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Evaluation of plant stage dependency of QTLs to homologous and heterologous rust pathogen isolates of barley

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A disease test at different leaf layers (plant stages) of homologous rust and heterologous rust species were studied. The result from homologous rust species showed that those quantitative trait loci (QTLs) (Rphq2 and Rphq11) which were effective at seedling stage were also effective across all plant stages with gradually decreasing effect as plants grew older. Rphq3 which had consistent effect in all leaf layers confirmed the same result, that it is a plant stage independent QTL. For heterologous rusts, the effect of Rnhq-V was studied on three rust species; P. hordei-murini (Phm), P. hordei-secalini (Phs) and P. triticina isolate 'Flamingo' at three stages (leaf layers). Infection frequencies are higher at seedling stage and dramatically decrease as plants grow older in all three rust species tested on both SusPtrit and Su-Rnhq-V. The difference between lines tends to be reduced with higher leaf layer in all three tested inappropriate rust species. However, this would be not because of less effectiveness of the Rnhq.

Key words: Plant stage, homologous rust, heterologous rust.

INTRODUCTION

Developmental conditions of the host plant may determine the outcome of pathogen infection; on the other hand, pathogen infection can change the developmental program of the host (Haffner et al., 2015; Grant and Jones, 2009). The influence of plant development on disease resistance is very crucial in understanding of plant-pathogen relationship. Resistance to infectious pathogens appears at different stages of host development, varies with plant age or tissue maturity, may be specific or broad-spectrum and is driven by diverse mechanisms, depending on plant pathogen interactions (Develey-Riviere and Galiana, 2007). These responses of plants to infectious pathogens include basal response through transcription of genes in response to pathogen-associated molecular pattern recognition, hypersensitive response at the site of infection, systemic acquired resistance making the entire plant resistant to infection, jasmonic acid response and non-host immunity (Boyajyan et al., 2014).

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Plants are generally more susceptible to disease in early stage than in late period and this could be due to the fact that there is an increase in resistance through time, with plants already resistance to a pathogen increasing their ability to overcome infection and colonization at a later growth stage (Develey-Riviere and Galiana, 2007). Many studies have been published on this phenomenon and reported for large number of crop plants.

So far different barley (*Hordeum vulgaris* L.) genotypes to *Puccinia hordei* were assessed in greenhouse tests at seedling growth stages and in the field at adult plant growth stages (Marcel et al., 2008; Yeo, 2008). For this barley leaf rust *P. hordei* isolate 1.2.1 was used to evaluate the level of partial resistance. Also, a number of quantitative trait loci (QTLs) were found to confer resistance to *Puccinia triticina (Pt), Puccinia hordei-murini (Phm),* and *Puccinia hordei-secalini(Phs)* when inoculated on three barley populations (L94xVada, Steptoe x Morex, and Oregon Wolfe Barley populations) (Bettgenhaeuser et al., 2014). From these, diversity of QTLs govern resistance in host system and also indicated that qualitative R genes are not involved in near nonhost resistance, however, genes conferring partial resistance to adapted pathogens may play role in nonhost resistance (Jafary et al., 2008; Niks, 2014).The effects of QTLs developed from those three barley populations, namely: *Rphq2*, *Rphq11* and *Rphq16* are plant stage dependent (Marcel et al., 2007), that they are effective only at seedling stage. *Rphq3* on the other hand has a strong and consistent effect at both seedling and adult plant stages. *Rnhq* is a QTL for nonhost resistance, and was effective to *Phm* and *Phs* at seedling stage (Jafary et al., 2006).

Near-isogenic lines (NILs) differing with regard to disease resistance QTLs provide valuable material for a more detailed study into the genetic and molecular dissection of the mechanisms underlies the emergence of disease resistance during host development. Such NILs allows the evaluation of QTL in a nearly uniform genetic background, overcoming the difficulties of identifying QTL phenotypes (Marcel et al., 2007). QTL-NILs do not only provide a better estimate for the effect of single QTL alleles, but also provide a better insight into QTL x pathogen and QTL x environment interactions.

Therefore, this study used the NILs developed for partial resistance QTLs *Rphq2, Rphq3, Rphq11,* and *Rphq16*, and a nonhost resistance QTL, *Rnhq* with the objective to evaluate whether these QTLs show plant growth stage dependency on resistance to homologous and heterologous rust pathogen isolates.

MATERIALS AND METHODS

Description of QTL parental lines

The QTLs that were used in this study were mapped in different barley mapping populations namely L94xVada recombinant inbred lines (RIL) mapping population (Neervoort and Parlevliet, 1978), Steptoe x Morex mapping population (Rasmusson and Wilcoxson,1979) and the Oregon Wolfe Barley (OWB) population (Costa et al., 2001).

Table 1. Quantitative trait loci near isogenic lines (QTL-NILs) used in this study.

QTL-NILs	Donor line
Rphq2-BC$_5$S$_1$	Vada
Rphq3-BC$_6$S$_1$	Vada
Rphq11-s.F$_2$.BC$_5$S$_1$	Steptoe
Rphq16-BC$_6$S$_1$	Dom
Qnh.L-F$_2$.BC$_5$S$_1$	L94
Qnh.V.F$_2$.BC$_5$S$_1$	Vada

Development of a research line and NILs

Earlier screens of barley accessions for susceptibility to *P. triticina* and *P. hordei-murini (Phm)* allowed identification of several accessions that showed some degree of susceptibility to these rust fungi. Crosses were made between these barley accessions which exhibited relatively high number of pustules and/or high infection types when infected with *P. triticina* and *Phm*. The F_2 lines for susceptibility to *P. triticina* and *Phm* at the seedling stage grown to adult plant stage and crossed between the two crossing combinations to obtain double cross (DC) plants. Each DC plant was grown to develop DC-S_1 lines by selfing. The most susceptible plants within the most susceptible DC-S_1 lines were selected and selfed for several cycles without selection. Later, susceptible DC-S_5 lines were challenged with *P. triticina* and *Phm*. The DC-S_5 line with the highest number of pustules per leaf and the highest infection type (IT) was selected and named SusPtrit (Sus = Susceptible, P = *Puccinia*, trit = *triticina*). SusPtrit was used as a recurrent parent in near isogenic lines (NILs) development program for the QTLs of our interest. (that is, PR QTLs - *Rphq2*, *Rphq3*, *Rphq11* and *Rphq16* and Nonhost resistance QTL- *Rnhq*).

As stated above, near isogenic lines (NILs) with SusPtrit genetic background (Table 1) and having resistance QTLs, *Rphq2*, *Rphq3*, *Rphq11, Rphq16* and *Rnhq* (*Rnhq-V* and *Rnhq-L*), were used for this study.

The parental lines for each respective NILs were used as a reference. For *Rphq2-BC$_5$S$_1$* and *Rphq3-BC$_6$S$_1$* besides SusPtrit and Vada were used as reference, L94, L94-NILs (L94-*Rphq2* and -*Rphq3*) and Vada-NILs (Vada-*rphq2*, and -*rphq3*) were included as well. The QTLs-NILs seeds were sown together with their respective reference lines. The sowing was done two times a week to ensure sufficient plants of each QTLs-NIL and reference lines at the required stage and was extended for eight weeks to have different plant stages. For each QTL-NIL and reference lines, 2 and 3 seeds, respectively were sown in a pot of 14 cm diameter. At the 8th week, the seeds were sown in boxes (39 cm × 37 cm). The plants were raised in the greenhouse compartments in three replications.

Inoculum

Eight different stages of plants for each partial resistance QTL-NIL were inoculated with barley leaf rust *P. hordei,* isolate 1.2.1. For Su-*Rnhq*, only three different growth stages (first, second and third leaf

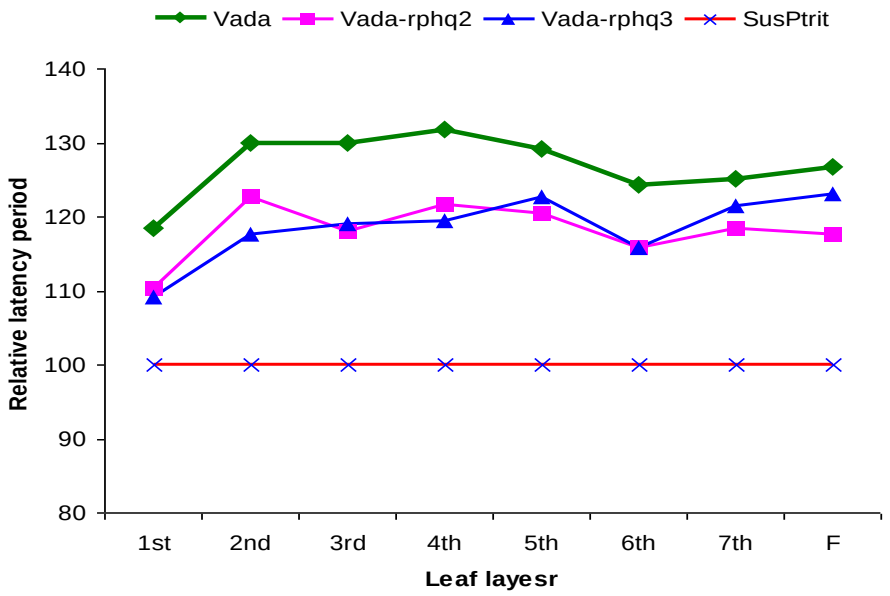

Figure 1. RLP50 of Vada and Vada-NILs relative to SusPtrit infected with *P. hordei*.

stages) were inoculated with *P. triticina (Pt)*, *P. hordei-murini(Phm)* and *P. hordei-secalini (Phs)* because the adult plants are resistant to these inappropriate rusts.

For inoculation of those plants at each stage, 1 mg (*P. hordei* 1.2.1) and 2 mg (heterologous rusts) of spores diluted 10 times with lycopodium spores were used as inoculum for each pot. Then, the inoculum was sprayed over the plants as uniformly as possible. The plants were then placed in a humidity chamber overnight (8 h) at 100% relative humidity in the dark at 18ºC to allow the spores to germinate. After incubation, the plants were transferred to a greenhouse compartment where the temperature was set at 14 ± 3°C with 30 to 70% relative humidity.

Data collection and analysis

Five to eight days after inoculation, when the infection flecks appear, observation zones containing proper density of flecks were delimited by marker. The observations started when the susceptible line showed the first mature pustules. The latency period (LP50) of three to five plants per QTL-NILs and two to three plants per parental line per stage in three replications and averages were considered to reflect the level of partial resistance for each QTL-NILs and donor lines. For all lines two leaves per pot per plant stages were scored.

For heterologous rusts, the frequency of visible infection sites (VIS; the number of both flecks and pustules per cm^2) and infection frequency (IF; the number of pustules per cm^2) following Jafary et al. (2006) were evaluated. Also, the latency period (LP) of the fungi on each plant was calculated. The data collected were analyzed using GenStat 12th edition statistical software.

RESULTS

Plant stage dependency of partial resistant QTLs

All lines tested show an increase in LP from the primary leaf up to the flag leaf. However, the LP of susceptible check, SusPtrit, was lower than the QTL-NILs and other parental lines which carry resistance genes.

LPs for SusPtrit were the shortest, averaging from 192 to 200 h from first leaf to forth leaf. Compared with SusPtrit, LP differences were slightly larger for QTL-NILs (201 to 240 h), Vada NILs (218 to 258 h) and L94-NILs (205 to 248 h) across all leaf layers (growth stages).

Rphq2

From first leaf to third leaf layers, *Rphq2* has longer RLP than *Rphq3* and up to second leaf layers compared to *Rphq11* on NILs with SusPtrit genetic background. Its effect starts to gradually decrease from second and fourth leaf layer onwards on L94-*Rphq2* and Sus-*Rphq2*, respectively. In general, the effect of *Rphq2* in Sus-*Rphq2* and L94-*Rphq2* is not significant above forth leaf layers (Figures 1 to 3). On the other hand, in Vada background NIL with *rphq3*, longer LP was observed after six leaf layer due to the presence of *Rphq2*. Its effect was lower than *Rphq3* on first, second and forth leaf layers.

Rphq3

The effect of this QTL is gradually increased after third leaf stage showing consistent effect in all developmental stages (Figures 1 to 3), except in Vada-*rphq2*. The effect of *Rphq3* was lower than *Rphq2* on L94-*Rphq2* from first to second leaf layers (Figure 2). It is also, lower than *Rphq2* and *Rphq11* from first leaf to third leaf layers on Su-*Rphq2* and Su-*Rphq11*, respectively (Figure 3). On the sixth leaf layer, its effect becomes equal to *Rphq2*

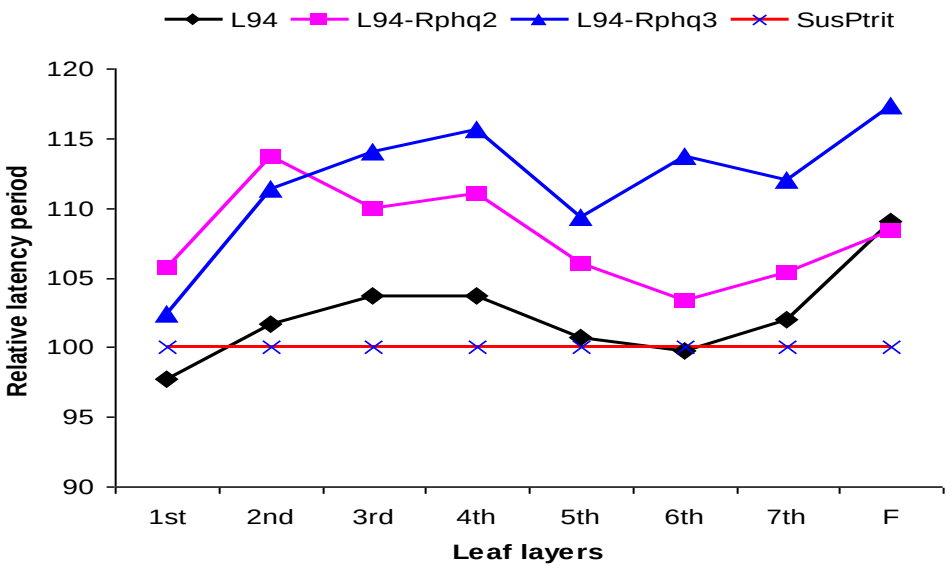

Figure 2. RLP50 of L94 and L94-NILs relative to SusPtrit infected with *P. hordei*.

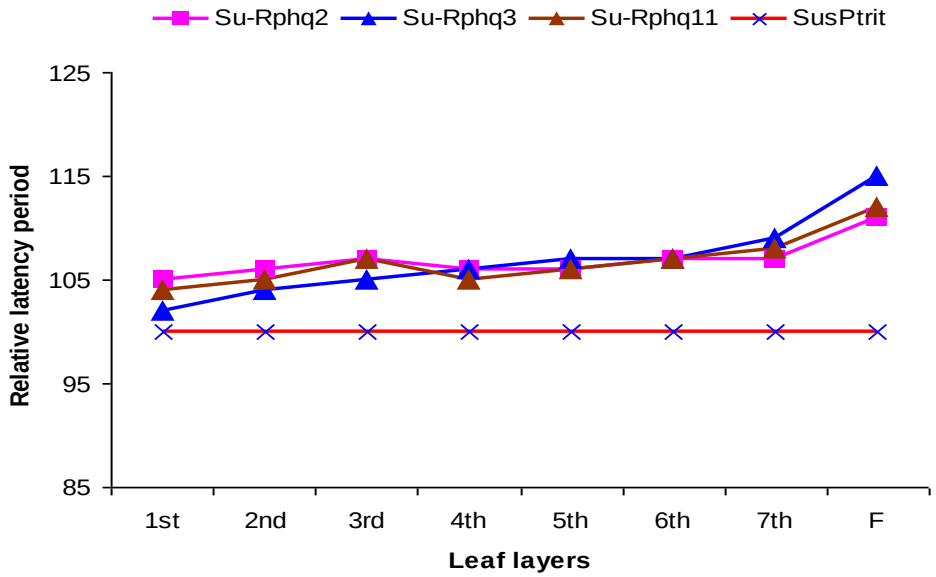

Figure 3. RLP50 of Su-*Rphq2,* Su-*Rphq3* and Su-*Rphq11* relative to SusPtrit infected with *P. hordei*.

and *Rphq11* on Su-*Rphq2,* and Su-*Rphq11,* respectively. However, its effect increases afterwards (Figure 3). In Vada-NILs with *rphq2*, the effect of *Rphq3* was higher than that with *rphq3* up to forth leaf layers though it has lower effect at third and sixth leaf layer afterwards.

Rphq11

The effect of *Rphq11* on Su-*Rphq11* was higher than Su-*Rphq3* from first leaf to third leaf layers (Figure 3). As plants grow higher, the RLP of Su-*Rphq11* increased as in other QTLs-NILs.

Plant stage dependency of nonhost resistance QTLs (*Rnhq-V*)

Effects of Su-*Rnhq-V* to the three inappropriate rust fungi were assessed by determining the relative latency period (RLP) and number of macroscopically visible infection sites. The level of infection established by inappropriate rusts rage from immune (no pustules and less than three flecks per cm^2) to susceptible. Infection frequencies are

Table 2. RIF of Su-*Rnhq*-V, SusPtrit, and L94 infected with *Pt, Phm* and *Phs*.

Lines	*P. Triticina* (Flamingo)			*P. hordei-murini*			*P. hordei-secalini*		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Su-Rnhq-V	66.3	38.0	33.0	37.5	22.0	18.0	39.7	33.7	25.0
Vada	2.1	-	-	-	-	-	6.0	2.0	-
L-94	73.1	47.0	39.5	63.0	57.0	53.0	77.3	69.0	63.0
SusPtrit	100.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 3. RLP50 of Su-*Rnhq*-V, SusPtrit, and L94 infected with *Pt, Phm* and *Phs*.

Lines	*P. Triticina* (Flamingo)			*P. hordei-murini*			*P. hordei-secalini*		
	1	2	3	1	2	3	1	2	3
Su-Rnhq-V	101	105	103	102	104	103	104	103	103
Vada	-	-	-	-	-	-	117	122	-
L-94	103	105	108	103	105	109	101	103	107
SusPtrit	100	100	100	100	100	100	100	100	100

higher at seedling stage and dramatically decrease as plants grow higher in all three rust species tested (Table 2). The uredia size of infected leaves was small and had chlorosis on Su-*Rnhq-V* NILs at seedling stage as compared to that of SusPtrit.

As shown in Table 2, for infection with *P. triticina,* effect of *Rnhq-V* showed significant effect on Su-Rnhq-V only at second leaf stage. In case of *Phm* at first leaf stage, the effect is not significant; however there was positive effect at second leaf stage. It had positive effect on RLP at first and second leaf stage for *Phs*. However, the effect seems to decrease at second and third leaf stages. In general, the effect of *Rnhq-V* on Su-*Rnhq-V* on RLP tends to decrease with increment in leaf layer in all three tested inappropriate rust species. Parental line 'Vada' showed no sporulating uredia on both *P. triticina* and *Phm* except very few on *Phs*, as a result RLP was not scored for this line on those two rust species (Table 3).

DISCUSSION

Adequate standardization of plant age, inoculum density and quality, and environmental conditions are required to recognize true differences in susceptibility. In this research, the environmental conditions during plant growth prior to inoculation, during exposure of inoculated plants to dew, and during post-dew development were sufficiently defined and controlled to provide an acceptable level of variation in disease development attributable solely to environmental factors.

Near-isogenic lines (NILs) differing with regard to disease resistance QTLs provide valuable material for a more detailed study into the genetic and molecular dissection of the mechanisms underlying the emergence of disease resistance during host development. Such NILs allows the evaluation of QTL in a nearly uniform genetic background, overcoming the difficulties of identifying QTL phenotypes (Marcel et al., 2007). QTL-NILs do not only provide a better estimate for the effect of single QTL alleles, but also provide a better insight into QTL x pathogen and QTL x environment interactions.

In this study, three most effective QTL-NILs with SusPtrit background, *Rphq2, Rphq3,* and *Rphq11* contributed in resistance to homologous rust were used to evaluate the effect of each QTL at different plant development stages.

As shown in Figure 3, *Rphq2* on Su-*Rphq2* had higher effect from first to third leaf layers as compared to *Rphq3* on Su-*Rphq3*. Its effect starts to relatively decrease after fourth leaf layers in SusPtrit background NILs. *Rphq2* is effective at seedling stage and gradually lose its effect as plant grows higher. The effect of *Rphq3* on Su-*Rphq3* consistently increases from first leaf layer to adult plant stage. This consistency in effect indicates that this QTL is stage independent. On the other hand, *Rphq11* on Su-*Rphq11* had no as such statistically significant difference from *Rphq2* and *Rphq3 o*n Su-*Rphq2* and *Su-Rphq3,* respectively. It tended to have an effect intermediate between *Rphq2* and *Rphq3* (Figure 3).

In previous studies (Niks et al., 2000; Marcel et al., 2008) it was reported that *Rphq2* had a strong effect in the seedling stage but almost no effect in adult plant stage, while *Rphq3* was effective in seedling and adult plant stages indicating that *Rphq3* is plant stage independent. Also it was reported (Yeo, 2008) that, *Rphq11* was effective in seedling stage. However, in present study, it was observed that those QTLs which were effective at seedling stage were also effective across all plant stages with gradually decreasing effects

as plants grew older. Previously, these QTLs were reported to be plant stage dependent because they were mapped at seedlings stage but not at adult stage. Here the QTL-NILs used for this study were only those in which QTLs of interest is in the plant material. So, that the QTLs which were reported to be plant stage dependent may not be as reported due to the fact that QTLs do function throughout the plant stage but its effect is smaller than other QTLs detected in a mapping population. However, the effect of *Rphq3* is consistent in all leaf layers observed, indicating that this QTL is plant stage independent as reported in previous studies. As far as *Rnhq* is concerned, Su-*Rnhq-V* had positive effect in resistance at first leaf layer; however, its effect seems to decrease as plants grew older.

Generally, as reviewed in detail in Develey-Rivière and Galiana (2007), resistance acquisition during development has been reported for a large number of crops from both monocots and dicots (wheat, rice, maize, soybean, common bean, tomato, grapevine, tobacco). This resistance to diseases associated with major transitions happening during plant life cycle (Kus et al., 2002; Poethig, 2003; Rusterucci et al., 2005; Baurle and Dean, 2006), function of the maturity of tissue or organ (Zeier, 2005), acquired resistance with development (Panter et al., 2002; Rusterucci et al., 2005), the functional regulation of plant resistance (R) genes (Panter et al., 2002; McDowell et al., 2005), induction of defense mechanisms (Cameron and Zaton, 2004; Dong, 2004; Hugot et al., 2004; Xu et al., 2006). In this study, we observed the same phenomenon of resistance at different developmental stage of nearly isogenic barley lines.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Baurle I, Dean C (2006). The timing of developmental transitions in plants. Cell 125:655-664.

Bettgenhaeuser J, Gilbert B, Ayliffe M, Moscou MJ (2014). Nonhost resistance to rust pathogens–a continuation of continua. Front. Plant Sci. 5(664):10-3389

Boyajyan A, Devejyan H, Haykazyan V, Avetisyan G, Khanoyan D (2014). Molecular mechanisms and mediators of the immune response in plants. J. Plant Sci. 2(1):23-30.

Cameron RK, Zaton K (2004). Intercellular salicylic acid accumulation is important for age-related resistance in Arabidopsis to *Pseudomonas syringae*. Physiol. Mol. Plant Pathol. 65:197-209.

Costa JM, Corey A, Hayes PM, Jobet C, Kleinhofs A, Kopisch-Obusch A, Kramer SF, Kudrna D, Li M, Riera-Lizarazu O, Sato K, Szucs P, Toojinda T, Vales MI, Wolfe RI (2001). Molecular mapping of the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. Theor. Appl. Genet. 103:415-424

Develey-Rivière M, Galiana E (2007). Resistance to pathogens and host developmental stage: a multifaceted relationship within the plant kingdom. New Phytol. 175:405-416.

Dong X (2004). NPR1, all things considered. Curr. Opin. Plant Biol. 7:547-552.

Grant MR, Jones JDG (2009). Hormone (dis)harmony moulds plant health and disease. Science 324:750-752.

Haffner E, Konietzki S, Diederichsen E (2015). Keeping Control: The Role of Senescence and Development in Plant Pathogenesis and Defense. Plants 4:449-488.

Hugot K, Riviere MP, Moreilhon C, Dayem MA, Cozzitorto J, Arbiol G, Barbry P, Weiss C, Galiana E (2004). Coordinated regulation of genes for secretion in tobacco at late developmental stages: association with resistance against oomycetes. Plant Physiol. 134:858-870.

Jafary H, Albertazzi G, Marcel TC, Niks RE (2008). High diversity of genes for nonhost resistance of barley to heterologous rust fungi. *Genetics* 178, 2327–2339. doi:10.1534/genetics.107.077552

Jafary H, Szabo LJ, Niks RE (2006). Innate nonhost immunity in barley to different heterologous rust fungi is controlled by sets of resistance genes with different and overlapping specificities. Mol. Plant Microbe Interact. 19(11):1270-1279.

Kus JV, Zaton K, Sarkar R, Cameron RK (2002). Age-related resistance in Arabidopsis is a developmentally regulated defense response to *Pseudomonas syringae*. Plant Cell 14:479-490.

Marcel TC, Varshney RK, Barbieri M, Jafary H, de Kock MJD, Graner A, Niks RE (2007). A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theor Appl. Genet 114:487-500.

Marcel TC, Gorguet B, Ta MT, Vels A, Niks RE (2008). The verification of QTLs for partial resistance to *Puccinia hordei* in NILs of barley confirms an isolate-specific effect. New Phytol. 177:743-755

McDowell JM, Williams SG, Funderburg NT, Eulgem T, Dangl JL (2005). Genetic analysis of developmentally regulated resistance to downy mildew (*Hyaloperonospora parasitica*) in *Arabidopsis thaliana*. Mol. Plant Microbe Interact. 18:1226-1234.

Neervoort WJ, Parlevliet JE (1978). Partial resistance of barley to leaf rust, *Puccinia hordei*. V. Analysis of the components of partial resistance in eight barley cultivars. Euphytica 27:33-39.

Niks RE (2014). How specific is non-hypersensitive host and nonhost resistance of barley to rust and mildew fungi? J. Integr. Agric. 13:244-254.

Niks RE, Fernández E, Van Haperen B, Bekele AB, Martínez F (2000). Specificity of QTLs for partial and non-host resistance of barley to leaf rust fungi. Acta Phytopathol. Entomol. Hung. 35(1/4):13-21.

Panter SN, Hammond-Kosack KE, Harrison K, Jones JD, Jones DA (2002). Developmental control of promoter activity is not responsible for mature onset of Cf-9B-mediated resistance to leaf mold in tomato. Mol. Plant Microbe Interact. 15:1099-1107.

Poethig RS (2003). Phase change and the regulation of developmental timing in plants. Science 301:334-336.

Rasmusson DC, Wilcoxson RD (1979). Registration of 'Morex' barley. Crop Sci. 19:293

Rusterucci C, Zhao Z, Haines K, Mellersh D, Neumann M, Cameron RK (2005). Age-related resistance to *Pseudomonas syringae* pv. *tomato* is associated with the transition to flowering in Arabidopsis and is effective against *Peronospora parasitica*. Physiol. Mol. Plant Pathol. 66:222-231.

Xu WH, Wang YS, Liu GZ, Chen X, Tinjuangjun P, Pi LY, Song WY (2006). The autophosphorylated Ser686, Thr688, and Ser689 residues in the intracellular juxtamembrane domain of XA21 are implicated in stability control of rice receptor-like kinase. Plant J. 45:740-751.

Yeo KSF (2008). Genetic Dissection of QTLs for partial resistance of barley to *Puccinia hordei.* MSc, Thesis, Wageningen University, Plant Breeding Department, The Netherlands.

Zeier J (2005). Age-dependent variations of local and systemic defence responses in Arabidopsis leaves towards an avirulent strain of *Pseudomonas syringae.* Physiol. Mol. Plant Pathol. 66(1):30-39.