

The constitutive expression and induction of three β -1,3-glucanases by bird cherry-oat aphid in relation to aphid resistance in 15 barley breeding lines

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Abstract In this study, 15 closely related barley genotypes were analyzed for the abundance of three β -1,3-glucanase transcripts immediately before and during infestation by the bird cherry-oat aphid (*Rhopalosiphum padi* L.). The barley lines are doubled haploid lines in backcross (BC) generations BC1 and BC2 from a cross between cultivar Lina and a wild barley accession. Previously, they have been characterized as susceptible (S) or resistant (R) to *R. padi* based on their ability to support nymphal growth. Here we also tested whether resistance was manifested as reduced aphid settling on the plants. Indeed, aphid numbers were lower on R than on S lines in all cases where there were significant differences between R and S lines. The choice of β -1,3-glucanase sequences is based on earlier results comparing two S and two R genotypes, suggesting that at least two of the three studied sequences are susceptibility factors. The comparisons of transcript abundance in plants with aphids showed for two of the β -1,3-glucanase sequences that there were several cases where an S genotype had significantly higher abundance than an R genotype, and in no case did an R line have significantly higher abundance than an S line. Thus,

there was some support for the idea that β -1,3-glucanase sequences are susceptibility factors in the interaction between barley and *R. padi*.

Keywords Aphid resistance · Aphid susceptibility · *Rhopalosiphum padi* · *Hordeum vulgare*

Introduction

Bird cherry-oat aphid (*Rhopalosiphum padi* L.) (Hemiptera: Aphididae) is a cosmopolitan cereal pest (Blackman and Eastop 2007). This aphid does not cause visible plant symptoms, but it can transmit viruses, such as barley yellow dwarf virus and under heavy infestation it reduces the yield by withdrawing nutrients (Riedell et al. 1999; 2003). One of the possible measures to reduce the damage made by *R. padi* is to grow resistant cultivars. However, to date there is no cultivar bred for resistance to this aphid. A plant's resistance level is dependent on several factors in the relationship between the insect and the host plant, classified as antixenosis (formerly nonpreference; Painter 1951), antibiosis and tolerance. Antixenosis is resistance that affects the insect's host selection behavior and makes the insect feed or reproduce less on the resistant compared to the susceptible plant. Antibiosis is resistance that affects the insect's physiology. It can reduce insect developmental rate, reproduction and life span and increase insect mortality. Tolerance is when the plant has the ability to withstand and recover from insect injury better than a susceptible plant (Smith 2005a, b).

Aphids feed passively on phloem sap. They penetrate the leaf with their stylet mostly intercellularly until they reach the sieve elements. During penetration in the cortical layers, aphids secrete gel saliva that forms a sheath. They

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also secrete watery saliva, both when probing into cells along the pathway and after penetrating the sieve elements with their stylet. The watery saliva contains calcium-binding proteins that suppress protein clogging and callose formation in the phloem, which would otherwise stop phloem transport (Giordanengo et al. 2010). Aphids induce various responses in the plants. Some of these are believed to be defense responses, whereas others may favor the aphid and thus rather be characterized as susceptibility responses.

In our study, we have focused on the putative role of β -1,3-glucanases in the interaction between *R. padi* and one of its host plants, barley (*Hordeum vulgare* L.). β -1,3-glucanases are one of 17 different families of pathogenesis-related (PR) proteins, which are induced upon microbial or herbivore attack (van Loon et al. 2006). The induction of β -glucanases by aphids has been studied at protein and transcript levels in several combinations of aphids and cereal species. Earlier studies at the protein level showed that β -1,3-glucanases were induced by aphids and suggested that they might be induced to a higher level in resistant genotypes. For example, it was shown in wheat that β -1,3-glucanase enzyme production and activity levels were induced by Russian wheat aphid (RWA; *Diuraphis noxia*, Kurdjumov) and that the induction was higher in three resistant cultivars carrying the *Dn-1* resistance gene than in the corresponding near-isogenic susceptible cultivars (van der Westhuizen et al. 1998). A study in barley showed four β -1,3-glucanases to be induced by *R. padi* in two barley lines, and the concentrations of two of them were higher in the line characterized as more resistant to this aphid (Forslund et al. 2000). More recent studies have been performed at transcript level. A β -1,3-glucanase transcript was induced in sorghum upon infestation by greenbug (*Schizaphis graminum* Rondani) (Zhu-Salzman et al. 2004; Park et al. 2006). Three reports on wheat indicate that β -glucanases might be susceptibility factors in the interactions with greenbug (Reddy et al. 2013) or RWA (Botha et al. 2010; Anderson et al. 2014). A possible mode of action of β -1,3-glucanases in susceptibility toward phloem feeders might be in the breakdown of induced callose in the sieve tube, as was shown in the interaction between the brown plant hopper and rice (Hao et al. 2008). In the case of β -1,3;1,4-glucanases, they may facilitate aphid probing and feeding by cell wall degradation as suggested by Anderson et al. (2014). We have results suggesting that β -1,3-glucanase sequences in barley might be susceptibility factors in the interaction with *R. padi*. A microarray study with four barley genotypes with varying susceptibility to *R. padi* showed that the induction of β -1,3-glucanases by the aphids varied between the genotypes and the pattern was different for different contigs (Delp et al. 2009). The contig1636_at was induced only in the two susceptible (S) lines, whereas contig1639_at was constitutively present only in S lines and

not induced by aphids (Delp et al. 2009). A third β -1,3-glucanase, contig1637_s_at, was induced by *R. padi* in both resistant (R) and S genotypes. The transcript levels were measured 48 h after infestation with *R. padi*. In a more recent study, we investigated the constitutive expression of putative resistance-related genes in 23 different barley genotypes with varying resistance to *R. padi* (Mehrabi et al. 2014). These genotypes are related to three of the four genotypes in the study by Delp et al. (2009). The study showed that the contig1636_at was present in all genotypes, as determined by genomic PCR, but constitutively expressed only in nine genotypes, seven of which are susceptible and two resistant to *R. padi* (Mehrabi et al. 2014). In the present study, we aim at obtaining further knowledge about especially the putative susceptibility-related sequences corresponding to contig1636_at and contig1639_at. The expression pattern before and after aphid infestation was therefore studied in a selection of 15 barley breeding lines with known aphid resistance characteristics based on the weight increase in aphid nymphs within 4 days.

The sequence corresponding to contig1637_s_at was included as a reference since it was induced by aphids both in R and S genotypes in the previous study by Delp et al. (2009). In this work, we also extend the characterization of the barley breeding lines with data concerning aphid host acceptance.

Materials and methods

Plant material

The barley (*H. vulgare* L.) genotypes used in this study were the BC1 lines 5172-28:4, 5172-39:9 and 5172-48:12 derived from an original cross between cv. Lina and a wild barley (*H. vulgare* ssp. *spontaneum*) accession Hsp5, and BC2 lines from further backcrosses to cv. Lina. The lines starting with number 51 are aphid-resistant parents of lines starting with number 66. All lines are doubled haploids. The crossing schemes and the characterization of the genotypes with regard to resistance or susceptibility to *R. padi* are shown in Supplementary Fig. 1.

Plant cultivation

Sterilized seeds on a moist filter paper were kept in darkness at 4 °C for 2 days followed by another 2 days in the light at room temperature. Then, the germinated seeds were transplanted to pots (8 cm diameter, 6 cm high) containing vermiculite and perlite 1:1. The plants were grown in growth chambers with 180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 16:8-h light/darkness at a temperature of 22 °C. The plants were watered with nutrient solution (Hamada and Jonsson 2013).

Aphid rearing

Rhopalosiphum padi had been collected from a field in the vicinity of Uppsala, central Sweden, and were reared on oat (*Avena sativa* L., cv. Kerstin) in a growth chamber at 22 °C with a photoperiod of 16:8-h light/darkness at 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Aphid host acceptance in a time course study

The middle part of the second leaf (first true leaf) of 14-day-old plants was confined in a plastic cylinder-shaped cage (4.5 cm length and 2.5 cm diameter) that was attached to a wooden stick for support. Using a fine painter's brush, 20 apterous adult aphids were carefully added to a plastic sponge used for closing the bottom of the cage. The top part of the cage was sealed with another sponge. Leaves of control plants were also equipped with cages but without aphids. The cages with aphids were applied starting 3.5 h after the beginning of the light period, and the number of settled aphids was counted at 6, 12, 24 and 48 h thereafter ($n = 4$ plants for each genotype). After adding aphids, plants with aphids were kept in another growth chamber than the control plants (without aphids). This was to avoid the risk of control plants being affected by aphid-induced volatile release from plants with aphids. The chamber growth conditions were the same for plants with and without aphids. The experiments were conducted separately with each family for logistic reasons.

mRNA extraction and cDNA synthesis

Immediately after each counting was finished, the aphids were brushed off from the leaves and the part of the leaves within the cage was cut out and frozen in liquid nitrogen and then stored at -80 °C. So were leaf pieces from control plants taken at each time point, as well as at time point 0 h. Each genotype had four replicates that were placed randomly in the block. Frozen leaf samples were grinded with pestle and mortar in liquid nitrogen. RNA was extracted using NucleoSpin[®] RNA Plant kit from Macherey–Nagel (Düren, Germany) according to the manufacturer's protocol and stored at -80 °C. Quality control of RNA, measuring of RNA concentration and cDNA synthesis were all carried out as in Mehrabi et al. (2014).

RT-qPCR

The procedures, conditions and calculations for RT-qPCR were the same as in Mehrabi et al. (2014). Because of the large sample size, only the leaves from three replicates per time point and treatment were included. The primers are shown in Supplementary Table 1.

Isolation and sequencing of primer products

For verification of the RT-qPCR products, DNA was isolated from the genotypes with the highest constitutive expression at time 0 h; from 6653-62 for contig1636_at and contig1639_at and from 5172-39:9 for contig1637_s_at. The primer sequences used in PCR are shown in Supplementary Table 1. The conditions and protocols were as described in Mehrabi et al. (2014) except for the PCR product purification that was carried out using Gene JET PCR Purification Kit provided by Thermo Scientific (Lithuania) according to the manufacturer's protocol. Sequencing was performed by Eurofins MWG Operon (Ebersberg, Germany). The sequences were blasted in NCBI database (<http://www.ncbi.nlm.nih.gov/>). The PCR product using primers for contig1636_at was 98 % identical to glucanase accession no X67099, and the product from primers for contig1639_at was 85 % identical to the same gene. Primers for contig1637_s_at resulted in a product that to 100 % matched the sequence with accession no AJ271367. This sequence is 99 % identical to M62907.1 and 98 % identical to M23548. The proteins corresponding to X67099 and M23548 have both been characterized as basic (1 \rightarrow 3)- β -glucan endohydrolases and named isoenzyme GIII (Wang et al. 1992) and GII (Hoj et al. 1989), respectively. Considering the similarity between contig1636_at and contig1639_at, we will present our data in the order contig1636_at, then contig1639_at followed by contig1637_s_at.

Statistical analysis

Analyses of differences in aphid settling or transcript abundance between genotypes and between time points in a family were carried out using two-way ANOVA (fixed factors "Genotype" and "Time points" and their interaction) and if the ANOVA showed significant differences ($p \leq 0.05$), Tukey HSD was performed as post hoc test, at $p \leq 0.05$. The statistical analyses were performed with StatPlus for Mac OS (version 2009) from AnalystSoft Inc. All data were checked for normal distribution before ANOVA analyses, and to satisfy the assumption of parametric data analysis, all data of transcript abundances were transformed (Table 2; Supplementary Table 2).

Results

Time course of aphid settling

The barley breeding lines used in this study have been characterized as moderately resistant (R) or susceptible

(S) based on a test where the weight of aphid nymphs was determined after 4 days of feeding (Mehrabi et al. 2014). Here we extended the characterization with a settling test. This study was important also in order to judge whether any differences in aphid-induced gene expression might be partly due to the number of aphids that had settled. Aphid settling was determined at 6, 12, 24 and 48 h after placing the aphids on the plants on three or four genotypes from each of three BC2 families and on two of the BC1 parent lines. The majority of the aphids had settled after 6 h (Fig. 1), and there is no reason to believe that there were major differences in transcript induction in the plants due to the number of settled aphids.

The comparison of numbers of settled aphids between genotypes including all the time points showed that there were significant differences between genotypes in all three families [6652: $df = (3,48)$, $F = 7.604$, $p < 0.001$; 6653: $df = (4,60)$, $F = 14.536$, $p < 0.001$; 6654: $df = (4,60)$, $F = 3.476$, $p = 0.012$]. Only in family 6654 was there a significant difference between time points [$df = (3,60)$ $F = 13.885$, $p < 0.001$]. There was no significant interaction between time and genotype in any of the families ($p > 0.05$). Post hoc Tukey HSD analysis (Table 1) revealed that in family 6652, the R line 6652-101 had lower aphid numbers than the S line 6652-79. Also, the R line 6652-179 had lower number of aphids than the S lines 6652-143 and 6652-79. In family 6653, the R line 6653-210 had lower aphid numbers compared to all other genotypes (i.e., the S lines 6653-150 and 6652-44 and R lines 5172-39:9 and 6653-62). In family 6654, the R line 5172-48:12 had lower aphid numbers compared to the S line 6654-123. Thus, in no case had an R line significantly higher aphid settling rate than an S line. In family 6654, the number of settled aphids was lower at 48 h compared to earlier time points (6, 12 and 24 h) (Table 1).

Does transcript abundance of β -1,3-glucanases relate to aphid susceptibility?

The transcript abundance of the three different β -1,3-glucanase sequences was examined in the barley genotypes before aphid infestation (0 h) and after aphid infestation at time points 6, 12, 24 and 48 h (Figs. 2, 3, 4). Immediately before aphid infestation, contig1636_at was expressed in five of the 15 genotypes from two of the three families (Fig. 2), which confirmed our earlier results that few of our genotypes express this transcript constitutively (Mehrabi et al. 2014). After aphid infestation, there was expression only in one additional genotype, the R line 6652-101, showing up first at 12 h, and with low levels of expression even at 48 h when transcript abundances of the other

Fig. 1 Aphid settling on two barley BC1 parents and four genotypes each in the BC2 family's 6652, 6653 and 6654 (average \pm SE, $n = 4$). Twenty apterous aphids were added in a small cage on the second leaf of 14-day-old plants. The number of settled aphids was counted after 6, 12, 24 and 48 h. Black bars represent R and white bars S genotypes

genotypes reached their highest (Fig. 2). The sequences corresponding to contig1639_at and contig1637_s_at were expressed in all 15 genotypes at all the time points (Fig. 3, 4).

We tested the hypothesis that transcript abundance of the investigated sequences would be higher in S genotypes than in R genotypes by comparing transcript abundances within families. The sequence corresponding to contig1636_at was excluded in this analysis, since this sequence was expressed in too few lines to make it meaningful. The first step in the analysis was done with two-way ANOVA investigating the factors time and genotype and their interaction (Supplementary Table 2). A second step was to apply Tukey HSD as post hoc test for the genotype differences, but only if there was no interaction between the factors time and genotype. The analyses were done for values both from plants without and with aphids as well as for the difference (estimating the induced abundances). The results where there were significant differences between lines are shown in Table 2.

Comparing the transcript abundances in plants, there were only one significant difference between genotypes for contig1639_at and six for contig1637_s_at. Among these differences, there were three concerning a pair of an R versus (vs) an S genotype: 6652-101 vs 6652-143; 6652-179 vs 6652-79 and 5172-39:9 vs 6653-150. There was no clear tendency, since in two of these comparisons, the abundance was higher in the R genotype than in the S genotype and in one it was lower (Table 2). Comparing the transcript abundances in plants with aphids, there were eight differences for contig1639_at and four for contig1637_s_at. Among them, there were eight concerning a pair of an R vs an S genotype. For contig1639_at, these were 6653-210 vs 6653-150; 6653-210 vs 6653-44; 5172-39:9 vs 6653-150; 6654-117 vs 6654-18; 6654-194 vs 6654-18 and 5172-48:12 vs 6654-18 and for contig1637_s_at: 6653-62 vs 6653-44 and 6653-210 vs 6653-44. In all these comparisons, the transcript abundance was higher in the S than in the R genotype. Comparing the levels of induced transcript (=the difference between plants with and without aphids), there was only one comparison that fulfilled our criteria and this concerned contig1637_s_at, which was higher in

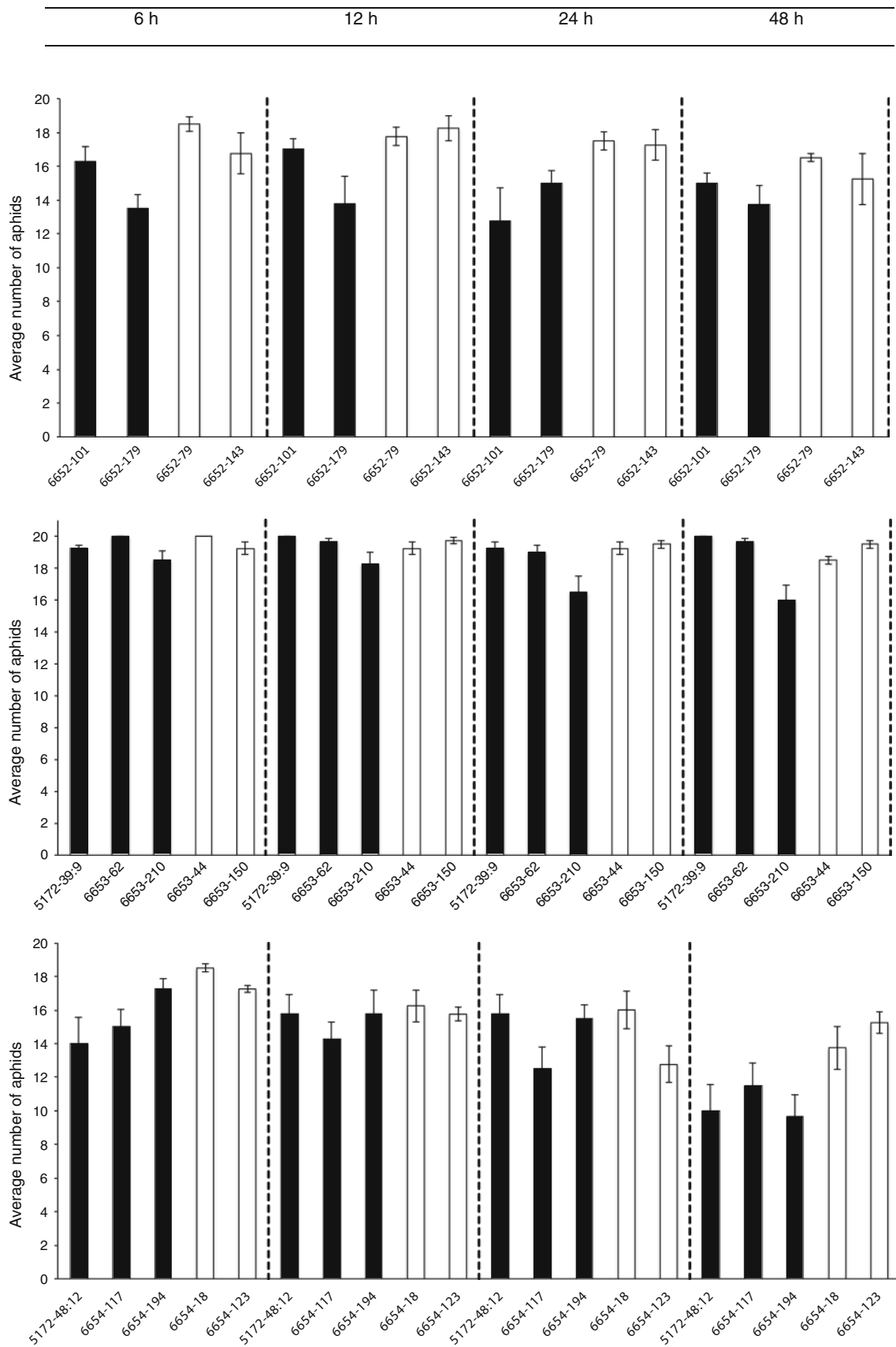


Table 1 Significant difference in aphid settling between genotypes within a family or between time points within family 6654

Groups	Difference	<i>p</i> value
Family 6652		
101 R versus 79 S	-2.312	0.035
179 R versus 143 S	-2.875	0.005
179 R versus 79 S	-3.562	0.000
Family 6653		
210 R versus 150 S	-2.187	0.000
210 R versus 5172-39:9 R	-2.312	0.000
210 R versus 44 S	-1.937	0.000
210 R versus 62 R	-2.312	0.000
Family 6654		
5172-48:12 R versus 123 S	-2.500	0.033
6 versus 48 h	4.550	0.000
12 versus 48 h	3.400	0.000
24 versus 48 h	3.550	0.000

Analysis by two-way ANOVA followed by Tukey HSD as post hoc test ($n = 4$). R resistant, S susceptible

the R line 6654-194 as compared to in the S line 6654-123 (Table 2). In summary, the analysis gave some support to the idea that β -1,3-glucanase sequences (contig1639_at and contig1637_s_at) were present at higher abundances in susceptible than in resistant genotypes, but only during infestation.

Time course of aphid induction of β -1,3-glucanase transcripts

To compare the temporal pattern of induction of β -1,3-glucanase transcripts in different genotypes, we calculated the relative induction for each time point: (the average with aphids minus the average without aphids)/the average at $t = 0$ and used the same criteria as in Delp et al. (2009) to determine whether the induction was considered significant or not (Table 3).

Contig1636_at was induced by aphids in four out of the five genotypes found to express the sequence constitutively. Two of the lines were of the R category and two of the S category. Contig1639_at was significantly upregulated after aphid infestation in 13 of the 15 genotypes. All three R parents, four out of the six R BC2 lines and all six S BC2 lines showed significant upregulation at one or more time points. In one of these genotypes, the S line 6653-44, there was significant downregulation at 6 h, but all later time points showed significant upregulation.

Contig1637_s_at showed a significant increase in transcript abundance in seven of the 15 genotypes after aphid infestation. Three of those genotypes were BC2 lines of the R category, and three were BC2 lines of the S category. One of the R parents did also show upregulation, whereas one S line, 6654-123, showed downregulation at 6 and 24 h. The only line that was not showing aphid regulation in any of the transcripts was the R BC2 line 6654-117,

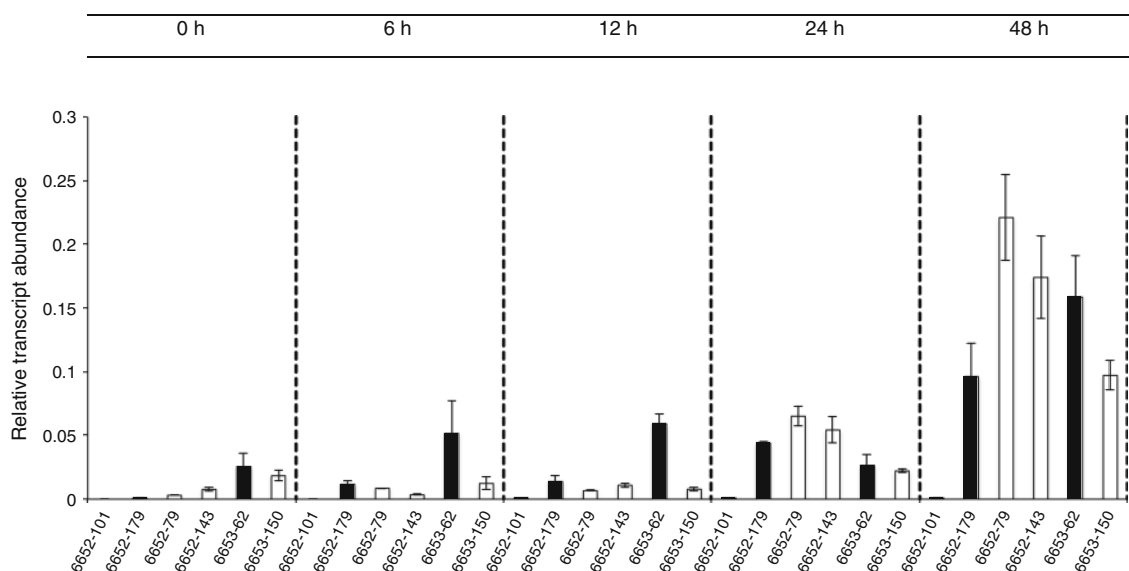


Fig. 2 The relative transcript abundance (average \pm SE) of the β -1,3-glucanase sequence corresponding to contig1636_at before and at different time points after aphid infestation. The sequence was found expressed only in six out of 15 investigated barley genotypes. Values

are from three biological replicates, analyzed with three technical replicates and normalized to the reference genes *HvHsp70* and *SF427*. Black bars R genotypes; white bars S genotypes

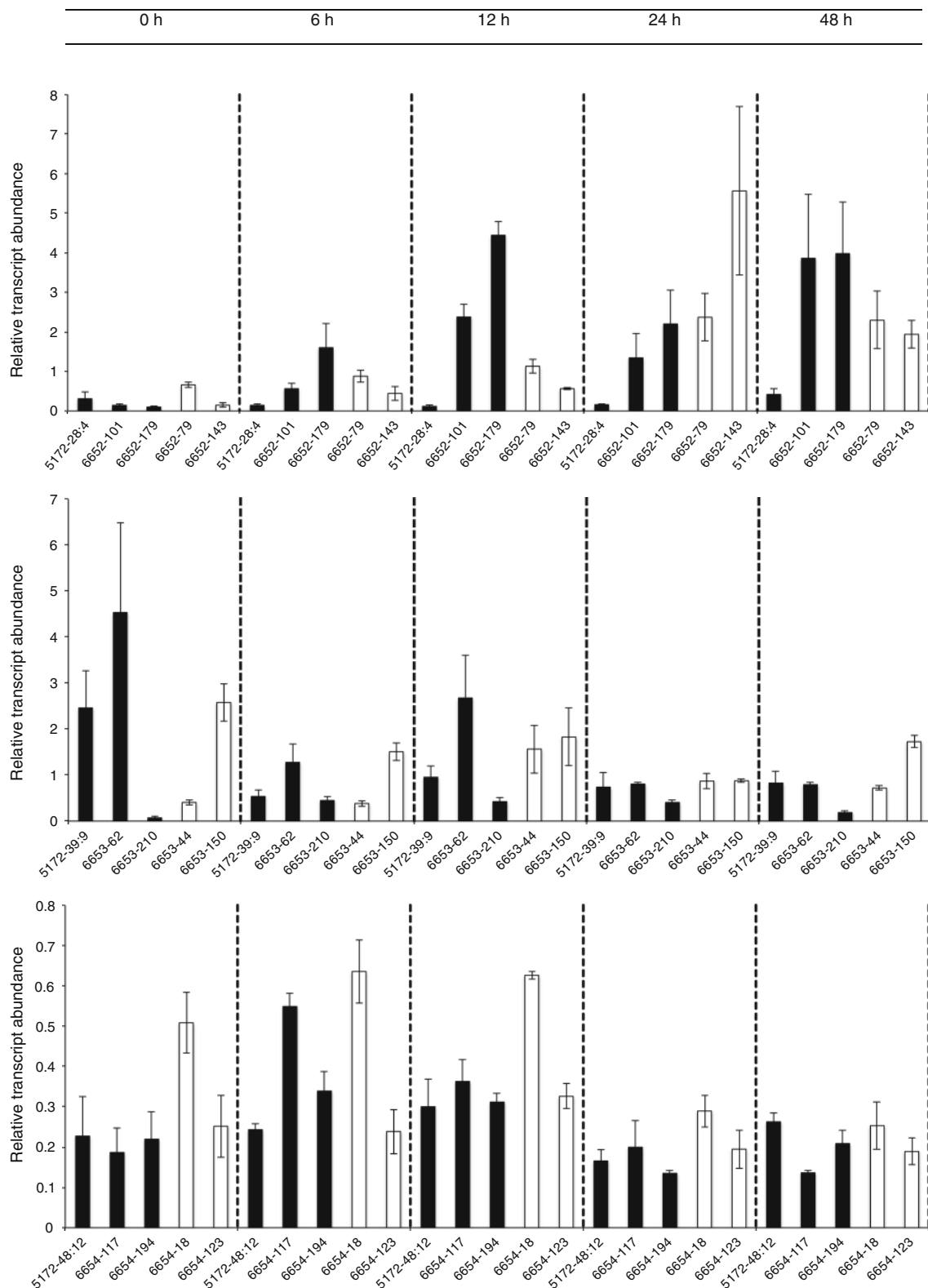


Fig. 3 The relative transcript abundance (average \pm SE) of the β -1,3-glucanase sequence corresponding to contig1639_at in 15 barley genotypes before and at different time points after aphid infestation.

Values are from three biological replicates, analyzed with three technical replicates and normalized to the reference genes *HvHsp70* and *SF427*. *Black bars* R genotypes; *white bars* S genotypes

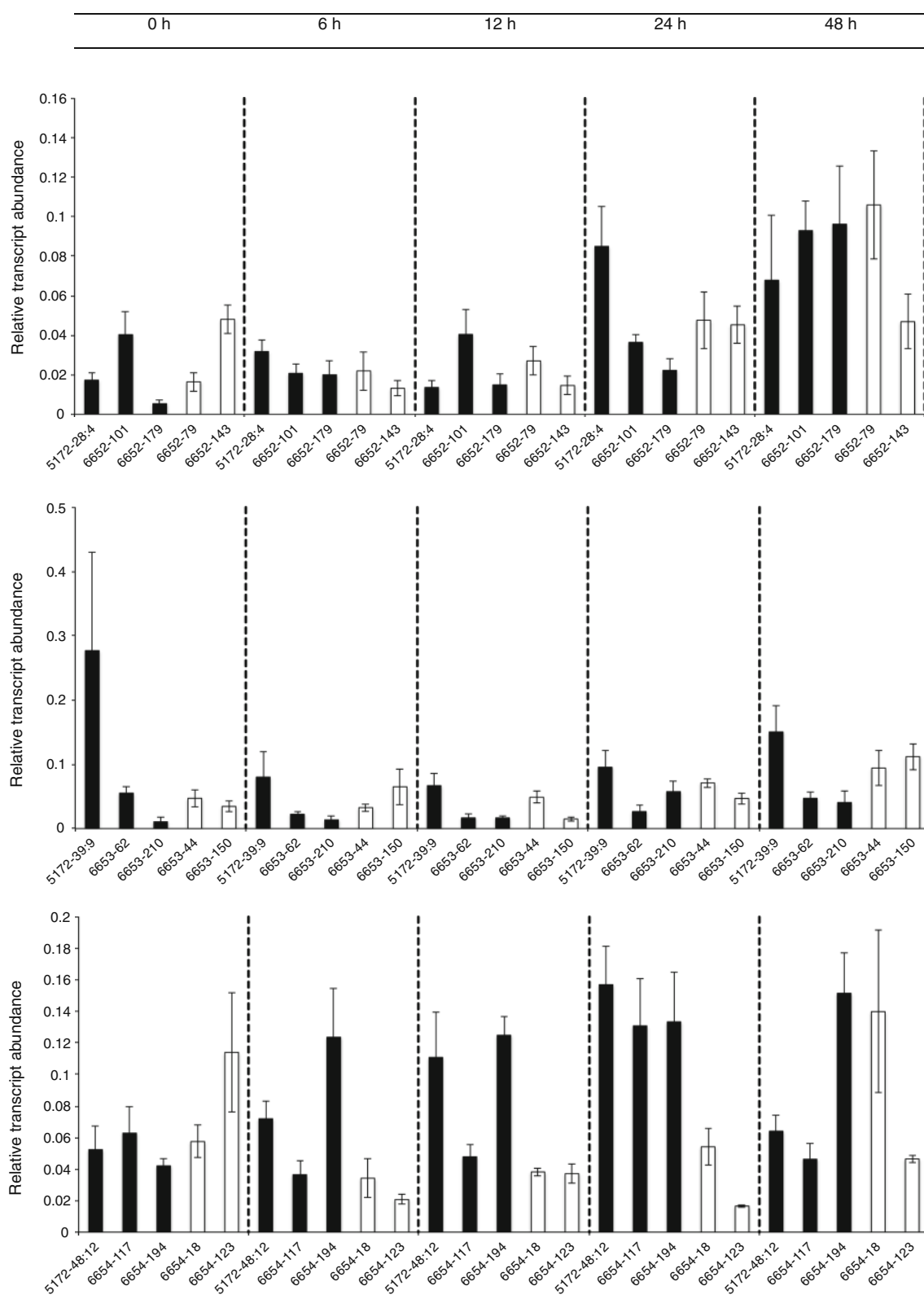


Fig. 4 The relative transcript abundance (average \pm SE) of the β -1,3-glucanase sequence corresponding to contig1637_s at in 15 barley genotypes before and at different time points after aphid

infestation. Values are from three biological replicates, analyzed with three technical replicates and normalized to the reference genes *HvHsp70* and *SF427*. Black bars R genotypes; white bars S genotypes

Table 2 Significant differences in transcript abundance between genotypes in each family without aphids, with aphids and the induced transcript abundance (with aphids minus without aphids)

	Groups	Difference	<i>p</i> -value
Without aphids			
<i>contig1639_at</i>			
Family 6654	123 S versus 18 S	-0.363	0.008
<i>contig1637_s_at</i>			
Family 6652	101 R versus 179 R	0.513	0.003
	101 R versus 143 S	0.384	0.044
	179 R versus 79 S	-0.477	0.007
Family 6653	62 R versus 210 R	0.579	0.041
	5172-39:9 R versus 210 R	0.925	0.000
	5172-39:9 R versus 150 S	0.571	0.045
With aphids			
<i>contig1639_at</i>			
Family 6653	210 R versus 62 R	-0.538	0.000
	210 R versus 150 S	-0.621	0.000
	210 R versus 44 S	-0.354	0.008
	5172-39:9 R versus 150 S	-0.358	0.007
Family 6654	123 S versus 18 S	-0.265	0.001
	117 R versus 18 S	-0.192	0.031
	194 R versus 18 S	-0.242	0.004
	5172-48:12 R versus 18 S	-0.240	0.004
<i>contig1637_s_at</i>			
Family 6653	62 R versus 5172-39:9 R	-0.542	0.002
	62 R versus 44 S	-0.393	0.043
	210 R versus 5172-39:9 R	-0.558	0.002
	210 R versus 44 S	-0.409	0.032
Difference (with aphids minus without aphids)			
<i>contig 1637_s_at</i>			
Family 6654	194 R versus 123 S	0.597	0.005

Analysis by two-way ANOVA (Supplementary Table 2) followed by Tukey HSD as post hoc test ($n = 3$), R resistant, S susceptible

whereas in some genotypes, all three β -1,3-glucanase sequences were induced. In summary, most genotypes showed upregulation in one or more β -1,3-glucanase transcripts during aphid attack although there was variation in timing and level between the genotypes, and there was no clear pattern related to the R and S categories.

Discussion

The present study was carried out to further investigate whether three different β -1,3-glucanase sequences in barley were related to aphid resistance or susceptibility, characteristics based on nymphal growth. Here we extended the characterizations to include also *R. padi* settling. In

all cases where there were significant differences between R and S lines, the R line had lower aphid numbers indicating behavioral effects of plant traits. However, both R and S lines are acceptable to the aphid and support aphid growth, although to a varying extent. In this sense, all lines can be considered susceptible. One of the three β -1,3-glucanase sequences, *contig1639_at*, was significantly induced by *R. padi* in the majority of the studied lines. This contig was more abundant in S than in R lines in all comparisons where there were significant differences between lines, suggesting that it is related to barley susceptibility to *R. padi*.

Based on previous investigations by Delp et al. (2009) using four different barley genotypes, we expected that the expressions of both *contig1636_at* and *contig1639_at* would relate to susceptibility. The sequence corresponding to *contig1637_s_at* was expected to be induced by aphids, but to exhibit no difference between resistant or susceptible lines. After the analysis of the induction of the three different β -1,3-glucanases upon aphid infestation in this much increased number of genotypes, the previous pattern was somewhat revised. To start with, the *contig1636_at* sequence had before been shown to be expressed constitutively only in nine out of 23 investigated barley genotypes (Mehrabi et al. 2014). In our present study, we show that aphid infestation caused expression in only one additional genotype. The induction pattern did not show any obvious difference between genotypes characterized as resistant or susceptible. The data for *contig1636_at* were not further analyzed due to the low number of genotypes expressing this sequence. Earlier studies of this gene have indicated that it is induced by salicylic acid (SA) and that there are sequences upstream of the promoter that might inhibit the expression (Li et al. 2005). Thus, low concentration of SA or the presence of inhibitory sequences may be the reason why it is not expressed in all genotypes.

The *contig1639_at* sequence was before in a selection of four genotypes found expressed constitutively only in two that are S (Delp et al. 2009). Here, the sequence was found expressed in all investigated genotypes and the transcript abundance increased upon aphid infestation in 13 out of 15 of these genotypes. The sequence corresponding to *contig1637_s_at* according to our criteria was induced only in 7 of 15 lines and repressed in one line (Table 3).

The analyses of aphid settling in the present study give additional suggestions about the resistance characteristics. The results showed that on four of the genotypes (6652-101, 6652-179, 6653-210 and 5172-48:12) there were lower numbers of settled aphids than on one or more of the other genotypes within the same family. All four genotypes belong to the category that has been characterized as R based on nymphal growth. This behavior might be caused by induced or already existing resistance factors.

Table 3 Relative transcript abundance of three β -1,3-glucanase sequences in 15 barley genotypes at different time points after aphid infestation

Genotype	6 h	12 h	24 h	48 h
contig1636_at ^a				
6652-101 R	b	b	b	b
6652-179 R	8.38	<i>13.93</i>	<i>53.02</i>	<i>115.09</i>
6652-79 S	-0.44	<i>0.45</i>	<i>19.45</i>	<i>68.82</i>
6652-143 S	0.13	<i>1.18</i>	<i>6.21</i>	<i>21.80</i>
6653-62 R	<i>1.17</i>	<i>1.98</i>	<i>0.74</i>	<i>5.88</i>
6653-150 S	0.08	0.17	0.85	4.51
contig1639_at				
5172-48:12 R	0.22	<i>0.28</i>	<i>0.44</i>	<i>1.09</i>
6652-101 R	<i>2.31</i>	<i>14.73</i>	<i>6.01</i>	<i>26.12</i>
6652-179 R	<i>9.55</i>	<i>39.01</i>	<i>15.45</i>	<i>36.43</i>
6652-79 S	<i>0.72</i>	<i>0.87</i>	<i>2.45</i>	<i>3.28</i>
6652-143 S	1.51	<i>2.95</i>	<i>33.89</i>	<i>12.59</i>
5172-39:9 R	-0.14	-0.14	0.18	<i>0.25</i>
6653-62 R	0.07	<i>0.42</i>	<i>0.05</i>	-0.02
6653-210 R	2.08	<i>2.25</i>	<i>5.16</i>	<i>2.34</i>
6653-44 S	-1.07	<i>3.06</i>	<i>1.77</i>	<i>1.49</i>
6653-150 S	0.20	0.16	<i>0.20</i>	<i>0.52</i>
5172-48:12 R	-1.32	0.52	<i>0.26</i>	<i>0.59</i>
6654-117 R	0.67	-0.07	0.58	0.30
6654-194 R	0.03	0.40	0.00	0.44
6654-18 S	0.11	<i>0.72</i>	<i>0.34</i>	0.14
6654-123 S	0.32	<i>0.42</i>	<i>0.57</i>	<i>0.52</i>
contig1637_s_at				
5172-48:12 R	0.35	-0.17	4.24	1.68
6652-101 R	-0.04	0.07	<i>0.74</i>	0.79
6652-179 R	<i>3.06</i>	0.96	<i>3.00</i>	<i>13.49</i>
6652-79 S	-1.38	-1.04	<i>2.13</i>	<i>4.84</i>
6652-143 S	<i>0.17</i>	<i>0.16</i>	<i>0.75</i>	<i>0.77</i>
5172-39:9 R	0.44	-0.57	-1.13	<i>1.52</i>
6653-62 R	-0.25	-0.53	-0.38	-0.18
6653-210 R	0.27	-1.39	-1.60	-1.60
6653-44 S	0.09	0.31	0.49	0.54
6653-150 S	0.76	-0.48	<i>0.78</i>	<i>2.10</i>
5172-48:12 R	0.15	0.98	0.85	0.26
6654-117 R	0.02	0.06	0.58	0.21
6654-194 R	<i>1.69</i>	<i>1.78</i>	<i>2.02</i>	1.62
6654-18 S	-0.30	0.00	0.10	1.04
6654-123 S	-0.60	-0.09	-0.12	0.00

The values indicate the average difference between aphid-infested and control plants ($n = 3$), normalized to the average at $t = 0$, set as 1.0. Numbers in italics fulfill the following criteria: There is increase or decrease in at least 8 of 9 pairwise comparisons between aphid-infested and control plants, and the average difference of log₂ values is at least +1 (italics) or -1 (bold)

^a This contig was expressed only in six lines

^b 6652-101 did not express contig1636_at at $t = 0$, and thus, it was not possible to calculate the relative induction

Alternatively, R lines have low amounts or lack one or more susceptibility factor. Both β -1,3-glucanase GIII (contig1636_at, contig1639_at) (Xu et al. 1992) and GII (contig1637_s_at) (Hoj et al. 1989) are extracellular proteins, and they may be secreted into the phloem. However, they were not found among proteins in phloem sap reported from barley (Gaupels et al. 2008). Increased levels of β -1,3-glucanases may be involved in the breakdown of induced callose (Hao et al. 2008). Our earlier studies have indicated that there was very little buildup of callose after infestation by bird cherry-oat aphid in a susceptible barley cultivar (Saheed et al. 2009). Callose was to a much stronger level induced by infestation by RWA, which however also caused much stronger induction of β -1,3-glucanases (among them 1637_s_at and 1636_s). These results were taken to suggest that the differences between RWA and bird cherry-oat aphid in causing callose were not likely to be caused by different levels of β -1,3-glucanases (Saheed et al. 2009). Still we cannot exclude the possibility that β -1,3-glucanases are instrumental in preventing callose accumulation as a response to bird cherry-oat aphid and thereby increasing susceptibility. This idea could be examined by studying callose accumulation in a selection of the genotypes used in this work. Another approach would be to inhibit the expression of the sequences related to contig1639_at and contig1637_s_at and study the consequences for aphid susceptibility.

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