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A Valuable QTL for Fusarium Head Blight Resistance from *Triticum turgidum* L. ssp. *dicoccoides* Has a Stable Expression in Durum Wheat Cultivars

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Langdon(Dic-3A)-10 line carrying the QTL *Qfhs.ndsu-3AS* from *T. turgidum* ssp. *dicoccoides* that confers Type II resistance to Fusarium head blight (FHB) was crossed with Argentinean durum wheat cultivars. F4 progeny were screened with the microsatellite locus *Xgwm2,* tightly linked to the *Qfhs.ndsu-3A* region. Reaction of these plants and parents to FHB was evaluated at 7, 14 and 21 days post-inoculation (dpi) with *F. graminearum; severity* (% symptomatic spikelets/spike) and *AUDPC* (area under disease progress curve) were calculated. F4 progeny carrying the resistance allele in heterozygous or in homozygous condition showed significantly lower scab damage at 21 dpi and slower progress of disease than cultivated parents. Our results indicate that the resistance *Qfhs.ndsu-3AS* has a stable dominance expression in genetic backgrounds of durum cultivars and demonstrate that the linked microsatellite is an effective molecular tool for resistance screening. This work offers valuable information for *Qfhs.ndsu-3AS* utilization in wheat breeding programs.

Keywords: durum wheat*, Fusarium graminearum*, resistance breeding, disease severity, marker-assisted selection

Abbreviations: AUDPC: area under disease progress curve; dpi: days post-inoculation; cv: cultivar; FHB: Fusarium Head Blight, QTL: quantitative trait locus.

Introduction

Fusarium Head Blight (FHB), caused primarily by *Fusarium graminearum* Schwabe, is a devastating fungal disease affecting common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) and durum wheat (*T. turgidum* L. ssp. *durum*, $2n = 4x = 28$, AABB). FHB epidemics occur mainly associated with warm weather conditions, precipitation and high humidity, during flowering and the early kernel development stages (Parry et al. 1995).

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The effects of FHB infection entail yield losses and a reduction in grain quality that includes contamination with mycotoxins which are unsafe for human and animal consumption (Wegulo 2012).

In recent decades FHB has re-emerged as a disease of worldwide significance because global climate change would favor FHB (McMullen et al. 1997, Chakraborty and Newton 2011). Increasing occurrence of FHB epidemics has also been associated with crop rotation involving susceptible crops such as corn and soybean, in combination with no-till agriculture that contributes to maintain FHB pathogens inoculum in the soil (Galich 1997; Broders et al. 2007). In Argentina, general estimates of yield losses due to FHB in common wheat ranged 20–30 % (Galich 1997; Moschini et al. 2002; Kikot et al. 2011), in some years causing unacceptable levels of deoxynivalenol toxin in the flour (Moschini et al. 2013; Martinez et al. 2014). Severe FHB epidemics also occurred in durum wheat, with crop losses as high as 70% (Moschini et al. 2004). Lack of FHB resistance in durum germplasm might be traced to the Mediterranean basin being the most important center of cultivar release for a long time, a region where FHB plays no major role as a disease (Miedaner and Longin 2014). A high FHB susceptibility of most durum varieties explains, in part, the present distribution of durum wheat in Argentina, confined to zones where FHB incidence is expected to be lower based on prevailing temperature and humidity conditions (Seghezzo 2015). An effective managing of FHB requires integration of many strategies, such as crop rotation, cultivars with improved behavior, biological control, chemical control and weather-based warning systems (Gilbert and Haber 2013). However, the utilization of varieties with improved disease resistance is considered to be the best economic and ecological strategy to manage the disease.

The progress of breeding for wheat FHB resistance has been quite limited because of scarce sources of resistance, a poor knowledge on the genetic basis and quantitative nature (Buerstmayr et al. 2009). In addition, environmental factors significantly influence the evaluation of resistance at field and make the phenotyping and breeding efforts harder. Two major mechanisms of wheat FHB resistance were first described by Schroeder and Christensen (1963): resistance to initial infection after spray inoculation (Type I) and resistance to fungal spread within the spike after point inoculation (Type II).

Because of durum wheat susceptibility, a great effort to identify sources of effective resistance to FHB has been made (Prat et al. 2014). An extensive screening of durum germplasm identified a few Syrian and Tunisian landraces as Type II resistance sources (Talas et al. 2011, Huhn et al. 2012). Tetraploid relatives have proved to be an important source of FHB resistance (Prat et al. 2014). QTLs conferring resistance were identified in *T. carthlicum* (Somers et al. 2006), *T. dicoccum* (Buerstmayr et al. 2012; Ruan et al. 2012; Zhang et al. 2014) and *T. dicoccoides* (Otto et al. 2002; Chen et al. 2007; Kumar et al. 2007; Buerstmayr et al. 2013), in some cases were mapped in similar positions as resistance QTLs described for hexaploid wheat.

Breeders targeted their search in even more distantly related species. A FHB resistance locus has been identified in the chromosome 1E of the perennial diploid wild grass *Lophopyrum elongatum* (Jauhar and Petersen 2008), consequently new resistant addition

and substitution lines were derived through hybridization with durum cv. Langdon (Jauhar et al. 2009; Jauhar and Peterson 2012; 2013).

The *T. triticum* ssp. *dicoccoides* (*AABB,* $2n = 4x = 28$) resistance source has been of great interest for breeding programs. A series of recombinant substitution lines were derived from crosses with *T. turgidum* ssp. *durum* cv. Langdon (Stack et al. 2002). In particular, the Langdon(Dic-3A)-10 line had the best performance regarding Type II FHB resistance. Using this recombinant line collection, Otto et al. (2002) identified the region *Qfhs.ndsu-3AS* on chromosome arm 3AS that explained 55% of the genetic variance for FHB resistance. Mapping analysis positioned *Qfhs.ndsu-3AS* within a chromosomal interval of 11.5 cM that is flanked by markers *Xfcp401* and *Xfcp397.2,* with the microsatellite locus *Xgwm2* tightly linked to *Qfhs.ndsu-3A* (Chen et al. 2007). A recent study saturated the *Qfhs.ndsu-3AS* region using 22 newly EST-derived markers (Zhu et al. 2015).

Durum wheat semolina is by preference the raw material used in production of pasta worldwide, because of its hard grain texture, amber color, and other grain quality traits related to endosperm protein (Sissons 2008). The development of resistant cultivars is considered the most economical and environmentally safe strategy to control FHB disease. In this way, the objectives of this study were to investigate the expression and stability of *Qfhs.ndsu-3AS* in new genetic backgrounds and evaluate the effectiveness of linked markers for selection of resistant genotypes The ultimate goal of this work is to incorporate the *Qfhs.ndsu-3AS* resistance region into durum wheat cultivars. This is the first report about performance of *Qfhs.ndsu-3AS* into adapted backgrounds.

Materials and Methods

Plant material

The FHB resistant line Langdon(Dic-3A)-10 carrying *Qfhs.ndsu-3AS*, and two moderately susceptible durum Argentinean commercial cultivars, Buck Esmeralda (http://www.bucksemillas.com.ar/productos/trigocandeal/) and Buck Candisur (http://genbank.vurv.cz/ewdb/asp/ewdb_d2.asp?accn=46850), were selected as parents to generate segregating populations. Seeds of Langdon(Dic-3A)-10 were kindly provided by Dra. Gabriela Tranquilli (INTA Castelar, Argentina) and seeds of cultivars were received from Ag. E. Lisardo González (Buck Seeds S.A. La Dulce, Argentina). Seeds were germinated in 10 L pots containing a mixture of compost and sand, fertilized with diammonium phosphate and placed in the greenhouse (25 ± 3 °C, 16 light hours). Replicated sowings were performed every~15 days in order to obtain plants with synchronized flowering.

A total of 22 controlled crosses (Miller et al. 1980) were conducted using Langdon (Dic-3A)-10 as both female and male parental. Pollinated spikes were immediately covered with a paper bag to avoid foreign pollen. Spikes were manually threshed and F1 seeds were kept in paper bags until planted. Then, three consecutive selfing generations (F2-F4) were performed.

Marker analysis

Individuals from F1 and F4 generations were genotyped at *Qfhs.ndsu-3AS* region using the linked SSR loci *Xgwm2* (Otto et al. 2002). DNA was extracted from leaf tissue following a modified CTAB method (Saghai-Maroof et al. 1984). The primer combination was L: CTGCAAGCCTGTGATCAACT and R: CATTCTCAAATGATCGAACA as reported by Röder et al. (1998). PCR reactions were performed in a total volume of 25 µL containing 0.25 µM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1X Taq buffer, 1 U *Taq* polymerase (Invitrogen, Brazil), and 75 ng DNA template. The PCR profile consisted of an initial denaturation step of 3 min at 94 °C, 45 cycles of 1 min at 94 °C, 1 min at 52 °C, 2 min at 72 °C, and a final extension step of 10 min at 72 °C. Amplification reactions were performed in a MyCycler Thermal Cycler (Bio-Rad, USA). Products were separated on a 6% denaturing polyacrylamide gel and silver-stained.

Seedlings of F4 progeny were genetically characterized as homozygous or heterozygous for *Qfhs.ndsu-3AS* region based on SSR marker and destined to severity assay.

Preparation of inoculum

A macroconidial suspension of *F. graminearum* strain KBC7 (kindly provided by Dr. Cecilia Farnochi, Universidad Nacional de Río Cuarto, Argentina) was cultured in a liquid mung bean medium for 7 days at 25 °C (Bai et al. 2000). The medium was then removed by two centrifugation steps in distilled water. The conidia concentration was quantified under an optical microscope with a Neubauer chamber and diluted to the desired concentration.

FHB evaluations

Spikes from each genotype were point inoculated with *F. graminearum* during early anthesis by injecting 10 µL of 10^5 conidia/mL suspension into the basal florets of the two central spikelets. After inoculation, the ears were wrapped in clear polythene bags and plants placed in a growth chamber with high relative humidity maintained by spraying with water. After three days, the bags were removed and the plant grew under greenhouse conditions. The infection score *severity* was estimated as the percentage of symptomatic spikelets per spike (Schroeder and Christensen 1963) and registered at 7, 14 and 21 days post-inoculation (dpi). To evaluate the spreading rate of the infection, *AUDPC* (Area under disease progress curve) was calculated with the formula:

$$AUDPC = \sum_i^{n-1} \left\{ \left[\left(Y_i + y_{i+1} \right) / 2 \right] \left(t_{i+1} - t_i \right) \right\}$$

where n = total number of observations, Y_i = visually symptomatic spikelets on the ith day and t_i is the day of the ith observation (Campbell and Madden 1990).

This study comprised two independent inoculation experiments as follows:

Experiment 1: Involved the resistant line Langdon(Dic-3A)-10, cultivars B. Esmeralda and B. Candisur and cultivar Langdon as susceptible control genotype. This experiment also included five F4 homozygous plants for resistant allele, five heterozygous plants and five homozygous plants for susceptible allele according to the previous molecular analysis. Statistical comparisons were first performed among lines (Series 1) and in a second step the parental lines and homozygous and heterozygous progeny (Series 2) were considered.

Experiment 2: Involved parental lines and ten F4 homozygous plants for resistant allele according to molecular analysis.

Statistical analysis

Mean values of *severity* and *AUDPC* were compared among genotypes using nested analysis of variance (ANOVA), with individual plants as replicates (*N*) and the spikes (*n*) nested under plants. To meet ANOVA assumptions, *severity* and *AUDPC* were transformed using the square root and natural log (ln) respectively. All statistical analyses were performed using Infostat software version 2010 (Di Rienzo et al. 2010).

Results

Inbred lines differed in level of infection and progress of disease

The FHB resistance level estimated via *severity* varied among inbred lines as shown in Table 1A for Series 1. At 7 dpi cv. Langdon (susceptible) and line Langdon(Dic-3A)-10 (resistant) were not significantly different in *severity* whereas cultivars B. Esmeralda and B. Candisur showed higher *severity* than the former two genotypes, with B. Candisur having the significant highest level of infection. At 14 dpi cv. Langdon was the most susceptible genotype. At 21 dpi a significantly higher disease score was observed in cv. Langdon in comparison with the recombinant line Langdon(Dic-3A)-10 indicating that the two genotypes varying in the *Qfhs.ndsu-3AS* region exhibited quite extreme FHB resistance phenotypes (Fig. 1a). Disease scores at 21 dpi in B. Esmeralda and B. Candisur had intermediate values (around 50–60%) in comparison to Langdon(Dic-3A)-10 and Langdon, although *severity* of B. Candisur did not significantly differentiate from cv. Langdon (Table 1A).

Regarding the rate of the pathogen spreading, differences in the development of disease were observed among genotypes along the period of evaluation (Fig. 1b). One week after inoculation, less than 9% of spikelets per spike showed symptoms of infections and differences between genotypes were not significant. Two weeks later, cv. Langdon rapidly increased the number of symptomatic spikelets and differentiated from the remaining genotypes. Three weeks after inoculation cv. Langdon reached the maximum level of infection in the experiment, Langdon(Dic-3A)-10 line had the lowest value and commercial genotypes were moderately infected. This time-course trend was statistically confirmed when means of total area under progress curve for each genotype were compared (*AUDPC*, Table 1A).

Table 1. Inoculation experiment 1. Mean values of *Severity* (% symptomatic spikelets/spike) at 7, 14 and 21 days post-inoculation (dpi) with *F. graminearum* and *AUDPC* (area under disease progress curve) for durum wheat genotypes: (A) parental lines and susceptible control Langdon (Series 1); (B) parental lines and genotype classes of F4 progeny being *M* the resistant allele and *m* the susceptible allele (Series 2). N = number of plants (replicates), *n* = number of inoculated spikes

A)

Genotype	N	N	*Severity*			*AUDPC*
			7 dpi	14 dpi	21 dpi	
Langdon(Dic-3A)-10	4	8	1.30^{a}	14.52^{a}	23.23^{a}	172.43^{a}
Langdon	5	9	1.69^{ab}	53.44^{b}	77.41^{c}	618.38^{c}
Buck Esmeralda	4	10	4.41^{bc}	23.91^{a}	49.56^{b}	330.30^{b}
Buck Candisur	3	6	8.35^{c}	17.31^{a}	57.61^{bc}	336.97^{b}

Values within a column followed by the same letters are not significantly different (Fisher's LSD test, $\alpha = 0.05$).

B)

Langdon(Dic-3A)-10 × Buck Candisur						
Genotype	N	n	*Severity*			*AUDPC*
			7 dpi	14 dpi	21 dpi	
Langdon(Dic-3A)-10	4	8	1.30^{a}	14.52^{b}	23.23^{b}	172.43^{bc}
F4 *MM*	3	9	1.46^{a}	5.71^{a}	14.67^{a}	83.93^{a}
F4 *Mm*	2	6	3.42^{a}	9.30^{ab}	16.00^{a}	111.05^{ab}
F4 *mm*	5	8	1.00^{a}	11.97^{b}	33.76^{c}	196.37^{c}
Buck Candisur	3	6	8.35^{b}	17.31^{b}	57.61^{d}	336.97^{d}
Buck Esmeralda × Langdon(Dic-3A)-10						
Genotype	N	n	*Severity*			*AUDPC*
			7 dpi	14 dpi	21 dpi	
Langdon(Dic-3A)-10	4	8	1.30^{a}	14.52^{a}	23.23^{a}	172.43^{ab}
F4 *MM*	6	7	1.00^{a}	11.63^{a}	18.84^{a}	127.74^{a}
F4 *Mm*	5	8	1.46^{a}	7.73^{a}	20.43^{a}	121.51^{a}
F4 *mm*	4	6	1.00^{a}	15.68^{ab}	45.43^{b}	257.38^{bc}
Buck Esmeralda	4	10	4.41^{b}	23.91^{b}	49.56^{b}	330.30^{c}

Values within a column followed by the same letters are not significantly different (Fisher's LSD test, $\alpha = 0.05$).

A microsatellite marker accurately identified allelic variants for resistance at Qfhs.ndsu-3AS

Amplification patterns of parental and F1 individuals at SSR locus *Xgwm2* are shown in Fig. 2a. Patterns were interpreted in terms of size fragments: the resistant allele *M* (210 bp) as the marker associated to parent Langdon(Dic-3A)-10 and the susceptible allele *m*

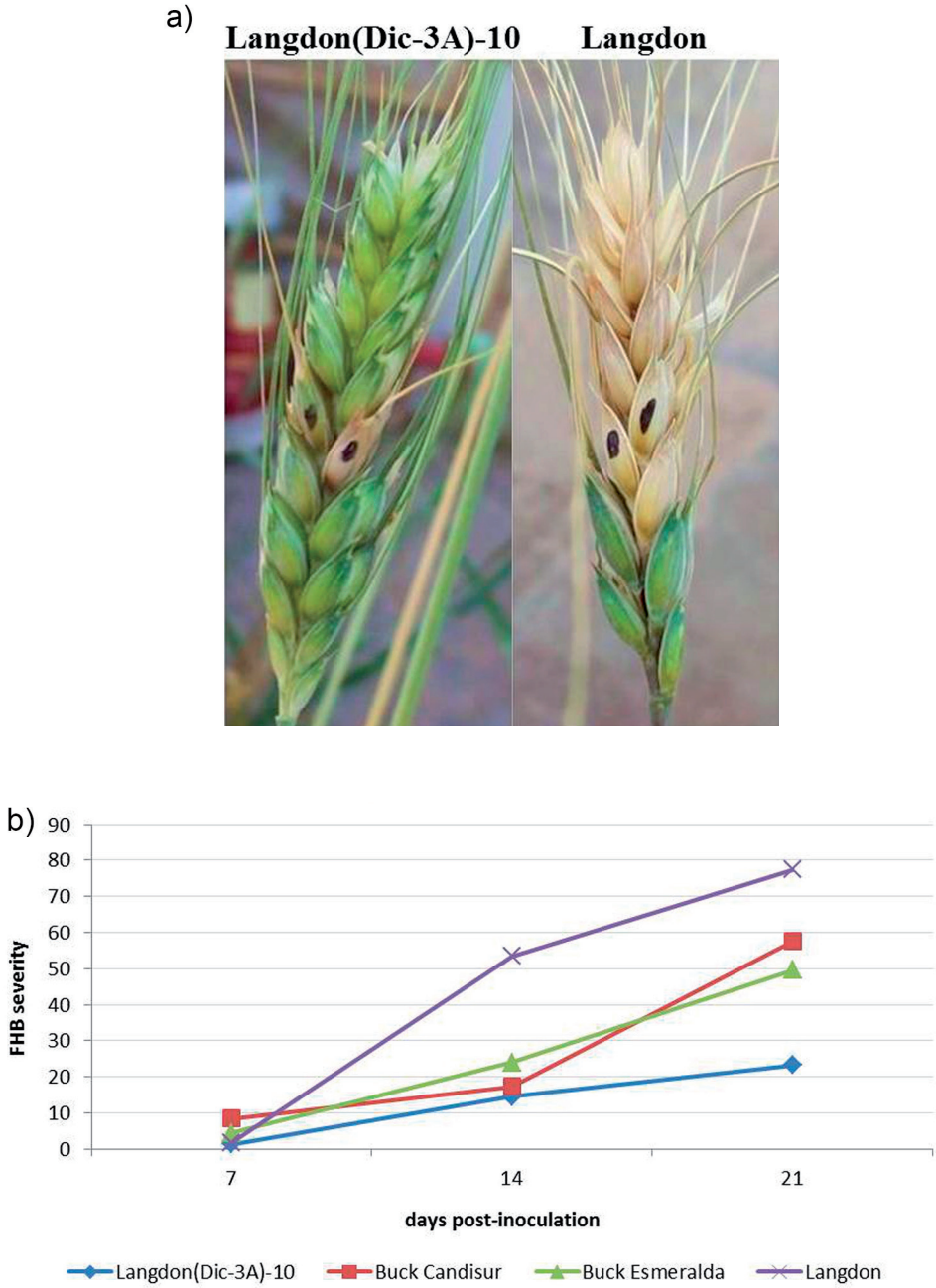

Figure 1. FHB resistance evaluation of durum wheat cultivars (a) Spikes at 21 days post-inoculation with *F. graminearum* in durum wheat genotypes Langdon(Dic-3A)-10 (resistant) and Langdon (susceptible). Black point indicates inoculated spikelets. (b) Progress curves of *Severity* after *F. graminearum* inoculation

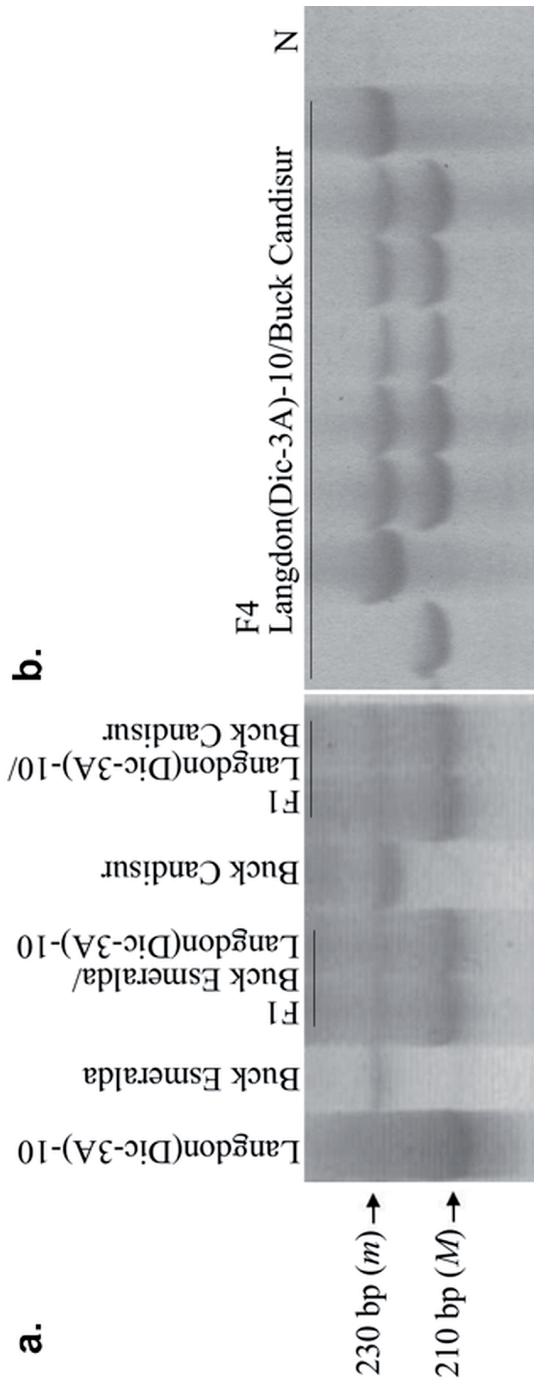

Figure 2. PCR amplification of the SSR marker *Xgwm2* linked to *Qfhs.ndsu-3AS*. (a) Amplification pattern in the parental lines Langdon(Dic-3A)-10, Buck Esmeralda, Buck Candisur and F1 plants. (b) Amplification pattern in the F4 plants. Susceptible genotypes present an amplicon of ~230 bp (*m*) and resistant an amplicon of ~210 bp (*M*). N: negative control

(230 bp) as the marker associated to parent B. Candisur or B. Esmeralda. Two F1 *Mm* plants from each cross were selected to advance to subsequent generations.

At F4 generation, a total of 100 seedlings from each cross (Langdon(Dic-3A)-10 × B. Candisur and B. Esmeralda × Langdon(Dic-3A)-10) were molecularly screened. Segregation of *Xgwm2 m*arker was clearly observed and F4 individuals could be unambiguously assigned to *MM*, *Mm* or *mm* classes (Fig. 2b).

A dominant effect of the Qfhs.ndsu-3AS region in F4 generation was observed

The evaluation of the spikes after point inoculation with *F. graminearum* demonstrated that the homozygous *MM* progeny displayed similar or even lower severity values in comparison to the resistant parent Langdon(Dic-3A)-10 and lower severity values than cultivated parents. These results were statistically demonstrated from *severity* at 21 dpi and *AUDPC* data in both crosses as shown in Table 1B and Table 2.

When all genotype classes were compared, the combined results of ANOVA and Fisher's LSD demonstrated that homozygous *MM* and heterozygous *Mm* did not significantly differ in their *severity* at 21 dpi as well as in their disease intensity over time (*AUDPC*). On the other hand, they both showed a significantly lower degree of disease in relation to individuals belonging to *mm* class as shown in Table 1B for Series 2. However, some differences between the relative performances of the progeny from the two crosses were observed. In the cross with B. Esmeralda, *MM* and *Mm* individuals reached levels of *severity* at 21 dpi similar to the resistant parent, whereas the same genotype classes performed better than the resistant parent when B. Candisur was used as cultivated parent.

Table 2. Inoculation experiment 2. Mean values of *Severity* (% symptomatic spikelets/spike) at 7, 14 and 21 days post-inoculation (dpi) and *AUDPC* (area under disease progress curve) for durum wheat genotypes: parental lines and genotype class *MM* of F4 progeny being *M* the resistant allele (Series 3). N = number of plants (replicates), *n* = number of inoculated spikes

Langdon(Dic-3A)-10 × Buck Candisur						
Genotype	N	n	*Severity*			*AUDPC*
			7 dpi	14 dpi	21 dpi	
Langdon(Dic-3A)-10	3	5	1.00[a]	7.73[a]	10.96[a]	85.63[a]
F4 *MM*	12	36	2.62[a]	10.96[ab]	17.47[a]	129.02[a]
Buck Candisur	3	6	4.41[a]	16.03[b]	50.27[b]	287.15[b]
Buck Esmeralda × Langdon(Dic-3A)-10						
Genotype	N	n	*Severity*			*AUDPC*
			7 dpi	14 dpi	21 dpi	
Langdon(Dic-3A)-10	3	5	1.00[a]	7.73[a]	10.96[a]	85.63[a]
F4 *MM*	10	29	1.21[a]	8.24[a]	16.56[a]	99.58[a]
Buck Esmeralda	3	5	1.99[a]	13.84[a]	43.16[b]	262.43[b]

Values within a column followed by the same letters are not significantly different (Fisher's LSD test, $\alpha = 0.05$).

In the case of *AUDPC* means, genotype classes of progenies and parental lines were not completely discriminated by Fisher's LSD test, likely due to some overlapping phenotypes (Table 1B).

When F4 plants within each genotype class were individually considered only one discrepancy between genotype based marker and phenotype was detected: one *MM* plant displayed similar *severity* to a *mm* plant within the B. Esmeralda × Langdon(Dic-3A)-10 progeny (data not shown).

Discussion

Discovering and transferring valuable QTLs from unadapted germplasm into elite breeding lines constitute a permanent objective of plant improvement. To our knowledge, this is the first study describing the expression of the resistance QTL *Qfhs.ndsu-3AS* from *Triticum turgidum* L. ssp. *dicoccoides* (Otto et al. 2002) in the genetic backgrounds of durum cultivars. This QTL region is not homoeologous to *fhb1*, a major FHB QTL derived from the common wheat cv. Sumai 3 (Chen et al. 2007).

Qfhs.ndsu-3AS region confers Type II resistance by slowing down or inhibiting the spread of the pathogen from the initial infection site (Stack et al. 2002). Visual assessment of *severity* is considered to be the most informative and the least environmental affected approach to evaluate FHB resistance (Bai and Shaner 1994) thus being the most widely used method in greenhouse screenings. In our study, the use of a single disease assessment at 21 dpi appears to have higher discrimination power than the integrated *AUDPC* data if one considers that statistical comparison of *AUDPC* means evidenced phenotypic overlaps between resistant Langdon(Dic-3A)-10 and *mm* progeny (Table 1b). These results have practical implications since, one, instead of three measures would be enough to obtain a reliable parameter of FHB susceptibility for each genotype. The disease progress curve (Fig. 1b) demonstrated that the evaluations of resistance at 7 and 14 dpi were unable to detect significant differences among genotypes that only became evident after three weeks. Anatomical studies in common wheat demonstrated that during the initial spread from infected floret hyphal grows up and down through the cortical cells and vasculature of the rachis although lateral and intercellular hyphal growth from the rachis into uninoculated spikelets only occur from 12 dpi (Brown et al. 2010).These observations could explain the inability of some spike inoculation tests to recognize proven differences in field resistance, due to the fact that elapsed time was no longer enough for fungal colonisation to be evident at the moment of disease assessment (Engle et al. 2003).

The comparison of disease progression between cv. Langdon and Langdon(Dic-3A)-10 line (Fig. 1b) demonstrated that the symptoms appeared earlier in Langdon. This observation reflects differences in its ability to resist the pathogen effect attributable to *Qfhs.ndsu-3AS* region. At 21 dpi, Langdon(Dic-3A)-10 showed severity levels around three times lower than cv. Langdon in accordance with Otto et al. (2002), and two- or five-fold lower than cultivars B. Candisur and B. Esmeralda across two experiments. Different mechanisms responsible for restricting the fungus spreading along the spike have been described. In resistant cultivars a rapid and intensive deposition of callose, lignin and

other materials conforming very thick layers of wall appositions play an important role as physical barriers (Kang and Buchenauer 2000). Once the infection is established, the sesquiterpenoid mycotoxins produced by *F. graminearum*, especially DON, interfere with the metabolism, physiologic processes and structural integrity of the host cell (Audenaert et al. 2013). On the other hand, the resistant cultivars achieve lower toxin concentration than susceptible due either to mechanisms that limit the hyphal colonization in the tissue (Kang and Buchenauer 2000) or the ability to turn deoxynivalenol into the less toxic metabolite deoxynivalenol-3-*O*-glucoside (Lemmens et al. 2005). As genomic data accumulates differences between FHB-resistant and susceptible wheat cultivars appear to be associated with early or delayed induction of defense genes involved in systemic, local responses and detoxification (Kosaka et al. 2015; Soresi et al. 2015). The early induction of *Fusarium*- and DON-responsive genes in resistant genotypes has been demonstrated not only in spike samples but also in seedling tissues (Walter et al. 2015), thus suggesting conserved mechanisms of resistance at both vegetative and reproductive stages. This feature of the disease allowed the development of an *in vitro* assay able to recognize genotypes carrying FHB resistance genes at seedling stage (Soresi et al. 2015).

Regarding the process of transferring the *Qfhs.ndsu-3AS* region from Langdon(Dic-3A)-10 to durum cultivars, all the F4 progeny of *MM* and *Mm* classes showed significantly higher resistance compared to the cultivated parent at 21 dpi, indicating that the resistance QTL from *T. turgidum* spp. *dicoccoides* is fully expressed in B. Candisur and B. Esmeralda genetic backgrounds. Moreover, these results demonstrated that the linked SSR locus *Xgwm2* is an effective molecular tool for resistance screening. Homozygous *MM* plants from two different F4 offspring had similar levels of *severity* across two greenhouse assays (Series 2 and 3), suggesting a quite stable expression of resistance. The lack of significant differences between *MM* and *Mm* genotype classes for both *severity* and disease intensity over time (*AUDPC)*, indicate that dominance effects mainly influence on QTL *Qfhs.ndsu-3AS* expression.

Genotyping of F4 plants based on SSR *Xgwm2* was successful, with only one discrepancy between allele marker and expected level of disease. It could have resulted from some non-controlled environmental effect on the inoculation experiment or originated in a recombination of events causing the resistance allele to be lost. New markers closer to *Qfhs.ndsu-3AS* have recently become available (Zhu et al. 2015). Within *MM* and *Mm* progeny, all except for three plants (one *MM* and two *Mm* plants from B. Esmeralda × Langdon(Dic-3A)-10 cross) exhibited lower disease scores than the resistant parent (data not shown). A combination of FHB-improving alleles at different loci with an additive effect is a plausible explanation for transgresive phenotypes of FHB resistance (Bai et al. 2003).

As a conclusion, the effect of the resistance allele at *Qfhs.ndsu-3AS* region was validated, thus demonstrating that it was the clear determinant of FHB resistance in different genetic backgrounds that might include common wheat. A linked marker for resistance gene could be used as an efficient tool for selection. Two rounds of field evaluation on agronomical traits have already been performed on MAS selected plants that hopefully will lead to new durum varieties with improved FHB resistance. Therefore, we are currently investigating the underlying molecular mechanism of this new source of resistance using RNAseq technology.

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