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Mapping and candidate gene identification of loci induced by phytohormones in barley (*Hordeum vulgare* L.)

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Abstract Phytohormones, a group of structurally unrelated small molecules are plant-signalling compounds that trigger induced resistance against certain pathogens and herbivores. The hormones jasmonic acid (JA), ABA, salicilic acid (SA) and ethylene (ET) are known to play major roles in regulating plant defence responses. In order to determine the changes in growth and in the chlorophyll content induced by the exogenous application of these elicitors, a set of DH lines of the Oregon-Wolfe Barley mapping population, previously screened to locate aphid resistant genes, was investigated. The aim of the current research was to map the induced defence genes and to reveal the relationship with aphid resistance. There were highly significant differences between controls and hormone treated plants in the aerial fresh and dry weights (AFW, ADW), the foliar area (FA) and the root dry weight (RDW). More than 15 JA and ET-induced lines exceeded the chlorophyll (Ch) values of their controls. Most of the plant traits were associated with the same genetic windows on chromosomes 3H, 5H and 7H in the controls and hormone treated plants. QTL(s) identified on chromosome 3H and 5H explained most of the variation of AFW, ADW, FA and RDW of controls and treated plants. QTL(s) located on chromosome 5H were associated with the variation of chlorophyll contents on JA-treated plants. The Ch in ET and ABA-treated plants was associated with two different regions on chromosome 7H. One of the latter genetic windows also explained the variation of RDW of ET- and ABA-treated plants. A sequence homology search was performed to derive the putative function of the genes linked to the QTLs. Several QTLs were identified located close to aphid resistance genes previously mapped. This is the first report of genes associated with hormone response in barley that could be involved with insect resistance. Those recombinant lines carrying the appropriate alleles could be useful for breeding barley to enlarge the genetic base of defence against stress.

Keywords Barley induced resistance · MAS · Candidate genes · Quantitative trait locus

Abbreviations

ABA	Abscisic acid
E	Ethylene

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SA	Salicylic acid
ADW	Aerial dry weight
FA	Foliar area
AFW	Aerial fresh weigh
JA	Jasmonic acid
Ch	Chlorophyll content
RDW	Root dry weight

Introduction

The plant hormones or phytohormones, a group of structurally unrelated small molecules, play important roles in diverse growth and developmental processes as well as various biotic and abiotic stress responses in plants (Santner et al. 2009). The jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA) and ethylene (ET) are known to play major roles in regulating plant defence responses against various pathogens, pests and abiotic stresses, wounding and exposure to ozone (Glazebrook 2005). These phytohormone signalling pathways are complex, often interacting antagonistically or synergistically with each other to allow the plant to fine-tune and activate attacker-specific responses (Bari and Jones 2009).

Whereas JA and ET are particularly well known as positive regulators of plant responses to herbivores and necrotrophic pathogens, SA has long been associated with resistance to biotrophic pathogens (Monaghan et al. 2009). Although SA and JA/ET defence pathways are mutually antagonistic, evidences of synergistic interactions have also been reported (Kunkel and Brooks 2002; Beckers and Spoel 2006). However, the role of SA in response to aphid feeding has been observed in many plant species such as wheat, barley and *Arabidopsis thaliana*. Kusnierczyk et al. (2008) revealed that a wide range of defense responses is dependent on SA signalling in *A. thaliana* attacked by *Brevicoryne brassicae*. Moreover, Chaman et al. (2003) reported SA accumulation occurring in two barley varieties in response to the damage caused by *Schizaphis graminum* with smaller aphid reproduction rate in one of these varieties, probably due to the greater presence of SA conjugated, or phenolics and other defensive compounds. Activation of the SA pathway may be a general mechanism of antibiosis or aphid repellence in resistant hosts, with a limited effectiveness in susceptible ones (Morkunas et al. 2011). Aphid-induced methyl salicylate (MeSA) is reported to be a strong aphid repellent that may deter aphids from settling on plants with already high aphid densities (Bernasconi et al. 1998; Preston et al. 1999). Many plant mitogen-activated protein kinases (MAPKs) play an important role in regulating responses to both abiotic and biotic stresses. BWMK1 was the first MAPK reported to be transcriptional activated by blast and wounding. Further experiments demonstrate than the expression of BWMK1 in rice was induced by cold, drought, dark and JA treatments, but it was suppressed by light and SA treatments (Hong et al. 2007). ABA is extensively involved in responses to abiotic stresses such as drought, low temperature, and osmotic stress, moreover ABA also governs a variety of growth and developmental processes. Exogenous applications of JA, SA and E are reported to induce plant defences. Phytohormones accumulation triggers both local and systemic plant responses, leading the production and accumulation of defence proteins and secondary metabolites in damaged and undamaged parts of the plant.

The plant defences can be constitutive or induced. The term "induced resistance" (IR) is used to describe plant defences that are elicitated by biotic or abiotic stresses. These plant defences are induced in the time they are needed, thus reducing maintenance costs compared to the constitutive defences, which are always enabled, whether or not necessary (Preston et al. 1999).

Among the inducible defence responses, the systemic acquired resistance (SAR) and the induced systemic resistance (ISR) can be identified. SAR is activated locally and systemically after infection by plant pathogens that cause necrosis. SAR activation provokes an endogenous increase of salicylate acid (Lawton et al. 1995). This hormone switches on regulatory genes with a consequence increase of regulatory proteins as NPR1/NIM and of transcriptional factors (TGAs) which control the expression of defence genes coding PR (pathogenesis related) proteins (Shah 2003). IR is also a systemic, broad-spectrum and durable resistance, dependant on ET and JA metabolic pathways (Grant and Lamb 2006).

Activation of IR against caterpillars and other chewing insects is dependent on induction of oxylipins such as JA and methyl jasmonate (Howe et al. 1996; McConn et al. 1997). These signalling compounds induce expression of plant defences such as proteinase

inhibitors (PI) and polyphenol oxidase, as well as volatile organic compounds that attract herbivore predators and parasitoids (van Poecke and Dicke 2004). Artificial induction of jasmonate-dependent defences can also deter phloem-feeding insects such as aphids (Cooper and Goggin 2005). JA treatment also reduced aphid reproduction in sorghum, although the transcript levels of JA-responsive genes were not altered following aphid infestation (Zhu-Salzman et al. 2004). The defense response in resistant varieties of wheat occurs within 1–2 h post infestation, and the observed hypersensitive response (HR) is later visible as necrotic lesions on the leaves of resistant plants. The HR is then followed by a SAR response that results in a prolonged resistance. In contrast, in susceptible varieties the recognition process does not occur, since no observable HR has been reported. This is compounded by delayed activation of the SAR (Van der Westhuizen et al. 1998a, b). Thus, the susceptible plant has no time to activate the appropriate machinery for cell maintenance. This leads to loss of energy production and cell death as a result of chlorophyll breakdown and a decrease in photosynthesis (Botha et al. 2006).

Barley and wheat tolerant cultivars to *S. graminum* maintained their growth after ET treatment, even at concentrations several times higher than exogenous ET levels induced by aphids (Castro et al. 1995). Aphid tolerant oat and barley genotypes showed no differences in growth or production when they were exposed to exogenous ET treatments (Castro et al. 1995). Chromosomes carrying genes for tolerance to *S. graminum* and *D. noxia*, in synthetic wheats, were associated with compensatory growth to exogenous hormone treatments (Castro et al. 2008).

Molecular marker-assisted selection (MAS) is an effective tool to accelerate production of cultivars with desirable traits (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). The DNA markers linked to the resistance QTLs are useful for MAS in barley breeding because phenotypic selection is limited due to time constraints and labour costs. Thus, indirect selection methods based on molecular markers would provide a strong advantage in practical efforts of breeding aphid resistance. Several QTLs triggered by hormones were significantly associated with growth traits located in the same regions than aphid resistance genes on the chromosome 6A in wheat (Castro et al. 2005), suggesting that these genes have the same or similar functions (Castro et al. 2008).

Two different QTLs providing tolerance to RWA (Russian Wheat Aphid) were mapped on chromosomes 1H and 2H in barley doubled haploid (DH) lines (Tocho et al. 2012) and on 1H and 3H in inbreed lines (Mittal et al. 2008). Also, greenbug resistance genes have been identified on chromosomes 2H, 5H and 7H (Tocho et al. 2013) in the barley DH mapping population mentioned before.

It is interesting to better understand the genetic bases of the plant growth responses under hormonal treatments and to reveal the relationship with aphid resistance. The purpose of this study was to determine whether exogenous application of hormones (JA, ABA, SA and ET) induces changes in growth or in the chlorophyll content that could be related with defence gene elicited, to map them and to identify candidate genes.

Materials and methods

Plant materials

A set of a doubled haploids (DH) mapping population derived from the cross between OWB_{DOM} and OWB_{REC} (Oregon Wolfe Barley, Wolfe and Franckowiak 1991) was used. The Oregon Wolfe Barley population (OWB) is a phenotypically polymorphic barley mapping population that was developed by Costa et al. (2001). Contrasting molecular markers between parental lines were developed and the DH lines were genotyped (transcript map) at the IPK Gatersleben, Germany (Stein et al. 2007). In order to perform a more precise phenotyping, only 79 DH lines and both parents were sown in a greenhouse and tested for hormone induction. Doubled haploid lines are ideal material for genetic analysis because they are completely homozygous and homogeneous.

Hormone solution

Hormonal solutions used, JA, SA, ABA and ET, were prepared in distilled water and Tween 20 (0.01 %, w/v). The doses sprayed were 10^{-5} M of JA, 50 mM of SA, 50 mM of Ethrel® and 10^{-5} M of ABA, which

were prepared following methods specified by the suppliers. Control plants were sprayed with distilled water and Tween 20 (0.01 %, w/v). The hormone doses were chosen according to preliminary tests (Castro et al. 2003).

Experimental procedures

Seeds of each DH and the parental lines were sown on vermiculite in vials (20 cc of volume) perforated at the base, with one seedling per vial, and placed in trays filled with 2 l of Hoagland's solution to enable a free supply of water and minerals. This volume was kept constant during all assays.

The trial was performed in a greenhouse (with 16:8 h day: night light regime, and 24–27 °C temperature regime), in La Plata, Argentina (34°55′S, 57°57′W) during 2010–2011. In both years plants of every genotype at the fully expanded 2nd leaf stage were subjected to exogenous sprayed of hormones using an atomizer to achieve a uniform application on the leaf surface. At least twenty plants of every genotype were used in the different treatments: JA, SA, ABA, ET and an untreated control sprayed with distilled water with 4–6 replicates on each treatment distributed in two blocks.

Seventy two hours after treatments, plants were harvested and tissues separated into aerial and root parts. The foliar area (FA in cm^2) was determined using a Licor Foliar area meter (Li3100). The aerial fresh weight (AFW in mg) was determined with a precision balance (Mettler Toledo). After washing the roots with water, each portion was oven dried at 70 °C until constant weight, and aerial (ADW in mg) and root dry weights (RDW in mg) were determined. Besides, the chlorophyll content (Ch), was measured by a non-destructive method using a hand-held chlorophyll meter (SPAD-52 Minolta, Camera, Osaka, Japan) on the middle of the second leaf. The SPAD meter readings correspond with the current 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). The mean value of every trait was the mean obtained from the experiments performed along the 2 years.The experiments were conducted as randomized complete blocks. The data were analysed by ANOVA using PROC GLM (SAS Institute 1998), and the Tukey Multiple Range Test was used to test the differences between means).

QTL mapping and EST annotation

For QTL detection, the single and multiple interval mapping (MIM) options provided by the MapMaker program (Lander et al. 1987) were used. 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 which resulted in a LOD threshold of 2.7 for AFW and Ch, 2.8 for ADW and RDW and 2.67 for FA 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 on the basis of the marker linkage map constructed by Kota et al. (2008). In total, 220 new SNP markers (most converted into CAPS) were mapped to the seven linkage groups spanning an overall genetic distance of 1,136 cM. As well as additional markers designated as GBR, GBM and GBS (for Gatersleben barley RFLP, microsatellite and SNP, respectively) (Stein et al. 2007).

A sequence homology search was performed to derive the putative function of the genes linked to the QTLs. For mapping population transcript maps consisting of 586 expressed sequences tag (EST)-based markers developed by Stein et al. (2007) and updated by Worch et al. (2011) are available. Annotation of the ESTs was performed by BLASTX (Basic Local Alignment Tool) similarity search against the public non-redundant protein database NRPEP (September 2012 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

Phenotypic differences

Statistical analyses revealed significant differences for each trait assessed on the DH lines between the

Table 1 ANOVAs for aerial fresh weight (AFW), aerial dry weight (ADW), foliar area (FA), root dry weight (RDW) and chlorophyll contents on the middle of the second leaf (Ch)

Source	df	Mean squares				
		AFW	ADW	FA	RDW	Ch
Genotypes	78	0.1484***	0.0015***	142.1216***	0.0002***	82.6747***
Treatments	4	0.0457***	0.0011**	46.1779***	0.0004***	96.4262**
G*T	312	0.0121***	0.0004ns	12.8931**	4×10^{-5}***	28.7896ns
Error	1047	0.0094	0.0003	10.1331	2×10^{-5}***	29.3166

*** $P \geq 0.001$, ** $0.001 \leq P \geq 0.01$, * $P \geq 0.05$, *ns* no significant

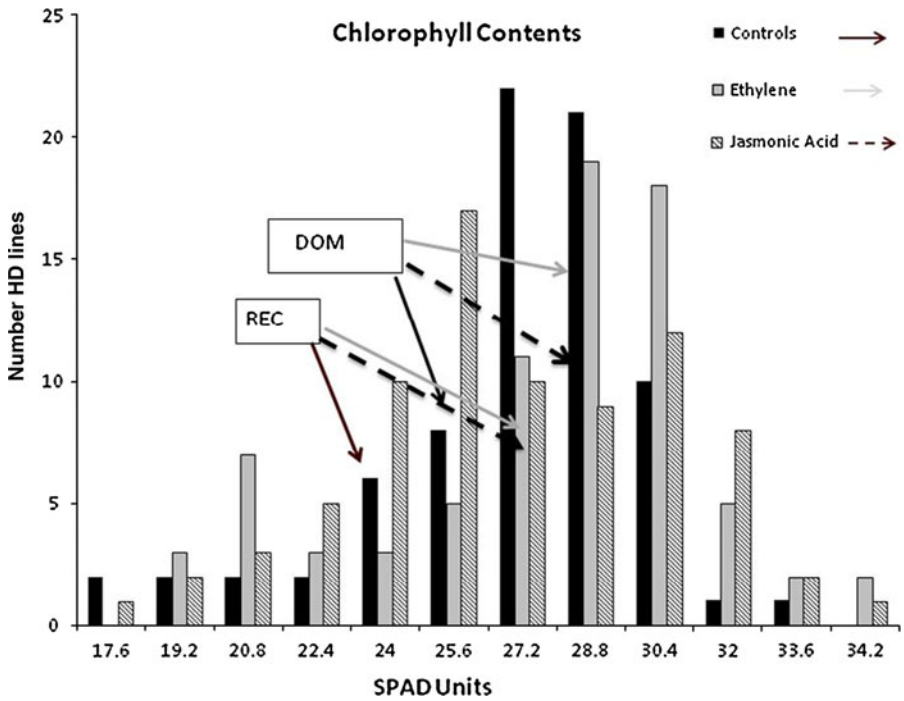

Fig. 1 Phenotypic distribution of chlorophyll contents on the middle part of the second leaves of controls, ethylene and jasmonic acid-treated plants of barley doubled haploid (DH) progeny of OWB_{DOM} and OWB_{REC}. Values of the parental lines OWB_{DOM} and OWB_{REC} are marked by *arrows*

genotypes, the treatments and in the interaction, except for ADW and Ch (Table 1).

The AFW, ADW, FA and RDW mean values of the parents and the DH lines showed no significant differences between controls, JA and ET induced plants. However, the OWR_{DOM} line had significantly higher Ch values on JA and ET induced plants, compared with their controls (Fig. 1). There were treated DH plants that showed higher chlorophyll contents than their control plants and exhibited a more intense dark green colour in the leaves. Besides, the DH population mean value for Ch showed significant differences in the ET treatment compared with the DH mean value on the controls (28.20 vs 24 SPAD units, respectively). The Ch was the most variable trait among the DH lines, more than 15 JA-induced lines exceeded the Ch values of their controls (Fig. 1). Simultaneously, the same DH lines increased their chlorophyll content after ET treatment. This would indicate that JA and ET treatments stimulate protective chlorophyll defence genes from oxidative stress. Consequently, those DH lines carrying the alleles with positive effects could have a greater photosynthetic capability compared with those ones carrying the opposite alleles.

There were no significant differences for most of the studied traits between the mean values of the control plants and the SA or ABA-treated plants of the parental and the DH lines (Table 2).The Ch mean value of the DH lines under SA treatment was the

Table 2 Mean values and standard errors of parental (OWB_{DOM}, OWB_{REC}) and DH lines, and range values for the aerial fresh weight (AFW), aerial dry weight (ADW), foliar area (FA), root dry weight (RDW) and chlorophyll content (Ch), in control (C) and treated plants with SA and ABA

Treatment	Trait				
	AFW	ADW $\times 10^{-2}$	FA	RDW $\times 10^{-2}$	Ch
C					
OBW_{DOM}	0.51 ± 0.05	4.30 ± 0.50	19.57 ± 1.80	1.70 ± 0.30	26.25 ± 0.29
OBW_{REC}	0.44 ± 0.04	3.30 ± 0.30	17.27 ± 1.15	0.89 ± 0.11	26.13 ± 0.92
Mean DH lines	0.57 ± 0.01	4.70 ± 0.30	21.7 ± 0.45	1.58 ± 0.07	24.55 ± 1.10
Range	0.22–1.04	2.00–8.50	8.92–41.10	0.57–3.17	18.36–32.43
SA					
OBW_{DOM}	0.55 ± 0.04	4.40 ± 0.60	20.42 ± 0.98	1.85 ± 0.49	27.60 ± 0.50
OBW_{REC}	0.49 ± 0.04	3.50 ± 0.40	19.92 ± 1.93	1.24 ± 0.08	25.82 ± 0.92
Mean DH lines	0.55 ± 0.01	4.30 ± 0.10	21.33 ± 0.39	1.59 ± 0.05	**27.06** ± **0.24**
Range	0.26–1.08	2.60–9.10	14.35–35.73	0.60–3.30	22.00–32.10
ABA	0.61 ± 0.07	5.20 ± 0.70	22.11 ± 1.93	1.30 ± 0.17	26.37 ± 0.57
OBW_{DOM}					
OBW_{REC}	0.52 ± 0.05	4.00 ± 0.30	20.03 ± 1.56	0.70 ± 0.05	26.01 ± 0.44
Mean DH lines	0.55 ± 0.01	4.60 ± 0.10	21.10 ± 0.35	1.35 ± 0.03	25.83 ± 0.35
Range	0.32–0.93	1.60–8.80	13.15–35.50	0.55–2.10	21.77–33.23

Bold underline value is the unique trait significantly different from that in the controls

unique trait significantly different from that in the controls (Table 2).

There were several DH lines that exceeded the parental lines values of every trait, in both the control and the ET, JA (Fig. 1), SA and ABA (Table 2) treated plants. These DH are significantly useful for breeding tolerance elicitated by phytohormones in barley.

Genetic analysis

QTL analysis allowed detecting significant associations between growth responses to the hormone treatments with molecular markers. QTLs accounting for a high proportion of trait variability were mainly located on chromosomes 3H, 5H and 7H (Table 3). Only ADW was associated with a region located on chromosome 2H. Every allele with positive effects was provided by OWB_{DOM}.

The aerial fresh weight values in the controls and hormone treated plants were associated with markers on chromosomes 3H and 5H (Table 3). The AFW was linked with the genetic window between 21.5 cM and 34.2 cM of chromosome 3H in the complete set of treatments (Fig 2a). This interval explained 85 % of AFW variability in control plants. Besides, AFW in controls, JA- and ET-treated plants was linked with the same region of chromosome 5H (between 3.3 and 16.5 cM) (Table 3). The AFW under the JA treatment was also associated with an interval placed between 27 cM and 35 cM on chromosome 5H (Fig. 2b), explaining 53 % of the trait variability (Table 3). The AFW variability was only partially explained under SA and ABA treatments (57 and 23 %, respectively) by the mentioned region of chromosome 3H (Table 3).

The ADW of control plants was not significantly linked to any chromosome. On the contrary, ADW was associated in every hormone treatment to the same interval (Fig. 2a) spanning 13 cM in chromosome 3H (Table 3). Besides, the dry weight in JA and ET treatments was associated with the same region of chromosome 5H (interval between 3.3 and 16.5 cM) (Fig 2b). Furthermore, the ADW when subjected to JA treatment was associated with the interval ranging from 119 and 125 cM of chromosome 2H (Table 3).

The foliar area was significantly associated with the mentioned genetic window located on chromosome 3H (Table 3), in the controls, ET- and SA-treated plants (Fig. 2a).

The chlorophyll content of controls was not associated with any chromosome. However, Ch in the JA

Table 3 QTL analysis with R^2 for aerial fresh weight (AFW), aerial dry weight (ADW), foliar area (FA), chlorophyll content (Ch) and root dry weight (RDW), found for the controls (C) and JA, ET, SA, and ABA hormonal treatments. CHR chromosome location; Position and interval in cM

Trait	Marker	CHR	Position	Interval	R^2 values				
					C	JA	ET	SA	ABA
AFW	*GBM1069*	3H	21.5	12.7 cM	0.85	0.17	0.55	0.57	0.23
	Bmac29	3H	24.9						
	OWB777dr	3H	27.5						
	GBR044	3H	34.2						
	GBS0408	5H	3.3						
	Bmag113c	5H	8.1	13.2 cM	0.15	0.19	0.45		
	GBM1001	5H	16.5						
	ABG391	5H	27.9						
	GBR304c	5H	34.2	6.3		0.53			
ADW	*Bmac125*	2H	119.1						
	Vrs1	2H	124.6	5.6 cM		0.29			
	GBM1069	3H	21.5	12.7 cM		0.17	0.22	0.21	0.19
	GBR044	3H	34.2						
	GBS0408	5H	3.3	13.2 cM		0.22	0.40		
	GBM1001	5H	16.5						
FA	*GBM1069*	3H	21.5	12.7 cM	0.36		0.21	0.22	
	GBR044	3H	34.2						
Ch	*GBR1474*	3H	203.3	2.6 cM				0.34	
	ABG460	3H	205.9						
	GBR0987	5H	0						
	GBR1640	5H	1.1						
	GBS0408	5H	3.3	8.1 cM		0.95			
	MWG602a	5H	7.5						
	Bmag113c	5H	8.1						
	GBM1359	7H	124.7						
	GBS0040	7H	127.3	9.9 cM			0.54		
	GBR1478	7H	134.6						
	GBS0591	7H	180.5						
	GBR074	7H	184.3	3.8 cM					0.30
RDW	*Bmac29*	3H	24.9						
	GBR044	3H	34.2	9.3	0.18			0.18	0.16
	GBM1001	5H	16.5						
	ABG391	5H	27.9	11.4 cM				0.33	
	GBM1359	7H	124.7						
	GBS0040	7H	127.3	11.1 cM			0.18		0.84
	GBR1478	7H	134.6						
	GBS0405	7H	135.8						

treatment was linked with the genetic window between 0 and 8.1 cM on chromosome 5H (Fig. 2b), explaining 95 % of the variability (Table 3). Chromosome 7H explained most of the variation of Ch when treated with ET and ABA (Table 3). However, two different regions of chromosome 7H were associated for each of the mentioned hormones (Fig. 2c). Under SA treatment, the Ch was linked with loci

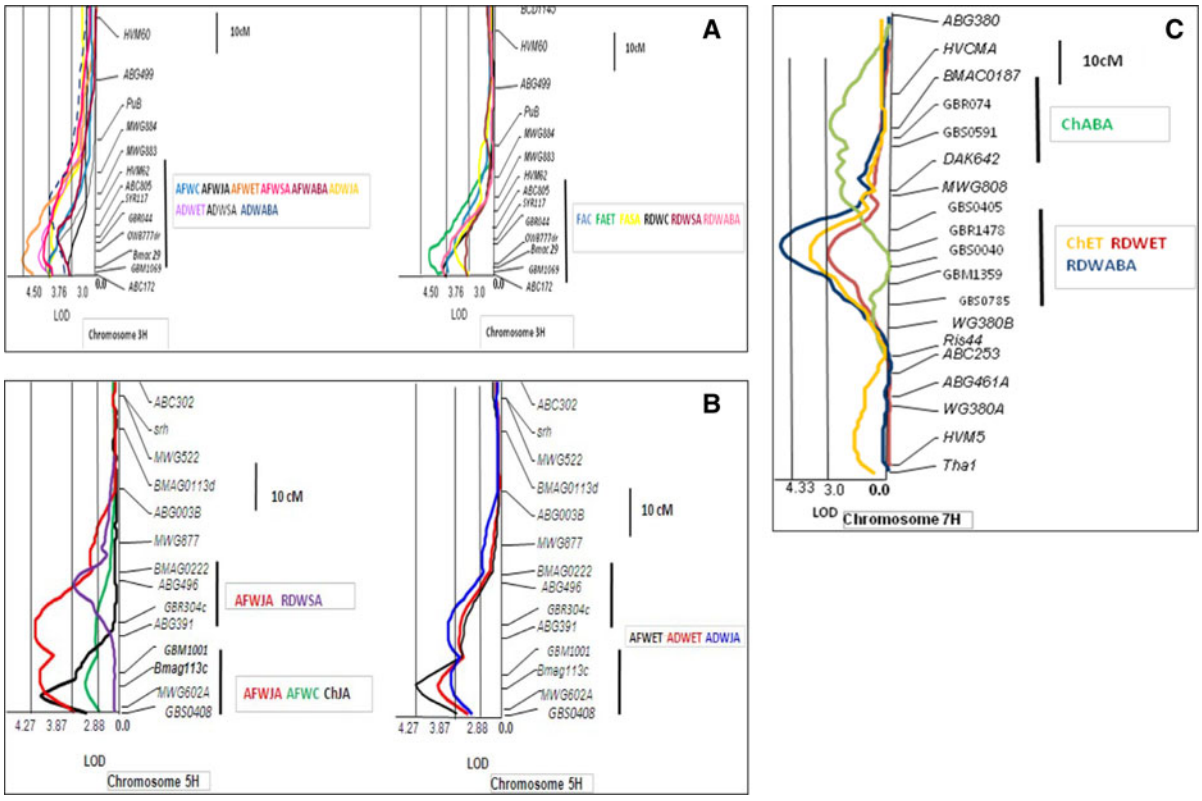

Fig. 2 QTL interval mapping results obtained for the OWB_{DOM} × OWB_{REC} population for AFDW, ADW, FA, Ch and RDW of control, JA, ET, SA and ABA-treated plants. **a** Chromosome 3H; **b** Chromosome 5H; **c** Chromosome 7H

located in a region of the chromosome 3H (interval ranging from 203 and 206 cM) (Table 3).

Root dry weight in the controls was associated with an interval located on chromosome 3H (between 24 and 34 cM) (Table 3), which also explained part of the variation of RDW under SA and ABA treatments (Fig. 2a). RDW of plants treated with JA, showed no association with any chromosome. The RDW in SA treatment was associated with the genetic window spanning 11 cM (between 16.5 and 27.9 cM) on chromosome 5H (Fig. 2b). In the ET and ABA treatments RDW was linked with the same interval (ranging from 124 and 136 cM) (Table 3) placed on chromosome 7H (Fig. 2c).

It is important to notice that one QTL was identified on chromosome 3H explaining AFW, ADW, FA and RDW variation in most of the treatments (Fig. 2a). Two QTLs mapped on chromosome 5H (Fig. 2b), explained the AFW of controls and the AFW and ADW of JA- and ET-treated plants, the Ch content on JA treatment and RDW of SA treated plants Another two QTLs were identified on chromosome 7H associated to Ch contents and RDW under ET and ABA treatments (Fig. 2c).

The QTLs on chromosomes 3H and 5H explained 100 % of the phenotypic variation in AFW of controls and ET-treated plants and 89 % of JA-treated plants (Table 3). Similarly, the 95 % of Ch content variation of plants under JA treatment was explained by QTL(s) on chromosome 5H (Table 3). Finally, the QTLs on chromosome 7H explained 84 % of the RDW variability of ABA-treated plants (Table 3).

Candidate gene identification

The identified candidates ESTs for hormone- induced responses have orthologs in rice, wheat and Arabidopsis with known functions in barley (Table 4). One of the regions of interest is on chromosome 3H close to *GBR044* marker loci. The candidate gene associated with the marker that map in that region on chromosome 3H (*EST AL509472*) is expressed as a

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

Marker	Chromosome	Hit_name	Functional annotation	Organism
GBR044	3H	*AL509472*	Bifunctional subtilisin/alpha-amylase inhibitor, RASI	*Oryza sativa*
GBS0408	5H	*AL510587*	Glutathione S-transferase	*Hordeum vulgare*
GBM1359	7H	*BQ460950*	Serine/threonine protein phosphatase PP2A-1 catalytic subunit	*Oryza sativa*
GBR1478	7H	*BQ464182*	Ethylene response element binding protein	*Triticum aestivum*
GBR074	7H	*AL510880*	Proteinase inhibitor Rgpi9	*Triticum aestivum*
GBS0040	7H	*AL502015*	Putative disease resistance protein	*Arabidopsis thaliana*
GBS0785	7H	*BQ468606*	Putative multiple stress-responsive zinc-finger protein	*Oryza sativa*

bifunctional subtilisin/alpha-amylase inhibitor in rice. This seed storage protein has evolved multiple functions and may be associated with a defence role (Yamasaki et al. 2006).

The candidate gene for *GBS0408* (*EST AL510587*), located on chromosome 5H, is a Glutathione S-transferase (GSTs). There are six functional markers located on chromosome 7H (Table 4). The candidate genes for markers *GBM1359* (*EST BQ460950)* is expressed as a serine/threonine protein phosphatase PP2A-1 catalytic subunit and for *GBR1478* (*EST BQ464182*) as an ET response element binding protein in *Triticum aestivum*. Another putative gene that is expressed in the same species is the candidate gene for *GBR074 loci (AL510880*) whose function is a proteinase inhibitor (Rgpi9). A putative gene for *GBS0040* marker (*EST AL502015)* is expressed as a disease resistance protein in Arabidopsis. Besides, there are functional markers located on chromosome 7H with orthologs in *Oryza sativa.* One of them is the candidate gene for *GBS0785* marker (EST: *BQ468606*) and is expressed as a putative multiple stress-responsive zinc-finger protein (Table 4).

Discussion

In the current study we identified QTLs distributed on chromosomes 3H, 5H and 7H with significant effects on the phenotypic variation of the responses to the hormone treatments in terms of AFW, ADW, FA, Ch and RDW. The responses to hormonal treatments are under multiple genes control in barley.

Stress signalling pathways are not independent. ABA, ET, SA and JA are signalling molecules for stress metabolism produced by plants under stress, and when exogenously applied, they induce a number of genes that respond to environmental or biotic stresses (Baldwin et al. 1994; Soriano et al. 2004; Yamaguchi-Shinozaki and Shinozaki 2005; Yamaguchi-Shinozaki and Shinozaki 2006). Nonetheless, the role of hormones in stress-responsive gene expression is not clear. Some genes can be induced by both biotic stress factors and exogenously applied hormones. Other genes are induced only by biotic factors or by hormones (Yamaguchi-Shinozaki and Shinozaki 2006). It is important to note that in our results some genes had a constitutive or an induced expression. The AFW was associated with the same genetic window in the controls and under hormonal treatment so this QTL should be considered as of constitutive expression for this trait. In contrast, the same interval should be considered as with inducible expression in relation to ADW, because it was associated only in the hormonal treated plants but not in controls. Moreover, it is important to notice that most of the variation of the AFW, ADW and Ch contents of JA treated plants were associated significantly with the same region of chromosome 5H (between 0 and 16 cM). In the mentioned region a major QTL (*RPHQ16),* providing partial resistance to *P. hordei*, has been mapped (Bouchon 2009). Several QTLs were previously mapped in the chromosomes 3H and 5H providing powdery mildew resistance (Aghnoum et al. 2010), one of these QTLs on chromosome 5H is close to those genes identified in the current research. Similarly, the genetic windows located on chromosomes 5H and 7H also explained part of the tolerance variability to greenbug (*S. graminum*) feeding (Tocho et al. 2013), in the same barley population. Moreover, the same interval associated with the ADW in JA-treated plants, located on chromosome 2H, also explained the RWA (*Diuraphis noxia*) tolerance (Tocho et al. 2012). The same genes elicitated by aphid feeding could have

been induced by JA treatment. Some QTLs regulated by final drought stress were mapping on linkage groups 3H, 5H and 7H for the OWB and Steptoe-Morex mapping populations (Worch et al. 2011). Several of these QTLs are close to those ones found in the present research.

It is interesting to notice that there were several DH lines with transgressive segregation in every trait. Although the favourable alleles for the different features were contributed by OWB_{DOM} parent, these transgressive segregants carry alleles with positive effects from both parents. Nonetheless, the contribution of OWB_{REC} parent was not addressed in the current analysis because the LOD values found in most of the traits were slightly lower than the LOD threshold.

The current results allowed the location of several QTLs and ESTs related with defence mechanisms induced by hormones, moreover there were chromosome intervals that also explained part of barley tolerance to aphids. These findings are valuable because it is the first report of genes associated with hormone response that also are involved with insect resistance in barley. Those recombinant lines carrying the appropriate alleles could be useful for breeding barley and to enlarge the genetic base of defense against stress.

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