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Mapping and candidate gene identification of loci determining tolerance to greenbug (*Schizaphis graminum*, Rondani) in barley

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Abstract Greenbug is one of the most aggressive pests of barley and wheat. In Argentina, yield losses of wheat, barley, oat and sorghum crops caused by greenbug are chronic and at times severe. Since Marker Assisted selection for greenbug resistance genes in barley is very limited, the purpose of the current study was to map greenbug resistance genes in doubled haploid (DH) lines and to identify candidate genes. A set of DH lines of the Oregon-Wolfe Barley (OWB) mapping population derived from the cross between OWB_{DOM} and OWB_{REC} and both parental lines were screened for tolerance to greenbug. There was significant variation among the DH lines in foliar area (FA), dry weight (DW) and chlorophyll contents (Ch) between infested and control DH lines. Three main QTLs were identified. These QTLs explained 82 % of the FA, 80 % of DW and 58 % of Ch variability of infested plants. The initial and final FA and DW of controls and final DW of infested plants were associated with the same molecular markers on chromosome 2H (*Vrs1*, *BmAc0144f*, *GBR259*, *GBS705*). The final FA of infested plants was significantly linked to molecular markers on chromosome 5H (*GBRO986*, *GBR518*, *GBM1483*, *GBR1082*). The positive alleles were provided by OWB_{DOM}. The content of chlorophyll of infested plants was associated with the marker loci *Ris44*, *GBR1608*, *GBR1637N* and *GBS0785* on chromosome 7H, with the positive alleles provided by OWB_{REC}. Both parents contributed to different tolerance traits. The QTLs found in this population are new greenbug resistance loci. A sequence homology search was performed to derive the putative function of the genes linked to the QTLs.

Keywords Marker assisted selection · Greenbug tolerance · QTLs · Candidate genes · *Hordeum vulgare*

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Introduction

Greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), is one of the main pests of wheat and barley worldwide. In Argentina, yield losses of wheat, barley, oat and sorghum crops caused by greenbug are chronic and at times severe. Greenbug damages the plant while feeding because of the toxic salivary enzymes injected, which induce chlorosis, thereby increasing the concentration of free amino acids around the feeding site (Dorschner et al. 1987).

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Greenbug damage is identified as chlorotic and necrotic spots, mainly in older leaves (Starks and Burton 1977).

Genetic resistance is considered one of the most important components of integrated pest management and is probably the best way to control this insect pest. The use of host plant resistance is an environmentally safe and cost-effective way to manage greenbug infestation. According to Painter (1951), it is possible to identify different categories of resistance, such as antixenosis, tolerance and antibiosis. Antixenosis, is the insect negative response to a particular plant that does not serve as a suitable host for food, shelter, or oviposition site. Tolerance is the ability of plants to withstand or recover from insect damage and antibiosis represents the adverse effects of a plant on the biology of the insect pest. The combination of different types of resistance has better effects than the individual ones (Smith 1989).

Molecular marker-assisted selection (MAS) is an effective tool to accelerate production of cultivars with desirable traits in particular with insect resistance (Young 1999; Yencho et al. 2000; Dekkers and Hospital 2002). MAS reduces the distortions associated with genotype x environment interactions, improves the selection efficiency, and facilitates combining different tolerance traits into a single genotype (Guo et al. 2008).

In an early work, Gardenhire et al. (1973) mapped the greenbug resistance genes in the centromeric region of barley chromosome 7H. Moharramipour et al. (1997) also identified a significant QTL on chromosome 7H conferring field resistance to a mixed population of corn leaf aphids (*Rhopalosiphum maidis* Fitch) and bird cherry-oat aphids as dominant species with minor presence of *S. graminum* and *Sitobion akebiae* (Shinji). Greenbug resistance in wheat is based on six genes that have been designated *Gb1*, *Gb2*, *Gb3*, *Gb4*, *Gb5* and *Gb6* and other three non-designated genes (*Gba*, *Gbx*, *Gbz*) (Zhu et al. 2004a). Greenbug resistance genes have also been found in the wild grass *Hordeum chilense* (Castro et al. 1994). The *Triticum aestivum/Hordeum chilense* disomic addition lines allowed the chromosomal location of greenbug antixenosis (1Hch), antibiosis (4Hch, 5Hch, 7Hch) and tolerance (7Hch) (Castro et al. 1996) which are expressed in wheat (Castro et al. 1998).

Nieto-Lopez and Blake (1994) found two regions of the barley chromosomes 1H and 2H associated with *Diuraphis noxia* (Kurdjumov) (RWA) resistance. Later, Mittal et al. (2008) mapped three QTLs for RWA resistance on chromosomes 1H and 3H, and a third locus with lower effects located on chromosome 2H. Two different QTLs providing tolerance to RWA were mapped on chromosomes 1H and 2H in barley doubled haploid lines (Tocho et al. 2012). On the other hand, Cheun et al. (2010) identified a novel QTL on chromosome 3H for bird cherry-oat aphid (*Rhopalosiphum padi*) resistance, located in a region different from those detected for RWA resistance on the same chromosome (Mittal et al. 2008; Tocho et al. 2012).

Resistance to greenbug on barley is based only on two genes (*Rsg1a* and *Rsg2b*) (Merkle et al. 1987; Porter et al. 2007). Therefore, it is imperative to find out new sources of resistance that will broaden the genetic base against this pest in barley. Since MAS for greenbug resistance genes in barley is very limited, the purpose of the current study was to map greenbug resistance genes in doubled haploid (DH) lines and to search for candidate genes.

Materials and methods

Plant material

A doubled-haploid (DH) mapping population derived from the cross between OWB_{DOM} and OWB_{REC} (Oregon Wolfe Barley, Wolfe and Franckowiak 1991; Costa et al. 2001) was used. Complete information on the "Oregon Wolfe Barley" population can be found at http://barleyworld.org/oregonwolfe. The population studied consisted of 83 DH lines and both parental lines. The plant material is kept at the IPK (Gatersleben, Germany). Contrasting molecular markers between parental lines were developed and the DH lines which were genotyped at the IPK (Stein et al. 2007).

Insect culture

Greenbugs were collected from wheat and barley fields in humid and sub-humid regions in Argentina from 2007 to 2010. Colonies were reared on the susceptible barley cultivar "Maltería Eda" in a plant growth cabinet kept at 20 °C, 50 % humidity, and 16:8 h day: night regime.

Testing procedures

Tolerance assay

Tolerance was determined by the Foliar Area (FA in cm^2), the Aerial Dry Weight (DW in mg) and the Chlorophyll content (Ch in SPAD units, mmol m^{-2}) of 83 DH and both parental lines, with and without infestation. Trays were maintained under natural conditions of light and temperature in a shelter, in La Plata, Argentina (34°55′S, 57°57′W). The experiment was repeated in two subsequent years (replicate in the first year: R1 and replicate in the second year: R2). Each experiment consisted of two treatments: infested and control plants.

Fifty germinating seeds of every DH line and the parents were sown in plastic trays filled with soil. At the second fully expanded leaf stage, half of the plants were infested with ten adult greenbugs (I) with a small, moistened, camel hair paint brush. The rest of the trays were kept uninfested as controls (C). At least 10–12 replicates of every genotype and treatment was assessed. The initial FA, DW and Ch were evaluated at the onset of infestation (day 0). Greenbug on infested plants were allowed to feed for 10 days, when susceptible plants were more than 50 % chlorotic. At that moment both, infested and uninfested plants were cut at the base (soil level) and insects feeding on infested plants were removed and destroyed. The foliar area was determined using a leaf area meter (Model LI-3100, Li-Cor). The aerial biomass of every plant was oven dried at 70 °C until constant weight, and then dry weights were determined with a precision scales (Mettler Toledo). Ch was measured by a non-destructive method using a hand-held chlorophyll meter (SPAD-52 Minolta, Camera, Osaka, Japan) on the apical (Ch^A) and basal (Ch^B) parts of the second leaf of every infested and control plant. The SPAD meter readings correspond with the actual chlorophyll content, and can thus be used to estimate the level of tolerance (Deol et al. 1997; Flinn et al. 2001; Lage et al. 2003).

All experiments were conducted as completely randomized blocks. Phenotypic summary statistics including means, standard error and analysis of variance (ANOVA) were generated using the StatSoft, Inc. (2005) program, and the Tukey Test was used to check the differences between means. The variation between replicates was not significant; therefore the graphics were obtained using the average values. Phenotypic distributions for FA, DW, Ch^A and Ch^B in the greenbug infested DH lines adjusted for the values of the respective control plants are showed together with both parental lines values (Fig. 1).

QTL mapping and EST annotation

For QTL detection, the MQM mapping was used to identify the QTLs for traits using the Map Maker program. To identify an appropriate threshold of the LOD (logarithm of the odds) score for declaring a significant QTL, a permutation test was conducted 1,000 times using the program, which resulted in a LOD threshold of 2.5 to declare the presence of a QTL. The positive values for additive effects indicate that the donor of the allele for the traits was OWB_{DOM}, whereas the negative values corresponded to OWB_{REC}. The percentage of phenotypic variation explained by each marker locus was calculated by the R^2 coefficient. The QTL analysis was performed for every year on the basis of the marker linkage map constructed by Kota et al. (2008).

For mapping population transcript maps consisting of 586 expressed sequence tag (EST)-based markers developed by Stein et al. (2007) are available. Markers were designated as GBR, GBM and GBS (for Gatersleben barley RFLP, microsatellite and SNP). Only markers in a $\pm$ 10 cM interval of the marker detected by single marker QTL analysis and having LOD values >3 were considered. Annotation of the ESTs was performed by BLASTX (Basic Local Alignment Tool) similarity search against the public non-redundant protein database NRPEP (September 2011 version), from NCBI (National Center for Biotechnology Information). Candidate orthologs were defined as those with hits with best high scoring pair (HSP) and significant E-value (Expected value) of\1.0E−10.

The sequence information of the barley ESTs are stored in the IPK Crop EST database, v1.5 (http://pgrc.ipk-gatersleben.de/cr-est).

Results

Tolerance assay

The ANOVA for FA and DW showed highly significant differences among genotypes and between the

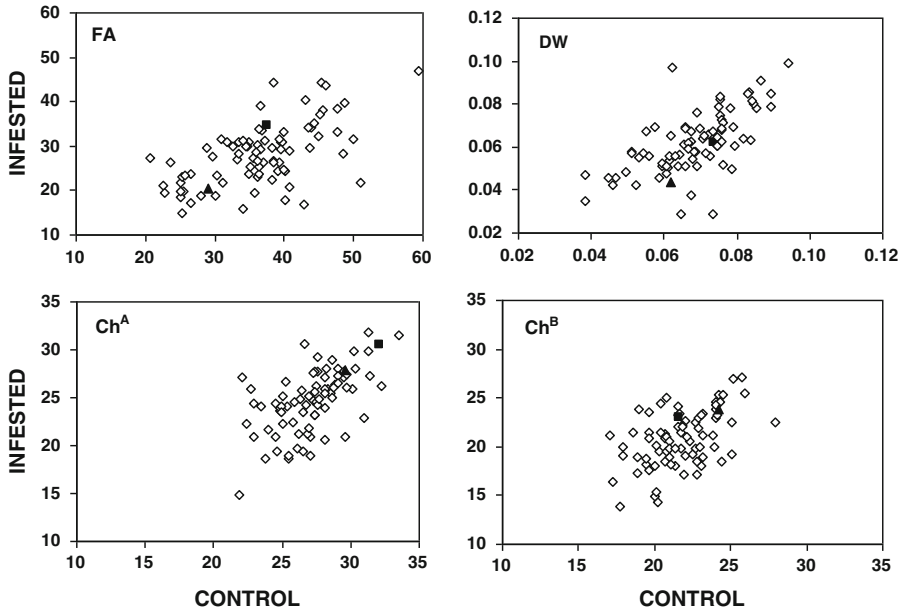

Fig. 1 Phenotypic distributions for final foliar area (FA), dry weight (DW), chlorophyll content in apical and basal regions (Ch^A and Ch^B) in the doubled haploid (DH) progeny of OWB_{DOM} and OWB_{REC}. The values for each trait under infestation with greenbug (*y*-axis) are plotted against the values of control plants (*x*-axis). *White rhombi*: DH lines; *Black squares*: OWB_{REC}; *Black triangles*: OBW_{DOM}

Table 1 ANOVA for final foliar area (FA), dry weight (DW) and chlorophyll contents in apical (Ch^A) and basal (Ch^B) foliar regions

Sources	FA			DW			Ch^A			Ch^B		
	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
Genotype	76	7.94	0.001	75	7.436	0.001	79	2.59	0.001	79	5.21	0.001
Treatment	1	188.03	0.001	1	9.036	0.003	1	0.79	0.37	1	75.17	0.001
Gen*Treat	76	1.29	0.070	75	0.996	0.501	79	2.55	0.001	79	2.76	0.001
Error	287			125			324			324		

treatments, and no differences in the interaction (Table 1). These results point out the differential behavior of the DH lines when subjected to greenbug infestation. In contrast, the Ch values were significantly different among the genotypes, between treatments and in the interaction for both leaf regions (Table 1).

The parental line OWB_{DOM} showed significantly lower values for final FA and DW under infestation compared with the controls in both replicates (Table 2; Fig. 1). However, there were no significant differences for Ch in both regions between infested and control plants.

In contrast, OWB_{REC} showed no differences in any of the parameters evaluated between infested and control plants, thus indicating that this line was more tolerant to *S. graminum* (Fig. 1). The mean values of the DH population, for all the traits when infested, were significantly different from those recorded in control plants (Table 2).

When data of FA, DW, and Ch of infested plants were adjusted for the values of the respective control plants, it was determined that the range for every trait of the DH lines exceeded the values recorded in the parental lines (Fig. 1). In this regard, the phenotypic distribution of the traits showed clear transgressive segregation under infestation (Fig. 1). Consequently, there were several lines with either lower or higher values compared to those ones recorded on parental lines. These facts point out a higher tolerance to greenbug assessed on several DH lines than that on parents. In this regard, few of the infested DH lines grew faster and retained higher chlorophyll contents in than their own controls (Fig. 1).

Table 2 Mean values for final foliar area (FA), dry weight (DW) and chlorophyll contents in apical (Ch^A) and basal (Ch^B) foliar regions in control (C), greenbug-infested (I) plants of 83 doubled haploids (DH) derived from the cross between OWB_{DOM} and OWB_{REC}, and parental lines

Trait	R	T	Parents		DH lines	
			OBW_{DOM}	OBW_{REC}	Range	Mean
FA	R1	C	**27.50 ± 0.57a**	35.23 ± 0.57a	18.29–59.99	32.68 ± 0.53
		I	**18.37 ± 0.57b**	32.26 ± 0.57a	11.25–49.39	23.92 ± 0.50
	R2	C	**30.42 ± 1.42a**	39.15 ± 3.44a	21.11–62.20	41.45 ± 0.63
		I	**22.25 ± 0.40b**	36.79 ± 0.98a	18.46–54.64	33.31 ± 0.62
DW	R1	C	**0.052 ± 0.005a**	0.058 ± 0.005a	0.024–0.086	0.052 ± 0.001
		I	**0.037 ± 0.005b**	0.052 ± 0.005a	0.026–0.095	0.041 ± 0.001
	R2	C	**0.072 ± 0.002a**	0.089 ± 0.003a	0.039–0.118	0.084 ± 0.001
		I	**0.050 ± 0.008b**	0.083 ± 0.003a	0.041–0.132	0.080 ± 0.001
Ch^A	R1	C	27.30 ± 0.31a	29.35 ± 0.50a	13.83–32.33	23.15 ± 0.27
		I	26.46 ± 1.74a	28.30 ± 1.25a	3.50–30.16	17.92 ± 0.25
	R2	C	31.87 ± 0.55a	34.87 ± 0.90a	23.93–36.83	30.85 ± 0.20
		I	29.42 ± 0.81a	32.62 ± 0.28a	21.93–39.00	30.95 ± 0.32
Ch^B	R1	C	21.76 ± 0.85a	20.01 ± 0.63a	13.02–28.23	20.88 ± 0.17
		I	22.52 ± 0.92a	19.78 ± 1.69a	2.33–26.00	16.86 ± 0.27
	R2	C	26.72 ± 1.36a	23.25 ± 0.91a	15.93–36.53	22.06 ± 0.27
		I	25.30 ± 0.31a	26.07 ± 2.25a	17.76–35.70	25.06 ± 0.28

The standard error (± SE) and range were included

Bold values indicate that there are significant differences ($P \geq 0.05$) between control and infested plants

R replicate experiment, *T* treatment

QTL mapping

Linkage analysis allowed detecting significant associations between the tolerance parameters with molecular markers. A QTL accounting for a high proportion of FA and DW phenotypic variation was identified on chromosome 2H (Table 3). An additional QTL also accounting for a high proportion of the variation in final FA was identified in infested plants (Table 3). Chlorophyll contents were scattered along chromosomes 1H, 3H, 6H and 7H.

The positive and negative additive effects at the different loci indicate that both parents contributed alleles for different traits; however, OWB_{DOM} alleles were more abundant.

The initial FA value was associated with the marker loci *BmAc0144f* and *Vrs1* and with the ESTs *GBR259*, *GBR773* and *GBS0705* (Table 3). All these markers, which explained a high proportion of phenotypic variation (99.5 %), were located in the same region of chromosome 2H. Similarly, final FA in the controls was associated with the same markers and with ESTs *GBR259,* explaining 48.60 % of total variation. In contrast, the final FA in the infested plants was associated with the ESTs markers on chromosome 5H (*GBR0986*, *GBR518*, *GBM1483 and GBR1082*) in both years, explaining 82.62 % of total phenotypic variation (Table 3). The alleles with positive effects were provided by OWB_{DOM}.

The initial DW was associated with the marker locus *Vrs1* and with the ESTs *GBS0705*, *GBR773* and *GBR259* located on chromosome 2H, explaining 95.64 % of the variation (Table 3). The final DW in the control plants was associated with the same markers, except for *GBR259,* as well as with the EST *GBS0008* (2H). These markers explained 95.28 % of the DW variation (Table 3). In the infested plants, the final DW was linked with the marker loci *Vrs1* and *Bmac144f* and with the ESTs *GBS0705*, *GBM1232* and *GBR1831*, all placed on chromosome 2H. In this case, 80.17 % of total phenotypic variation was explained, with OWB_{DOM} providing the alleles with positive effects for this trait (Table 3).

The initial chlorophyll contents on the apical and basal (Ch^A and Ch^B) foliar regions in both years were associated with the ESTs *GBR1438* and *GBR1553*

Table 3 Linkage analysis of the tolerance traits final foliar area (FA), dry weight (DW) and chlorophyll content on the apical and basal foliar regions (Ch^A and Ch^B) with the molecular and physiological markers mapped for initial values, control and greenbug-infested plants on a set of 83 doubled haploids (DH) derived from the cross between OWB_{DOM} and OWB_{REC}

Trait	Marker	Ch[a]	LOD	Additive effect[b]	R^2(%)[c]
Initial values					
FA	*BmAc0144f*	2H	4.26	1.8000	24.15
	GBR259	2H	3.67	1.9700	25.68
	Vrs1	2H	3.12	1.5500	17.18
	GBR773	2H	2.99	1.5700	17.2
	GBS0705	2H	2.84	1.4700	15.28
Dw	*Vrs1*	2H	5.08	0.0001	26.30
	GBS0705	2H	4.44	0.0001	24.16
	GBR773	2H	3.53	0.0001	20.99
	GBR259	2H	3.37	0.0001	24.19
Ch^A	*GBR1438*	1H	2.95	−0.9900	16.78
	GBR1553	1H	2.75	−0.9700	15.73
Ch^B	*ABG458*	6H	3.21	1.3300	18.12
	GBR1438	1H	2.95	−0.9890	16.78
	GBR1553	1H	2.75	−0.9700	15.75
	GBR1681	1H	2.59	−0.9100	14.53
Control plants					
FA	*Vrs1*	2H	3.09	3.5300	16.69
	BmAc0144f	2H	2.98	3.4900	16.95
	GBR259	2H	2.66	0.9000	14.96
DW	*Vrs1*	2H	5.92	0.0001	30.16
	GBS0705	2H	4.95	0.0001	25.86
	GBR773	2H	3.98	0.0001	20.33
	GBS0008	2H	3.42	0.0001	18.93
Ch^A	*HVM60*	3H	3.11	−1.2100	19.78
	GBR0788	3H	3.11	−1.1200	19.72
Infested plants					
FA	*GBR0986*	5H	3.90	3.67	21.53
	GBR518	5H	3.58	3.68	20.98
	GBM1483	5H	3.53	3.65	22.15
	GBR1082	5H	3.22	3.38	17.96
DW	*Vrs1*	2H	3.27	0.0001	17.10
	Bmac144f	2H	3.23	0.0001	17.40
	GBS0705	2H	2.89	0.0001	15.83
	GBM1232	2H	2.61	0.0001	15.78
	GBR1831	2H	2.52	0.0001	14.06
Ch^A	*Ris44*	7H	4.68	−2.8900	23.60
	GBR1608	7H	3.23	−2.5300	17.77
	GBR1637	7H	3.14	−2.4600	17.12
Ch^B	*Ris44*	7H	3.63	−0.9100	14.53
	GBS0785	7H	2.73	−0.9500	17.00
	GBR1608	7H	2.71	−2.0700	15.16

[a] Chromosome

[b] Negative values indicate effects from OBW_{REC} and positive values indicate effects from OBW_{DOM}

[c] Percentage of phenotypic variation explained by each marker locus

located on chromosome 1H. In addition, Ch^B was associated with *GBR1681* (1H) and *ABG458* situated on chromosome 6H (Table 3). The parental line OWB_{REC} provided the positives alleles, except for the 6H marker.

The final chlorophyll content in the apical part of the leaves (Ch^A) of controls was associated with the marker locus *HVM60* and with EST *GBR0788*, both located on chromosome 3H (Table 3). Meanwhile, the final contents of Ch^A in the infested plants were associated with *Ris44*, *GBR1608* and GBR1637, and those ones of Ch^B were linked with *Ris44*, *GBS0785* and *GBR1608*, all placed on chromosome 7H. The total variation explained by these markers was 58.49 % for Ch^A and 46.69 % for Ch^B and OWB_{REC} provided the alleles with positive effects (Table 3, Fig. 2).

Candidate gene identification

The candidate ESTs identified in the regions associated with QTLs for tolerance to greenbug have orthologs in rice, *Arabidopsis* and rye known functions in barley (Table 4). Four putative genes are associated with the QTLs that explained most of the FA and DW variation in the region of interest on chromosome 2H. The EST *GBR259* is expressed as a C13 endopeptidase protein. Another putative gene (*GBR733*) is expressed as a DNA repair protein (RAD23-3) in *Arabidopsis thaliana*. The candidate gene for marker *GBS0008* is a putative UV-B-resistance protein. The EST *GBS0705* is expressed as a protein with unknown function (Table 4).

The functional markers located on chromosome 5H, linked to the final FA of infested plants, have orthologs in *Oryza sativa*. The markers *GBR0986* and *GBM1483* are expressed as leucine-zipper proteins, *GBR518* is expressed as a histone H3, and *GBR1082* as a putative calcium-binding protein (Table 4).

The candidate gene for marker *GBR1608* on chromosome 7H (which was associated with Ch^A and Ch^B on infested plants) has an ortholog in rye, expressing a heat-shock protein, and *GBR1637* has an unknown function (Table 4). The only EST on chromosome 3H has an unknown function (Table 4).

Discussion

Schizaphis graminum infestation caused a differential growth rate on both parents: OWB_{DOM} was more susceptible than OWB_{REC}, and as a result, the DH lines segregated different levels of tolerance. Several lines displayed a tolerance degree higher than that of both parents. This phenomenon is known as transgressive segregation. Transgressive inheritance is based on the fact that both parents carry different alleles for the genes involved in the traits of interest that can end up together, enhancing the tolerance of these lines (Aghnoum and Nicks 2011). Transgressive

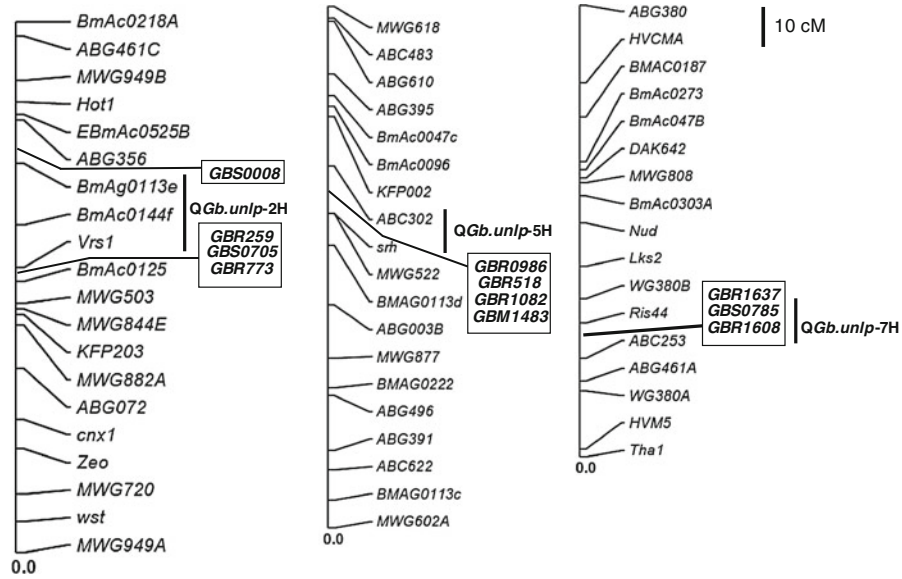

Fig. 2 QTL linkage map obtained for the OWB_{DOM} x OWB_{REC} population for final FA and DW of control and greenbug-infested plants (chromosomes 2H and 5H) and for final chlorophyll contents of infested plants (chromosome 7H) obtained from the mean values of both years. The most significant QTLs are marked with a *vertical line*. Markers associated with candidate genes are *boxed*

Table 4 Biological function of candidate ESTs having significant E-value (\1.0E−10)

EST-marker	Ch	Hit_name	Functional annotation	Organism
Vrs1	2H		Six rowed spike	*Hordeum vulgare*
			tRNA aminoacylation for protein translation; valyl-tRNA aminoacylation	
GBR259	2H	AL510729	C13 endopeptidase NP1 precursor	*Hordeum vulgare*
GBR773	2H	AL511111	DNA repair protein RAD23-3 (RAD23-like protein 3)	*Arabidopsis thaliana*
GBS0008	2H	AL509087	Putative UVB-resistance protein	*Oryza sativa*
GBS0705	2H	BU977495	Unknown protein	
GBR0986	5H	AL502820	Homeobox leucine-zipper protein	*Oryza sativa*
GBR518	5H	AL512248	Histone H3	*Oryza sativa*
GBM1483	5H	BU998555	Homeodomain leucine zipper protein	*Oryza sativa*
GBR1082	5H	AL501796	Putative calcium binding protein	*Oryza sativa*
GBR1608	7H	CA005592	Heat-shock protein	*Secale cereale*
Ris44	7H	–		
GBR1637	7H	CK085363	Unknown protein	
GBR0788	3H	AL511235	Unknown protein	

Ch chromosome

inheritance is common in many species for complex characters.

Greenbug reduces the aerial growth of susceptible barley plants (Castro and Rumi 1987). In the current work, the tolerant and susceptible infested DH plants showed a differential aerial growth. The susceptible lines suffered an inhibition under infestation, in agreement with previous reports (Castro et al. 1988). In contrast, tolerant DH lines showed a significantly higher growth rate and chlorophyll contents, in agreement with that found by other authors (Burton et al. 1986; Castro et al. 1994; Van Emden 2007; Ricciardi et al. 2010). The assessment of these lines might thus allow the development of barley commercial cultivars with resistance genes against greenbug.

Linkage analysis indicated that tolerance to *S. graminum* is explained by multiple genes rather than a major gene in barley. In the current study, a total of five QTLs, located on chromosomes 1H, 2H, 3H, 5H and 7H were found to be associated with tolerance to greenbug.

It is important to note that most marker loci on chromosome 2H for FA and DW were common to both conditions (control and stressed plants). Therefore, these QTLs may be considered of constitutive expression for these traits. However, the QTLs located on chromosomes 5H for FA and 7H for Ch were detected only under greenbug challenge and should be considered as inducible defences.

Aerial biomass in control and infested plants, in terms of FA and DW, was significantly correlated with marker loci located in the same region of chromosome 2H. The *Vrs1* morphological marker was shared by every trait, except for the FA on the infested plants. This marker, which determines inflorescence row type (two or six rows) and is widely mentioned in the literature, has been associated with resistance to FHB (Fusarium head blight) with a low concentration of DON (Massman et al. 2011). Moreover, this locus is connected with grain and agronomic traits, such as yield, kernel plumpness, and test weight (Marquez-Cedillo et al. 2001). Since *Vrs1* has pleiotropic effect on grain size it may have an effect on the seedling performance, the QTL detected on chromosome 2H may be caused by a pleiotropic effect of *Vrs1*, nevertheless it appears as a QTL for FA and DW.

On the other hand, the chlorophyll content of control plants was associated with the marker locus *HVM60* located on chromosome 3H. This marker has been reported to increase the percentage of doubled haploid green plants. In cereal anther and microspore culture, regeneration of albino plants is a limitation to the efficient use of doubled haploids in breeding programs (Muñoz-Amatriaín et al. 2008). The same authors found that two-row cultivars have significantly higher values of green plant percentage than six-row ones. However, in our work, the six-row parental

OWB_{REC} was the donor of the alleles with positive effects for the chlorophyll contents. Moreover, in the infested plants, this trait was not linked with 3H markers instead, chlorophyll content was associated with chromosome 7H.

The present work, four candidate genes were associated with markers detected in one of the region of interest of chromosome 2H, near the *Vrs1* gene, between 70 and 96 cM, according to the barley SNPs consensus map (Kota et al. 2008). These genes have also been reported as candidates for tolerance against RWA (Tocho et al. 2012).

In the current study, most of the variation in the tolerance to greenbug was associated with chromosomes 2H, 5H and 7H. The QTLs (genes) identified in the present research are new resistance loci, which should be designated as Q*Gb.unlp*-2H, Q*Gb.unlp*-5H and, Q*Gb.unlp*-7H. These novel genes providing tolerance to greenbug could be transferred to barley cultivars already carrying other genes will result in gene pyramiding to enlarge the genetic base of defense against greenbug.

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