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 F2 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 F2-derived F3 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 (Jilibene 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 (Sth1 to Sth15) have been reported (http://www.shigen.nig.ac.jp/wheat/komugi/mgenes/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 F2 progeny and F2-derived F3 families from two crosses between the durum wheat resistant...
parent ‘Agili’ and the durum wheat susceptible parents ‘Karim’ and ‘Khiar’ and BC1F1 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 F2 generation, seedlings and adult plants of both crosses were tested to isolate ‘Tun6’ of *M. graminicola* whereas for BC1F1 progeny only adult plants were scored. F2 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 ($\chi^2$) 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 ‘Khiar’ 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).

**Segregation analysis of the F2 progeny for STB resistance**

F2 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 F2 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 F2 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 F2 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 BC1F1 population which fits closely to the expected 1: 1 ratio (Table 1).

**Segregation analysis of the F3 families for STB resistance**

To confirm the single control of resistance in local durum wheat ‘Agili’, the resistance of F2 derived F3 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 pycnidia whereas the entire individual within the susceptible families had leaves covered with pycnidia. F3 families in both crosses segregated in

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**Table 1** Phenotypic segregation of F2 and BC1F1 populations for reaction to Septoria tritici blotch caused by isolate ‘Tun6’ of *Mycosphaerella graminicola*.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Total plants</th>
<th>Observed</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Agili’/‘Karim’ F2 (seedlings)</td>
<td>58</td>
<td>48:10</td>
<td>3:1</td>
<td>0.46 (1.39)</td>
<td></td>
</tr>
<tr>
<td>‘Agili’/‘Karim’ F2 (adult plants)</td>
<td>100</td>
<td>69:31</td>
<td>3:1</td>
<td>0.48 (1.44)</td>
<td></td>
</tr>
<tr>
<td>‘Agili’/‘Khiar’ F2 (seedlings)</td>
<td>74</td>
<td>58:16</td>
<td>3:1</td>
<td>0.19 (0.33)</td>
<td></td>
</tr>
<tr>
<td>‘Agili’/‘Khiar’ F2 (adult plants)</td>
<td>95</td>
<td>67:28</td>
<td>3:1</td>
<td>0.25 (0.76)</td>
<td></td>
</tr>
<tr>
<td>‘Agili’/‘Karim’/‘Karim’ BC1F1 (adult plants)</td>
<td>71</td>
<td>42:29</td>
<td>1:1</td>
<td>1.19 (1.19)</td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2$ critical values at $P = 0.05$ and 0.1 with 1 degree of freedom are 3.84 and 2.71, respectively.

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**Table 2** Phenotypic segregation of F3 population at adult plant stage for reaction to isolate ‘Tun6’ of *Mycosphaerella graminicola*.

<table>
<thead>
<tr>
<th>Source of F3 population</th>
<th>Total number of F3 families</th>
<th>STB-homozygous resistant</th>
<th>Segregating</th>
<th>STB-homozygous susceptible</th>
<th>$\chi^2$ (1:2:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Agili’/‘Karim’</td>
<td>48</td>
<td>24</td>
<td>11</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>‘Agili’/‘Khiar’</td>
<td>57</td>
<td>29</td>
<td>14</td>
<td>0.017</td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2$ critical values at $P = 0.05$ and 0.1 with 2 degrees of freedom are 5.99 and 4.61, respectively.
effective at both plant stages. The present results are consistent with those of Wainshilbaum and Lippis (1991) and Somasco et al. (1996), who found good correlation between resistance at seedling and adult plant stages respectively in F₃ progenies derived from the crosses ‘Tadima’/ ‘Yecora rojo,’ and ‘Tadima’/‘Inia66R’ and on two soft red winter wheat cultivars (AGRA GRBSS and Caldwell). Whereas, Kema and Silhout (1997) and Gieco et al. (2004) found no significant reaction between ‘Agili’ and pycnidia was observed on durum wheat ‘Agili’ 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.

ACKNOWLEDGEMENTS

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