wilson 1985). To date 15 major genes for resistance to *M. graminicola* of hexaploid wheat varieties (*Stb1* to *Stb15*) have been reported (http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene/2008) and 13 have been identified and mapped (Goodwin 2007; Jing *et al.* 2008). Molecular markers flanking some of these genes were identified in order to facilitate resistance gene pyramiding which may slow or prevent the breakdown of resistance in the field. However, wheat lines carrying multiple STB resistance genes can lead to selection pressure on *M. graminicola* populations, which may result in a rapid development of virulence to individual or particular combinations of the resistance genes (Cowger *et al.* 2000). The emergence of fungal isolates harbouring mutations in avirulence genes matching plant isolate-specific resistance genes could rapidly lead to the break-down of resistance. For this reason there is a continuing need to identify new sources of resistance possibly possessing novel resistance genes.

The traditional growing of durum wheat in Tunisia has led in rise to adaptation of Tunisian *M. graminicola* isolates to this subspecies (Kema *et al.* 1996; Medini and Hamza 2008), which prompted Tunisian breeders to search for several new sources of resistance in durum local germplasm collection. To date little studies describing resistance to STB in durum wheat sources has been described. High level of resistance derived from a durum wheat cv. Coulter to *M. graminicola* was previously reported (McCartney *et al.* 2002), however this resistance is specific to Canadian races because Tunisian isolates were virulent to this cultivar. The present work consists on the study of the genetic inheritance of the high resistance level to *M. graminicola* of local durum wheat 'Agili'. The experiment involves the most virulent pathotype 'Tun6' characterized on a differential series composed of 4 durum and 4 bread wheat cultivars (Medini and Hamza 2008).

MATERIALS AND METHODS

Plant materials

The work reported here used an F_2 progeny and F_2-derived F_3 families from two crosses between the durum wheat resistant

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parent 'Agili' and the durum wheat susceptible parents 'Karim' and 'Khiar' and BC_1F_1 progeny derived from the cross 'Agili'/'Karim'/'Karim' to *M. graminicola* isolate 'Tun6'. These different progenies were tested respectively during 2007-08 and 2008-09 growing season at the INRAT research station at Oued Beja (Tunisia).

Inoculum preparation and plant inoculation

Inoculum was prepared from virulent pathotype 'Tun6' (Medini and Hamza 2008), by inoculating 250 ml of liquid yeast-glucose medium (10 g of yeast extract and 30 g of glucose in 1 liter of distilled water) in 500 ml Erlenmeyer flasks with fresh *M. graminicola* colonies in solid yeast glucose medium containing agar (20 g/l). Erlenmeyer flasks were incubated for 7 to 10 days with shaking (100 rpm). The resulting inoculum suspensions were filtered and adjusted to 10^7 spores per ml with distilled water. Ten drops of Tween 20 (polyoxyethylene-sorbitan monolaurate) were added per liter of spore suspension to reduce surface tension. Plants were inoculated twice at the three-leaf stage (seedlings stage) and at stem elongation (Zadoks scale 37) with a hand operated sprayer.

Disease assessment

Symptoms of STB were assessed at 28 days post inoculation (dpi). Susceptibility and resistance were measured using a qualitative scale i.e. plants were scored as susceptible if leaves the plants were covered by necrotic lesions bearing pycnidia, or as resistant if leaves of the whole plant had no pycnidia (Kema *et al*. 2000; Brading *et al*. 2002). For the F_2 generation, seedlings and adult plants of both crosses were tested to isolate 'Tun6' of *M. graminicola* whereas for BC_1F_1 progeny only adult plants were scored. F_3 families were classified as homozygous resistant when all plants within the family were resistant, heterozygous when the family segregate for resistance, and homozygous susceptible when all plants within the family were susceptible. Observed data was tested for goodness of fit to specific genetic ratios using the standard chi-squared (χ^2) test.

RESULTS

Level of 'Agili' resistance to *M. graminicola* in field condition

Over the period 2007–09, the three wheat varieties, 'Karim' and 'Khiar' and local durum wheat 'Agili' were evaluated in field experiment done on the Oued Beja Research farm after artificial inoculation with pathotype 'Tun6'. All the plants of the durum wheat varieties 'Karim' and 'Khair' developed typical STB disease symptoms i.e. leaves covered with necrotic lesions bearing abundant pycnidia indicating a high level of disease pressure (**Fig. 1A**). By contrast, no visible disease symptoms (lesion containing pycnidia) were ever found on the green leaves of 'Agili' (**Fig. 1B**). Therefore, the resistant response and incompatible interactions were defined as absence of pycnidial formation, whereas the formation of pycnidia containing pycnidiospores indicated a susceptibility response and a compatible interaction (Brading *et al*. 2002).

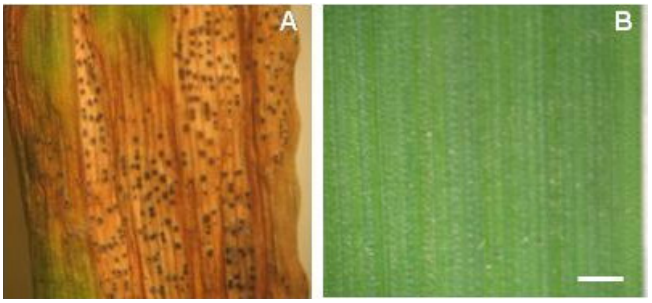

Fig. 1 Examination of resistance and susceptibility of durum wheat to virulent *Mycosphaerella graminicola* isolate 'Tun 6' under field conditions. Representative leaf segments showing the development of macroscopic symptoms on susceptible durum wheat variety 'Karim' (**A**) and on resistant durum wheat 'Agili' (**B**), at 28 dpi with *M. graminicola* isolate Tun6. Bar: 25 mm.

Segregation analysis of the F_2 progeny for STB resistance

F_2 population involving the resistant parent 'Agili' and susceptible parents 'Karim' and 'Khiar' segregated in a 3: 1 at seedling and adult stages (**Table 1**). At the seedling stage, over 58 F_2 progeny derived from the cross 'Agili'/'Karim', 10 plants exhibited lesion with pycnidia (susceptible) and 48 plants did not developed pycnidia (resistant). At adult stage, over 100 F_2 progeny evaluated for reaction to the virulent isolate 'Tun6', 69 were resistant and 31 were susceptible. This segregation fits into 3: 1 ratio (**Table 1**). The reaction of the F_2 progeny for the cross 'Agili'/'Khiar' at the seedling and adult stages provided the same segregation ratio as observed for the cross 'Agili'/'Karim' indicating that at both stages 'Agili' resistance is controlled by a single dominant gene. These results were supported by the data of BC_1F_1 population which fits closely to the expected 1: 1 ratio (**Table 1**).

Segregation analysis of the F_3 families for STB resistance

To confirm the single control of resistance in local durum wheat 'Agili', the resistance of F_2 derived F_3 families was assessed at adult stage in field conditions after inoculation with the same pathotype 'Tun6'. In both crosses the resistant families were uniformly resistant as all the plants within the family did not develop pynidia whereas the entire individual within the susceptible families had leaves covered with pycnidia. F_3 families in both crosses segregated in

Table 1 Phenotypic segregation of F_2 and BC_1F_1 populations for reaction to Septoria tritici blotch caused by isolate 'Tun6' of *Mycosphaerella graminicola.*

Cross	Generation	Total plants	Observed[a]	Expected ratio	χ^2
'Agili'/ 'Karim'	F_2 (seedlings)	58	48:10	3:1	0.46 (1.39)
'Agili'/'Karim'	F_2 (adult plants)	100	69:31	3:1	0.48 (1.44)
'Agili'/'Khiar'	F_2 (seedlings)	74	58:16	3:1	0.19 (0.33)
'Agili'/'Khiar'	F_2 (adult plants)	95	67:28	3:1	0.25 (0.76)
'Agili'/'Karim'/'Karim'	BC_1F_1 (adult plants)	71	42:29	1:1	1.19 (1.19)

χ^2 critical values at $P = 0.05$ and 0.1 with 1 degree of freedom are 3.84 and 2.71, respectively.
[a] Resistant/susceptible for F2 data.

Table 2 Phenotypic segregation of F_3 population at adult plant stage for reaction to isolate 'Tun6' of *Mycosphaerella graminicola.*

Source of F_3 population	Total number of F_3 families	Number of F_3 families			χ^2 (1:2:1)
		STB-homozygous resistant	Segregating	STB-homozygous susceptible	
'Agili'/'Karim'	48	13	24	11	0.016
'Agili'/'Khiar'	57	14	29	14	0.017

χ^2 critical values at $P = 0.05$ and 0.1 with 2 degrees of freedom are 5.99 and 4.61, respectively.

a 1:2:1 (homozygous resistant /segregating/ homozygous susceptible) ratio confirming that the resistance of 'Agili' is governed by a single dominant gene (**Table 2**).

DISCUSSION

Over three 3-year field evaluation under high disease pressure by inoculation with a virulent pathotype, no necrosis and pycnidia was observed on durum wheat 'Agili', indicating that the resistance was effective against Tunisian *M. Graminicola* pathotype 'Tun6'. This also indicated that among *M. graminicola* populations in the field, a new pathotype that would overcome 'Agili' resistance did not occur yet. This level of resistance in 'Agili' resemble the resistance phenotype observed on *T. monococcum* where in most interactions examined, no pycnidia formation was observed and were considered to be incompatible interaction (Jing *et al.* 2008).

The inheritance in F_2 and F_3 progenies in both crosses 'Agili'/'Karim' and 'Agili'/'Khiar', showed that resistance to isolate 'Tun6' of *M. graminicola* is conferred by a single dominant gene in the local durum wheat 'Agili'. These results are consistent with previous findings of qualitative inheritance of resistance to STB in hexaploid wheat *T. aestivum* (Rosielle and Brown 1979; Wilson 1979; Lee and Gough 1984; Wilson 1985; Somasco *et al.* 1996; McCartney *et al.* 2002). The qualitative inheritance of resistance of the durum wheat cv. 'Coulter' to Canadian races MG2 and MG96-36 was also reported (McCartney *et al.* 2002). However, this source of resistance is susceptible to 'Tun6' pathotype (Medini and Hamza 2008) indicating that the resistant gene in 'Agili' is different from the genes found in cv. 'Coulter'. Some of the lines in F_2 progeny of the cross 'Coulter'/4B1149 (resistant/susceptible) showed an intermediate resistance (reaction type 3) associated with heterozygous individuals, whereas in the present study the resistant (homozygous and hetererozygous) individuals in F_2 populations displayed no pycnidia formation as the parent 'Agili'. This indicates that the resistance carried by 'Agili' is controlled by a complete dominant gene which is in accordance with gene for gene pathosystem where only a single incompatible interaction is required to provide a full host resistance to the pathogen. The same discrimination criteria presence/absence of pycnidia was used to analyze the monogenic inheritance of a high resistance to *M. graminicola* in a *T. monococcum* accession (Jing *et al.* 2008), which suggest that the resistant level in 'Agili' is as high as the level of resistance found in *T. monococum*. However, the experiments on *T. monococcum* F_2 progeny were conducted using high disease pressure i.e. inoculum infiltration, and therefore we cannot exclude that under this condition the resistant F_2 progeny of 'Agili'/'Khiar' and 'Agili'/'Karim' crosses would manifest pycnidial formation. The qualitative disease scoring (presence/absence of pycnidia) used in our study was not applied to analyze the genetic inheritance of the identified *Stb* genes in hexaploid wheat varieties because a minimal pycnidial formation (5 to 20% of leaf surface covered with pycnidia) was assigned to the resistant lines (Brading *et al.* 2002; Adhikari *et al.* 2004; Chartrain *et al.* 2005; Raman *et al.* 2009). This indicates that the resistance level found in hexaploid wheat varieties is lower than that observed in tetraploid wheat 'Agili'. Moreover, the high level of resistance found in 'Agili' led to the use of a qualitative scale to analyze genetic inheritance in the field. In fact, by using this quantitative scale, variability of symptom expression in the field was described, which complicates the scoring of inoculated plants and therefore uncertain data for genetic analysis of the resistance (McCartney *et al.* 2003; Goodwin 2007). Further experiments to introgress this resistant gene into susceptible lines could be then conducted in the field since accurate phenotype of the segregating generations can be assigned.

The data recorded in F_2 progeny at seedling stage agree with those occurred at heading stage. These data suggest that the resistance gene identified in this study may be effective at both plant stages. The present results are consistent with those of Wainshilbaum and Lipps (1991) and Somasco *et al*. (1996), who found good correlation between resistance at seedling and adult plant stages respectively in F_3 progenies derived from the crosses 'Tadinia'/'Yecora rojo', and 'Tadinia'/'Inia66R' and on two soft red winter wheat cultivars (AGRA GR8SS and Caldwell). Whereas, Kema and Silfhout (1997) and Gieco *et al*. (2004) found significant difference in the reactions of bread wheat lines at seedling and adult plant stages to two of three *M. graminicola* isolates, indicating differential expression of resistance at the seedling and adult plant stages.

In conclusion, a high resistance level to *M. graminicola* isolate 'Tun6' controlled by a single dominant gene was found in old local durum wheat 'Agili.' The resistance level is similar to resistance found in *T. monococcum* accessions. Whether the resistant gene in 'Agili' has evolved from *T. monococcum Stb* gene (*Tmstb1*) is to be analysed. Genetic mapping and the co-localization of 'Agili' resistant gene within the same chromosomal region as *TmStb1* will confirm this hypothesis. A complimentary molecular analysis is also needed to confirm the genetic results recorded, by locating and identifying flanking markers to the resistance locus that can be used for screening breeding lines. This suggestion will be the aim of the next study.

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