Investigation of the relation between foliage and tuber late blight resistance under laboratory and field conditions

Master thesis

Menno van der Zweep

December 2014

Supervisors:

prof. dr. Edith Lammerts van Bueren,
Wageningen University, PBR

dr. Ronald Hutten,
Wageningen University, PBR

WAGENINGEN UR
For quality of life

Table of contents

Abstract

Phytophthora infestans is a devastating disease that infects both tubers and foliage of potato. The purpose of this study was to investigate the relation between tuber and foliage late blight infection and the relation between late blight analysis in the laboratory and in the field. Desiree transformants with one or more resistance genes originated from wild Solanum species were used in the different tests. This set of genetic modified Desiree plants with single *R* genes in an identical genetic background provided accurate characterization of the performance of the *R* genes. High variation in resistance was observed between the different transformants. *VNT1* showed high tuber and foliage resistance in the laboratory tests and under field conditions. *CHC1* showed susceptibility in the leaf test in the laboratory, while foliage resistance was found in the field and tuber resistance in the laboratory. *BLB3* showed isolate-specific resistance for both the tubers and foliage. *BLB1, STO1* and *PTA1* showed foliage specific resistance, with intermediate resistance in the foliage and almost complete susceptibility in the tubers. *EDN2* showed a good level of resistance in the field, but was susceptible in the tuber and foliage in the laboratory tests. *MCQ1, R3a* and *R3b* were susceptible in both foliage and tubers. Influence of stacking *R* genes was observed for the combination of *BLB3+STO1*, which performed better compared to *BLB3* or *STO1.* Influence of the genetic background for the transgenic potatoes was observed by comparing several cultivar x construct combinations containing the same *R* gene.

Next to the transformants also a set of cultivars and breeding lines were screened for late blight resistance in the foliage and tubers. Although most of the cultivars were susceptible in both foliage and tubers, some cultivars were found with tuber specific resistance. Breeding lines with *BLB2* showed foliage resistance in the field, but tuber susceptibility and partial foliage resistance in the laboratory. *R8* and *EDN2* showed a good level of foliage resistance in the field, but showed susceptibility in the tubers and isolate-specific foliage resistance in the laboratory.

In general the tuber and foliage late blight analysis went well. However, only marginal variation in tuber infection was found between the genotypes in the field. No correlation was found between the results of the tuber tests in the laboratory and the field analysis. However, significant correlation was found between the foliage test in the laboratory and the foliage analysis in the field.

Although good infection levels were observed during the experiments, it is suggested to repeat the experiments, because variation was observed in the different tests within the results of genotypes.

1 Introduction

1.1 Potato

Potato *(Solanum tuberosum)* belongs to the *Solanaceae* family and shares the genus Solanum with 1,000 other species like tomato, pepper, tobacco and eggplant. Potato has 12 chromosomes and different ploidy levels, ranging from diploid (2x=24) to hexaploid (6x=72). The cultivated potato cultivars in Europe are *Solanum tuberosum* L., tertraploid with 48 chromosomes. Wild potato species are often diploid.

The potato originates, with some exceptions, from South America. About 8000 years ago the potato was already domesticated in the southern part of Peru. In this region still a wide variety of wild relatives of the domesticated potato can be found (Spooner et al., 2005). In the 16th century the potato was introduced in Europe and from here it was spread to the rest of the world (Hawkes & Francisco-Ortega, 1993). At the beginning potato was grown as an ornamental and it took about one century before potato was accepted as a food crop.

Nowadays potato is the third most important food crop in the world after wheat and rice. Potato is an adaptable plant widely grown over the world in temperate, subtropical an tropical regions and will grow without ideal growing conditions. Potatoes are grown by planting seed potatoes. Usually about two tonnes of seed tubers are used for one hectare of land. Under good conditions one hectare can yield between 40 and 50 tonnes of fresh potatoes, especially under temperate conditions like in Northern Europe and North America. (http://www.fao.org/potato-2008/en/potato/cultivation.html). In 2012 China was leader in the total production of potatoes (85,9 million tonnes) followed by India (45 million tonnes) and Russia (30 million tonnes) (FAOSTAT 2012). Potatoes are a good source of dietary energy by a high concentration of carbohydrates. About 80% of a potato is water and 20% dry matter and about 80 percent of the dry matter consists of starch. Next to that, potatoes are low in fat, and a good source of some micronutrients and dietary antioxidants, like Vitamin C, which benefits health (Kolosa, 1993).

1.2 Late blight

Late blight, caused by *Phytophthora infestans (P. infestans)*, is the most important disease in the cultivation of potato and can destroy leaves, stems and tubers. *P infestans* has a hemibitrophic lifestyle, with both a biotrophic phase and a nectrophic phase (Perfect and Green, 2001).

Infection of the leaves by *P. infestans* can cause wilting of the crop, leading indirectly to a reduced harvest of potatoes. Infection of the tubers cause directly a reduced harvest by browning of the potatoes.

P. infestans is not a fungus, but belongs to the oomycetes and is taxonomically closely related to golden-brown algae (Judelson and Blanco, 2005). Oomycetes produce asexual sporangia on the plant surface or sexual oospores within the host tissues (Hardham, 2007). The sporangia are transported over large distances by wind. The sporangia are able to germinate directly by forming branching hyphae with feeding structures known as haustoria (Kamoun et al., 1999). The hyphae will penetrate the host surface and expand the infection to the neighboring cells when a plant is susceptible. A resistant plant will show a hypersensitive reaction which is discussed later on. Sporangia can also germinate indirectly by forming motile zoospores. Motility is achieved by the action of the two flagella and is important to bring root and foliar pathogens to infection sites such as stomata. In favorable conditions the oomycete can spread rapidly by the development of multiple generations of

conidia in a short time (Frinking and Van der Stoel, 1987).The potato disease cycle is shown in Figure 1.

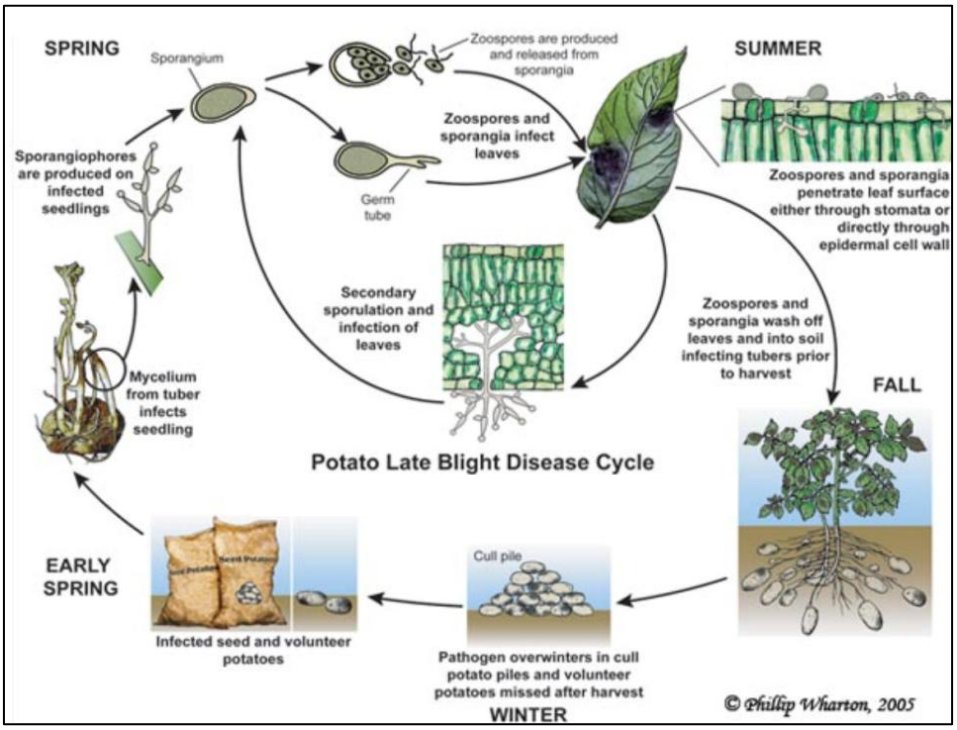


Figure 1. The potato late blight disease cycle (www.potatodiseases.org).

1.3 Immune system

Plants defend themselves against pathogens in different ways. General or basal defence is active to a broad range of pathogens. General defence is divided in a constitutive part and an induced part. The constitutive part is caused by a natural barrier to the pathogen such as a thick wax layer on the leaves and anti-microbial compounds in the leaves. Defence is induced when PAMPs (pathogen-associated molecular patterns), e.g. the chitin cell wall compounds of fungi, are recognized by plants receptors. Such perception will cause PAMP-triggered immunity. Successful pathogens could suppress the PAMP-triggered immunity by effector proteins which interfere with the defence reaction. However, the effector proteins (Avr factors) from the pathogen could be recognized by the plant receptors (*R* genes) to activate a pathogen- specific defence.

Resistance to late blight in the current potato cultivars is mostly based on these single *R* genes and is also called vertical resistance, complete resistance or qualitative resistance. These *R* genes provide a high level of resistance but a drawback is the durability of the resistance with respect to pathogens with a high adaptability as is with *P. infestans*. The *R* gene mediated resistance is based on the genetic interaction between the pathogen avirulence genes (*avr* genes) and the corresponding *R* genes in the plant, also called gene for gene interaction. When the *R* gene and the *avr* gene match, a hypersensitivity response (HR) will follow. This will cause immediately programmed cell death of the plant tissue close to the pathogen (Poland et al., 2009).
The recognition of the pathogen by the plant probably takes place at the plant cell membrane. If the *avr* gene (effector) of the pathogen is changed by a mutation there will be no avirulance gene product and recognition by the plant is not possible and no hypersensitivity response occurs. The plant becomes susceptible. Due to the high adaptability of *P. infestans* and the single gene resistance

in potato, breaking down of resistance against *P. infestans* is a major problem. Breaking down of resistance occurs often when on a large scale cultivars are cultivated with the same resistance gene. Selection pressure of the pathogen will cause that the frequency of rare pathogen genotypes with a dysfunctional *avr* gene will increase in the population (Niks et al., 2011).
Late blight resistance has been identified in different wild Solanum species originated from South America. Currently, a large number of diploid and polyploid Solanum species exist from the southern part of Argentina to the southern United States (Jacobs et al., 2008). Eleven late blight resistance genes (*R1-R1*1) have been distinguished in *Solanum demissum,* a common species in central Mexico (Black and Mastenbroek, 1953). Later on, late blight resistance have been discovered in other wild *Solanum* species summarized by Vleeshouwers et al. (2011): *Solanum hjertingii* (*Rpi-hjt1*); *Solanum schenckii* (*Rpi-snk1*); *Solanum edinense* (*Rpi-edn1*); *Solanum bulbocastanum* (*Rpi-BLB1*), (*Rpi-BLB2*); *Solanum stoloniferum* (*Rpi-STO1*); *Solanum papita* (*Rpi-PTA1*); *Solanum venturii* (*Rpi-VNT1*); *Solanum chacoense* (*Rpi-CHC1*). Next to late blight *R* genes, several QTLs (quantitative trait loci) conferring resistance to late blight have been reported in domesticated and wild potatoes.

1.4 Foliage and tuber late blight

P. infestans infects foliage as well as tubers of potato. Foliage infection by late blight causes an indirect yield loss by limited tuber production. Infection of tubers directly reduces yield or even complete loss of yield. Not only the infection of *P. infestans* is a problem, but also the colonisation of the infection patches by soft rotting bacteria, leading to a slimy breakdown of stored tubers.
The tubers become infected when the disease is growing down to the lower stem, through the stolons to the tubers. Tubers can also become infected when sporangia and spores are washed from the leaves into cracks of the soil. The potato hills provide an ideal humid environment for infection. Especially tubers which grow shallow in the hill, or tubers which grow in cracked and eroded hills have the greatest risk of becoming infected (http://www.plantpath.wisc.edu/). Potatoes could also become directly infected by infection of oospores. Oospores can survive for three to four years in the soil.
Foliage resistance, which prevents the spread and reproduction of the disease, is an important way to reduce the risk of tuber infection. Besides that, tuber blight resistance could be used to prevent tubers to become infected.
Different components protects the tubers against pathogens such as late blight (Pathak and Clarke, 1987). Several layers of phellem, known as periderm, forms the first defence barrier. Tubers can only become infected through natural openings of the periderm such as stomata, or wounds and cracks. The outer cortex cell layers form the second defence barrier. These layers reduce the hyphal growth. The storage tissue of the tuber, the medulla, forms the third defence barrier.

Genetics of tuber blight is not extensively studied, although it is an important trait. In general plants with a high level of foliage resistance show good tuber resistance (Collins, 1999). However, this is not always the case and the relationship between tuber and foliage resistance remains unclear. Several genes conferring late blight resistance have been detected and incorporated in *Solanum tuberosum* cultivars. It seems there is not always a positive correlation between foliage and tuber resistance (Roer and Toxopeus,1961; Park et al., 2005; Collins et al., 1999; Oberhagemann et al., 1999; Simko, 2006; Mayton et al., 2010). Park et al. (2005) found that *Rpi-abpt* and *R3a* are foliage specific *R* genes

and do not confer resistance in tubers. Another foliage specific *R* gene is *Rpi-BLB1* (Halterman et al., 2008).
Besides dominant *R* genes, also quantitative trait loci (QTL) involved in late blight resistance are identified. A QTL on chromosome 5, associated with foliar resistance and tuber susceptibility, was found by Oberhagemann et al. This QTL is located close to the QTL for foliage maturity type on chromosome 5 and both traits are genetically linked to each other (Collins et al., 1999; Oberhagemann et al., 1999; Visker et al., 2003)
Negative correlation between foliar and tuber resistance was also found in another study (Mayton et al., 2010). Three markers were found to be associated with a QTL for foliar blight resistance, but marginally associated with tuber blight resistance. This negative correlation was confirmed by Simko et al. (2006). They found that most QTLs involved in foliage resistance are different from QTLs involved in tuber resistance.
Some studies found a positive correlation (Stewart, 1994, Platt and Tai, 1998), indicating that foliar and tuber resistance are determined by the same genes or by different closely linked genes. Positive correlation was found for *R1* or *R1* like genes that act both in foliage and tubers (Park et al, 2005; Pel, 2010). It was concluded that the expression of resistance for tubers or foliage late blight depends on the *R* genes. Besides *R1*, also *R3b*, *Rpi-phu1* and *Rpi-ber* confer resistance to both tubers and foliage (Park et al., 2005; Sliwka et al., 2006; Pel, 2010; Mayton et al., 2011).

1.5 Importance

The current way to prevent tuber late blight is by using fungicides to restrict the pathogen to reproduce and spread spores to the tubers in the ground. However, in the organic potato production the use of fungicides is forbidden and the problem of tuber late blight is even bigger compared to the conventional potato production. Looking at the importance of tuber blight in commercial potato production, the identification of genes involved in durable tuber blight resistance is crucial for the development of tuber resistant cultivars (Mayton et al., 2010). However, also in the conventional production of potatoes a durable form of genetic resistance is important, because of the restriction in fungicides and the development of fungicide resistant *P. infestans strains.* (Goodwin et al., 1996). In case of organic production the genes involved in tuber blight resistance have to be incorporated by classical breeding, and may be assisted by molecular markers. However, by the use of genetically transformed tubers in this study it is possible to study the effect of several *R* genes in the same genetic background. The effect of several *R* genes can be analysed and compared in this way. The effect of several *R* genes together in one cultivars can be studied by stacking the *R* genes. For that reason transformants can be used to obtain information about the *R* genes which could be used to incorporate valuable *R* genes by classical breeding.
The developments in molecular genetics provided a rapid discovery of novel *R* genes involved in late blight resistance (Vleeshouwers et al., 2011). In this study several of these *R* genes are studied in different genetic backgrounds with different isolates. Besides that, a set of cultivars and breeding lines are tested on foliage and tuber blight in the laboratory and in the field.

In this study, several transformants, cultivars and breeding lines are used to investigate the relation between tuber and foliage blight resistance. This study should lead to answers for the following research questions.

1.6 Research questions:

- Which genes confer resistance to late blight in tubers and foliage?
- Which genes confer foliage specific resistance?
- What is the influence of the genetic background in which the foreign gene is introduced on the level of resistance in tuber and foliage?
- What is the influence of stacking genes on the effectivity of the resistance genes in tuber and foliage.
- How do the field and laboratory late blight trials correlate?
- What is the influence of different isolates on the level of resistance of the material that is tested.

2 Material and methods

Different tests were performed during this thesis project to investigate the effect of resistance genes originating from wild *Solanum* species on late blight resistance of foliage and tubers in current *S. tuberosum* cultivars. For part of the material the genes were introgressed by classical breeding and the other part by transformation. Also a set of current cultivars was tested.
Tubers and foliage were tested for late blight resistance in the laboratory and in the field. The following tests were performed:

1) a detached leaf assay (DLA) in the laboratory (score 1-8)
2) a tuber slice assay in the laboratory (% relative to Desiree)
3) and 4) a tuber (% infected tubers) and leaf analysis (score 0-10) under field conditions

A tuber slice assay and a detached leaf assay were conducted in the laboratory. The late blight field trial was conducted to investigate foliage and tuber late blight resistance under field conditions. Molecular marker analysis was conducted to determine the presence of several known *R* genes in cultivars and breeding lines.

2.1 Plant material

Transformants of the cultivars Desiree (42 transformants), Premiere (6), Aveka (8) and Atlantic (7) were used for the different experiments. Desiree and Atlantic contain no *R* genes, Premiere contains *R10* and Aveka *R3*. The non-transformed cv. Desiree was included in all the tests as a reference. The following late blight *R* genes cloned from wild Solanum species were brought into Desiree by genetic transformation and used in this study:

Rpi-BLB3 from *S. bulbocastanum* (Lokkousou et al., 2009)
Rpi-BLB1 from *S. bulbocastanum,* ***Rpi-STO1*** from *S. stoloniferum* and ***Rpi-PTA1*** from *S. papita (*van der Vossen et al., 2003; Vleeshouwers et al., 2008*)*
Rpi-VNT1 from *S. venturii (*Pel et al., 2009; Foster et al., 2009*)*
Rpi-EDN2 from *S. edinense* (Vetten et al., 2011)
R3a and R3b from *S. demissum* (respectively Huang et al., 2005; Li et al., 2011)
Rpi-CHC1 from *S. chacoense* (Vossen et al., 2009)
Rpi-MCQ1 from *S. mochiquense* (Smilde et al., 2005)

Some of these genes were also introgressed in other cultivars, such as Premiere, Aveka and Atlantic. One or more *R* genes were introgressed in the material, in order to investigate the influence of stacking *R* genes. When available, three events per cultivar x construct combination were used. In this way three repetitions of the same cultivar x construct combination were tested in this study, in order to conduct a proper statistical analysis.
Besides the transformants, also 15 cultivars and 26 breeding lines were included in the tests. The resistance genes *Rpi-BLB2 (S. bulbocastanum), R2, R3a, R3b* and *R8 (S. demissum) and Rpi-EDN2 (S. edinense)* were introgressed in this material by classical breeding. The complete list of tested genotypes can be found in Appendix 1. All the genotypes in Appendix 1 were tested for tuber and foliage late blight resistance in the laboratory and the field. For the cultivars Anuschka and Lady Anna it was not possible to test them in the field, because not enough tubers were available of these cultivars for planting them.

2.2 Isolates

The isolates Katshaar, Dinteloord, IPO-C and IPO-0 were used for the inoculation of the experiments. These isolates differ in virulence spectra and aggressiveness, which make them suitable for characterising the different *R* genes. The isolates have the following virulence spectra to *S. demissum R* genes:

- Dinteloord: 1,2,4
- Katshaar: 1,3,4,7,8,10,11
- IPO 0: 3b
- IPO C: 1,2,3,4,5,6,7,10,11

These isolates were used for the tuber and leaf tests in the laboratory. Every week the isolates were propagated on rye agar plates to provide sufficient and vital spores for inoculation.

2.3 Tuber slice assay

Tubers harvested in 2013, from a conventionally grown yield trial, were assessed for tuber blight resistance in the laboratory. Intact tubers were washed in water, sterilized in 0.5% bleach solution for 5 min and rinsed thoroughly with tap water. Five tubers per genotype and eight genotypes in total were used per tuber test. In every test Desiree was included as a susceptible control. The non-transformed cultivar was included as a control in the tuber tests with transformants of cultivars Premiere, Aveka and Atlantic. One tuber slice was cut from one sterilized tuber for an assay with one isolate.

The tuber slices were randomly placed in a plastic tray with watered blotting paper.

The inoculum preparation was done by washing the sporangia from a agar plate by adding 10ml tab water. The agar plates were used for the multiplication of the different isolates. The inoculum was then poured in a plastic tube and incubated for two hours at 7 C˚. Each slice was inoculated with four droplets of 10 µl inoculum and the tray was covered with a plastic bag. The inoculated tuber slices were incubated at 15C˚ for 9 days in the dark. After 9 days the tuber slices were scored for the amount of produced sporangia. Tuber slices were rinsed in 10 ml tab mater for 30 seconds to remove the sporangia. The samples were counted for the number of sporangia by making use of a counting chamber under the microscope. The number of sporangia was divided by the amount of sporangia produced on the susceptible control Desiree to end up with a percentage of sporangia relative to Desiree. Some pictures of the preparation of a tuber slice assay can be seen in Figure 2.

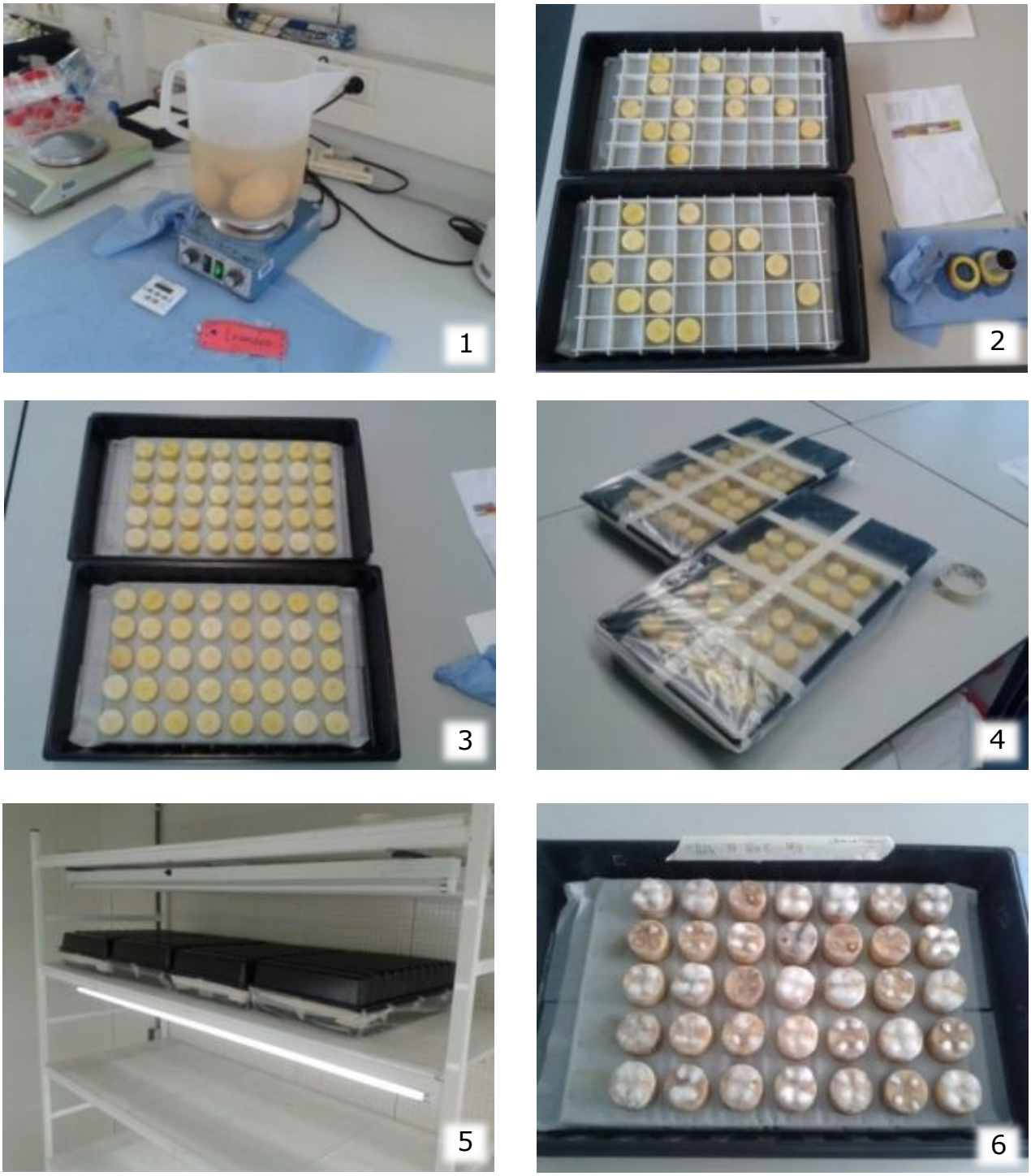


Figure 2. Description of the different steps of the tuber slice assay
1. Intact tubers were washed in water, sterilized in 0.5% bleach solution for 5 min and rinsed thoroughly with tap water.
2. Tuber were dried and slices were made from the middle of the tubers and randomly placed in the tray following a schedule. The trays were made in duplo for the inoculation with two different isolates.
3. One tray with tuber slices was inoculated with isolate Katshaar and the other tray was inoculated with isolate IPO C.
4 and 5. The trays were covered with plastic and incubated for 8 to 9 days at 15 C˚ in the dark.
6. After 8-9 days the trays were scored by counting the sporangia per tuber slice under the microscope.

2.4 Detached leaf assay

Foliage blight resistance was assessed by a detached leaf assay. Leaves were collected from the field at the end of May and placed in small pieces of watered oasis (Figure 3). Two plants per genotype and two leaves per genotype were used for inoculation. The genotypes were randomly distributed in plastic trays with watered blotting paper to ensure a high relative humidity. The leaves were inoculated by one 10 µl droplet of inoculum on 4 places of the abaxial side of the leaves. Isolate IPO C was inoculated at the left side of the leaf vain and isolate Katshaar on the right site of the leaf vein. The inoculum was prepared in the same way as for the tuber slice tests. The leaves were incubated in a climate room at 15C˚. The symptoms were scored from 1-8 seven days after inoculation by using the following scoring form:

Table 1. Scoring form for the detached leaf assays (Wageningen UR)

score	Description
8	Sporulation very intensive
7	Sporulation not as intensive as score 8
5	Sporulation only clearly visible through binocular
4	Sporadic sporulation, only visible through binocular
4	Necrosis bigger than or equal to 10 mm diameter
2	HR spot between 3 and 10 mm diameter
1	HR as big as inoculation spot
1	Seemingly not inoculated

Figure 3. Arrangement of the potato leaves in a detached leaf assay. Two leaves of two plants were used per genotype and put in watered foam blocks. Two genotypes were tested per box. The black spots on the leaves represent the hypersensitive response.

2.5 Late blight trial field

Seed tubers were planted on May 1st at an outdoor location with sandy soil near Wageningen. The cultivars and breeding lines were planted in three plots of four plants per genotype. The transformants were planted separately from the cultivars and breeding lines in two plots of six plants per genotype. The plots were randomly distributed and encircled by rows of the susceptible cultivar Bintje to separate the plots and to encourage the late blight infection (Figure 4).
The plan was to inoculate the field July 1st with isolate IPO-C. However, the potato plants were already infected by that time due to the high late blight infection pressure and the first symptoms were visible on the susceptible plants. It was chosen not to inoculate the field with IPO C. The field was watered twice a day with sprayers to ensure a high humidity to encourage the natural late blight infection.
The late blight analysis on the leaves was started when the first symptoms were clearly visible on the susceptible control plants. The analysis continued weekly for about six weeks.
The plants were scored for percentage infection from 0-10. Plants that were scored with a 1 were for about 10% visible affected by late blight. Plants that were scored with a 9 were for about 90% affected by late blight. Complete susceptible plants were scored with a 10, complete resistant plants were scored with a 0.

Figure 4. The late blight field trial near Wageningen. The genotypes were randomly distributed over the field. The susceptible cultivars Bintje and Nicola were planted in the border rows. On the picture is a susceptible row of cv. Bintje visible with the transformants around.

The potato leaves were sprayed and killed when the leaf observation was completed. The potatoes were harvested by hand two weeks after spraying. The potatoes were harvested per genotype plot and collected in plastic boxes. Infected and rotten tubers were counted and left in the field (Figure 5). In total about 330 filled boxes were collected and stored outside under a roof. The tubers were sorted for a minimum size of 2,5 cm and counted. After four weeks the amount of infected tubers per box was counted and a percentage infected tubers was calculated per box and per genotype.

Figure 5. Rotten tubers were counted and a percentage infected tubers was calculated per genotype.

2.6 Data analysis

Statistical analysis of the data obtained in these tests was performed with the statistical programmes Genstat 16th edition and IBM SPSS statistics 20.

The statistical analysis of the transformants was mainly done by performing an ANOVA using Genstat 16th edition. Formely a non-parametric test had to be used such as a Kruskal Wallis test, because most of the data were not normally distributed and the data lack homogeneity of variances. However, it was not possible to make proper multiple comparisons with a Kruskal Wallis test (using IBM SPSS statistics 20), due to the amount of genotypes. Therefore it was chosen to use the parametric ANOVA test and to perform a Scheffé's post hoc test. This is the most conservative post hoc test giving maximum protection against making a type 1 error (false positives). By doing both the statistical tests, it was found that for these data the results were quite comparable between both tests.

Two sample T tests were done for the comparison between the infection levels for two isolates. Mainly the non-parametric Mann Whitney U test (using Genstat) was used, because most of the data were not normally distributed and the data lack homogeneity of variances.

Correlation tests (using SPSS)were done for investigating the association between two testing methods. A Kendall's tau-b correlation test was used for data with ties, while a Pearson correlation test was used for data without ties.

2.7 Molecular marker analysis

In August a selection of the material was tested for the presence of the *R* genes *R1, R3a, R3b, Rpi-BLB1, Rpi-BLB2, Rpi-BLB3, Rpi-CHC1, R8* and *Rpi-EDN2* by molecular marker analysis. From a selection of the material that was tested in this study it was known that they contain one or more *R* genes. The goal of testing the material with markers was to confirm the presence or absence of these *R* genes.

3 Results

The results of this study are divided to two parts. The first part will present the results found in the different experiments performed with the transgenic potatoes. Most of the experiments were conducted with transformants of the cultivar Desiree and some with transformants of the cultivars Aveka, Atlantic and Premiere. Each transformant contained one or more late blight resistant genes. The second part will show the results with different cultivars and breeding lines. In the next chapter the results will be discussed. The results per genotype can be found in Appendix 2. Photos of the experiments can be found in Appendix 4.

The isolates IPO C and Katshaar were used for the inoculation of the assays in the laboratory. The intention was to use also the isolates Dinteloord and IPO 0, but due to failure of infection in the tubers slice assays, it was chosen to exclude these isolates from this study.

3.1 Transformants

3.1.1 Detached leaf assay in the laboratory

Fifteen different Desiree x construct combinations were analysed in a detached leaf assay in the laboratory to test for late blight tuber resistance. These 15 combinations are unique combinations with one or more inserted *R* genes in the same genetic Desiree background.

Detached leaves of the genotypes were cut from the field and inoculated with the isolates Katshaar and IPO C. The infection level was analysed on a scale from 1-8 with a score 1 for no infection and score 8 for complete infection. The results in Figure 6 represent the average score of 48 observations per *R* gene or combination of *R* genes (4 observations per leaf, four leaves per genotype, and three transformation events (clone numbers) per *R* gene).

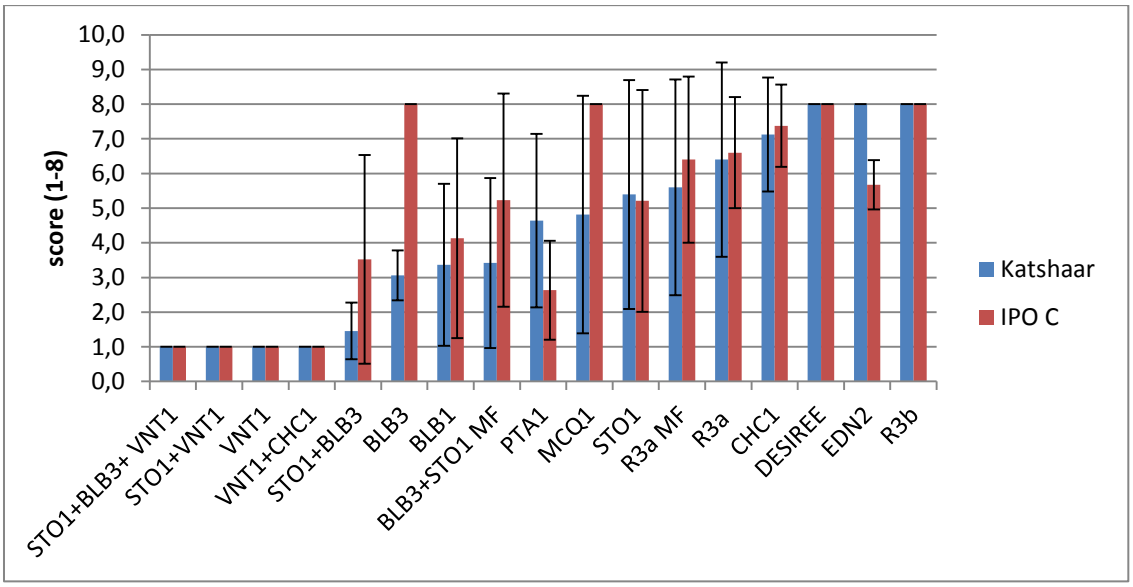


Figure 6. Results from the detached leaf assay performed with Desiree transformants containing one or more late blight *R* genes. The mean leaf infection level per genotype is presented for the isolates Katshaar and IPO C (score 1 = no infection, score 8 = complete infection).

Table 2 shows the results of an ANOVA test with a post hoc Scheffe's test for the different Desiree transformation combinations.

Table 2a and b. Results of the analysis of variance (ANOVA) for the different Desiree transformation combinations containing one or more late blight *R* genes. The score present the mean leaf infection level (score 1-8) per genotype for the isolates Katshaar (a) and (b) IPO C (score 1 = no infection, score 8 = complete infection). Genotypes that do not have the same letter differ significantly in level of infection.

Katshaar		
Genotype	score	Sig.
STO1+BLB3+ VNT1	1.0	a
STO1+VNT1	1.0	a
VNT1	1.0	a
VNT1+CHC1	1.0	a
STO1+BLB3	1.5	a
BLB3	3.1	ab
BLB1	3.4	abc
***BLB3+STO1* MF**	3.4	abc
PTA1	4.6	abc
MCQ1	4.8	abc
STO1	5.4	bc
R3a	6.4	bc
CHC1	7.1	bc
DESIREE	8.0	c
EDN2	8.0	c
R3b	8.0	c

IPO C		
Genotype	score	Sig.
STO1+BLB3+ VNT1	1.0	a
STO1+VNT1	1.0	a
VNT1	1.0	a
VNT1+CHC1	1.0	a
PTA1	2.6	ab
STO1+BLB3	3.5	abc
BLB1	4.1	abc
STO1	5.2	bc
***BLB3+STO1* MF**	5.2	bc
EDN2	5.7	bc
R3a	6.6	c
CHC1	7.4	c
BLB3	8.0	c
DESIREE	8.0	c
MCQ1	8.0	c
R3b	8.0	c

Variation in infection level was observed for both isolates (Figure 6). An analysis of variance (ANOVA) on these scores yielded significant variation among the genotypes for Katshaar ($F = 21.35$, $p < .001$) and IPO C ($F = 23.73$, $p < .001$). The results of isolate Katshaar showed that the genotypes with the *VNT1* gene had a mean score of 1 and differed significantly from *STO1*, *R3a*, *CHC1*, Desiree, *EDN2* and *R3b* (Table 2). Significance difference was also observed between these genotypes and *STO1*+*BLB3* with a mean score of 1.5. The genotypes *BLB3*, *BLB1*, *BLB3*+*STO1* MF, *PTA1* and *MCQ1* did not differ significantly from the group of genotypes with the *VNT1* and STO+*BLB3*. Although a mean infection level of 3.1 to 4.8 was scored for these genotypes, no significant difference was found with Desiree. Comparable results were found when analysing the results with isolate IPO C. The genotype group with the *VNT1* gene was found to be significant different from *STO1*, *BLB3*+STOMF, *EDN2*, *R3a*, *CHC1*, *BLB3*, Desiree, *MCQ1* and *R3b*. Genotype *PTA1* differed significantly from *R3a*, *CHC1*, *BLB3*, Desiree, *MCQ1* and *R3b*. The genotypes *STO1*+*BLB3* (score 3.5) and *BLB1* (score 4.1) were not found to be significantly different with the *VNT1* group, but did also not differ significantly from Desiree.

Influence of the isolates

Significant differences between the infection levels of the isolates Katshaar and IPO C per genotype was analysed by doing a two sample t test. Because the data were not normal distributed it was chosen to use a non-parametric two sample t test, the Man Whitney U test (Table 3). With this test the null hypothesis was tested that a Desiree x construct combination has the same infection level for both isolates. A significance level lower than 0.05 rejected this hypothesis.

Table 3. The results of a Mann Whitney U test between the leaf infection levels for the isolates Katshaar and IPO C for the different Desiree transformant combinations containing one or more late blight *R* genes. A p-value <0.05 (above the bold line) indicates significant difference in infection level between the two isolates.

Genotype	p value
EDN2	< 0.001
MCQ1	0.014
BLB3	0.029
PTA1	0.037
STO1+BLB3	0.074
BLB3+STO1 MF	0.169
R3a	0.441
BLB1	0.741
STO1	0.785
CHC1	0.856
DESIREE	1
VNT1	1
STO1+BLB3+ VNT1	1
STO1+VNT1	1
VNT1+CHC1	1
R3b	1

Significant different infection levels were found between Katshaar and IPO C for the genotypes *EDN2*, *MCQ1*, *BLB3* and *PTA1*. Inoculation with the isolate IPO C caused a higher infection level compared with Katshaar for the genotypes *MCQ1* and *BLB3*. Both genotypes were completely susceptible (score 8) when inoculated with IPO C, while *BLB3* scored on average a 3 and *MCQ1* a 4.8 when inoculated with Katshaar. The genotypes *EDN2* and *PTA1* were found to have a higher infection level when inoculated with Katshaar. *EDN2* was completely susceptible (score 8) when inoculated with Katshaar, while this genotype scored a 5.7 when inoculated with IPOC. *PTA1* scored a 4.6 with isolate Katshaar, while a 2.6 when infected with IPO C.

Premiere, Aveka and Atlantic transformants

Atlantic, Aveka and Premiere transformants were included in the detached leaf assay to look for the influence of genetic background on the level of resistance for several constructs with resistance genes *BLB1*, *VNT1*, *CHC1* and *STO1*. The results of Desiree and Desiree transformants were included in the figure to compare with the results of Aveka, Atlantic, Premiere and their transformants. The leaves were inoculated with Katshaar and IPO C. The statistical analysis was performed with a non-parametric Kruskall Wallis test.

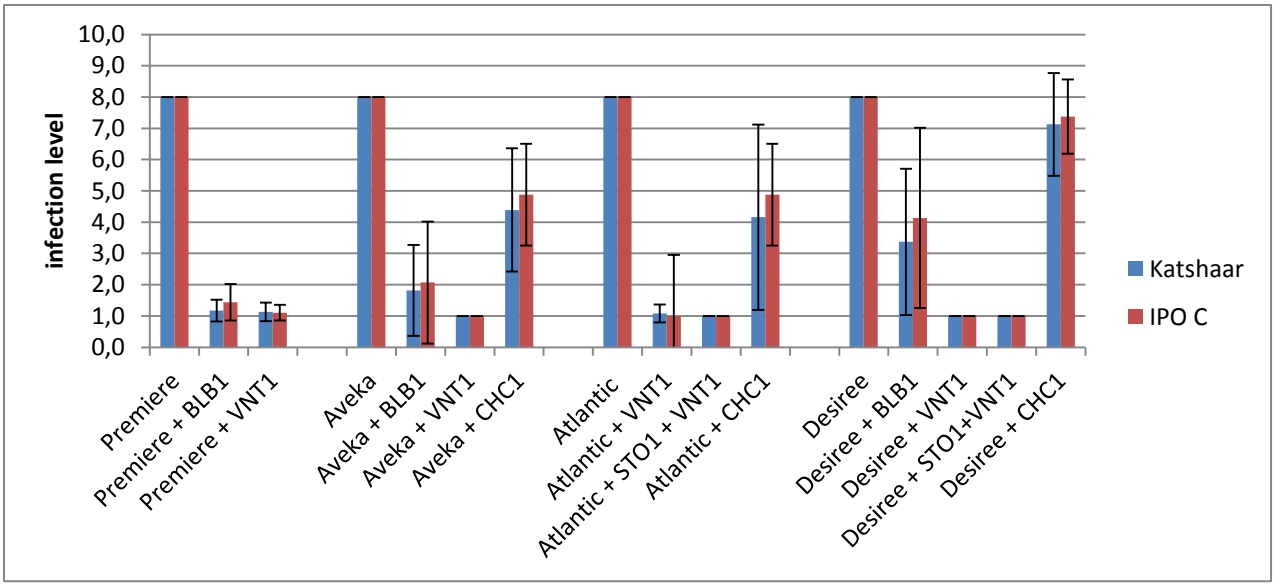


Figure 7. Results from the detached leaf assay performed with cultivar x construct combinations of the cultivars Atlantic, Aveka, Desiree and Premiere containing one or more late blight *R* genes. The mean leaf infection level per combination is presented for the isolates Katshaar and IPO C (score 1 = no infection, score 8 = complete infection).

No difference was observed between the infection levels of the non-transformed cultivars (Figure 7). Premiere, Aveka, Atlantic and Desiree were scored with the highest infection level for both isolates. Desiree+*BLB1* showed a significant higher infection level compared to Aveka+*BLB1* and Premiere+*BLB1* for isolates IPO C (p=0.001) and Katshaar (p=0.002). No difference was observed between the cultivars with the *VNT1* and *STO1*+*VNT1* constructs. All these combinations scored the lowest infection level for both isolates. Significant difference in infection level was observed for the cultivars Aveka, Atlantic and Desiree with the *CHC1* construct for isolates IPO C (p=0.003) and Katshaar (p=0.009). Desiree with *CHC1* showed a significant higher infection level compared to Atlantic with *CHC1* and Aveka with *CHC1* for both isolates.

<u>Results within each cultivar and their transformants</u>

Premiere with *BLB1* and *VNT1* showed a significantly lower infection level compared to the non-transformed Premiere for isolates IPO C and Katshaar (p=0.001). Aveka with *BLB1* and *VNT1* were found to differ significantly for their infection level from the non-transformed Aveka. Aveka with *VNT1* scored significantly lower compared to Aveka with *CHC1* for isolates IPO C and Katshaar (p=0.000). Atlantic with *VNT1* and with *STO1*+*VNT1* were significantly different from Atlantic and Atlantic with *CHC1* for both isolates (p=0.000).

3.1.2 Tuber slice assay in the laboratory

The same genotypes that were used for the detached leaf assay, were used for the tuber slice assay. Tuber slices were inoculated with IPO C and Katshaar and scored for the infection level by counting sporangia. The amount of sporangia was converted in a percentage infection relative to the non-transformed cv. Desiree.

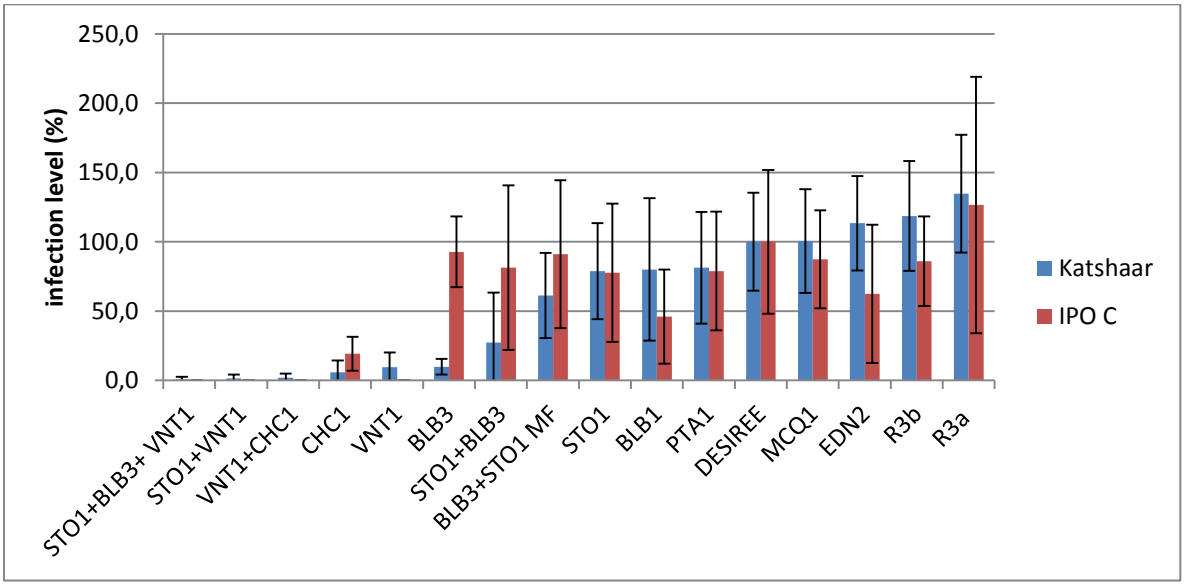


Figure 8. Results from the tuber slice assay performed with Desiree x construct combinations containing one or more late blight *R* genes. The mean tuber infection level per genotype relative to cv. Desiree is presented for the isolates Katshaar and IPO C.

Figure 8 shows the percentage infection of the different Desiree x construct combinations relative to cv. Desiree. An analysis of variance (ANOVA) on these scores yielded significant variation among the genotypes for Katshaar ($F = 35.19$, $p < .001$) and IPO C ($F = 14.96$, $p < .001$). Table 4 shows the results of an ANOVA test with a post hoc Scheffe's test for the different genotypes. Genotypes that do not have the same letter differ significantly in level of infection.

From the results with isolate Katshaar variation between the genotypes was observed. The group of genotypes with the *VNT1* gene had an infection level lower than 10% relative to Desiree and were significantly different from cv Desiree. Figure 9 shows the clear resistance of Desiree tuber slices containing *VNT1.* No mycelium growth was visible for these tuber slices. *CHC1* (6%), *BLB3* (10%) and *STO1*+*BLB3* (27%) were other combinations that differed significantly from Desiree. The genotypes *STO1* (79%), *BLB1* (80%) and *PTA1* (81%) performed comparable in infection level and did not differ significantly from Desiree. The genotypes *MCQ1*, *EDN2*, *R3b* and *R3a* were higher in percentage infection relative to Desiree, but did not differ significantly.

The genotypes with the *VNT1* gene were not infected when these were inoculated with IPO C and differed significantly from Desiree. Desiree with *CHC1* had an infection level of 19% and differed also significantly from Desiree. The other genotypes performed not significantly different compared to Desiree. The genotype with the *R3a* gene had an infection level higher than Desiree, but did not differ significantly.

Table 4 a and b. Results of the analysis of variance (ANOVA) for the different Desiree x construct combinations containing one or more late blight *R* genes. The score present the mean tuber infection level per genotype relative to cv. Desiree for the isolates Katshaar (a) and IPO C (b). Genotypes that do not have the same letter differ significantly in level of infection.

Katshaar		
Genotype	**score**	**Sig.**
STO1+BLB3+ VNT1	1	a
STO1+VNT1	2	ab
VNT1+CHC1	2	ab
CHC1	6	ab
VNT1	9	ab
BLB3	10	abc
STO1+BLB3	27	abc
BLB3+STO1 MF	61	bcd
STO1	79	cde
BLB1	80	cde
PTA1	81	cde
DESIREE	100	de
MCQ1	101	de
EDN2	113	de
R3b	119	de
R3a	135	e

IPO C		
Genotype	**score**	**Sig**
STO1+BLB3+ VNT1	0	a
STO1+VNT1	0	a
VNT1	0	a
VNT1+CHC1	0	a
CHC1	19	ab
BLB1	46	abc
EDN2	62	abc
STO1	78	abc
PTA1	81	abc
STO1+BLB3	81	abc
R3b	86	bc
MCQ1	87	bc
BLB3+STO1 MF	91	bc
BLB3	93	bc
DESIREE	100	c
R3a	127	c

Influence of the isolates

The level of influence of the isolates on the infection level of the genotypes can be observed in Figure 8. Significant differences between the infection levels of the isolates Katshaar and IPO C per construct were analysed by doing a two sample t test. Because the data were not normally distributed it was chosen to use a non-parametric two sample t test, the Man Whitney U test (Table 5). A significance level (p-value) lower than 0.05 indicates significant difference between the infection levels of IPO C and Katshaar.

Genotypes above the bold line differed significantly in infection level when the isolates Katshaar and IPO C were compared. The genotypes *STO1+BLB3*, *BLB3*, *CHC1* and *BLB3+STO1* MF showed a significant lower infection level for isolate Katshaar compared with isolate IPO C. The genotypes *VNT1*, *VNT1+CHC1*, *EDN2*, *BLB1* and *R3b* showed a significant higher infection level for isolate Katshaar compared with isolate IPO C. The genotype *BLB3* showed the biggest difference in infection level between the isolates. Inoculation with IPO C caused a higher infection level (93%) compared with isolate Katshaar (10%) for the genotype with the single *BLB3 R* gene. The genotypes below the bold line did not show significant difference in infection level between the isolates.

Table 5. The results of a Mann Whitney U test between the tuber infection levels of the isolates Katshaar and IPO C for the different Desiree transformants containing one or more late blight *R* genes. A p-value <0.05 (above the bold line) indicates significant difference in infection level between the two isolates.

Genotype	p-value
STO1+BLB3	< 0.001
VNT1	< 0.001
VNT1+CHC1	< 0.001
EDN2	0.003
BLB3	0.008
CHC1	0.019
R3b	0.026
BLB1	0.041
BLB3+STO1 MF	0.046
STO1+VNT1	0.1
STO1+BLB3+ VNT1	0.224
R3a	0.305
MCQ1	0.325
STO1	0.632
PTA1	0.967

Figure 9. Tuber slices inoculated with isolate Katshaar. Tubers with the *VNT1* gene showed clear resistance. The susceptible tubers showed mycelium growth.

Aveka and Atlantic transformants

Atlantic and Aveka transformants were included in the tuber slice assay to look for the influence of genetic background on the level of resistance for several constructs with resistance genes *BLB1*, *VNT1*, *CHC1* and *STO1* (Figure 10). This figure shows only the results of tuber slice assays inoculated with Katshaar, because the assays with IPO C failed.

The results of Desiree and Desiree combinations are included in the figure to compare with the results of Aveka and Atlantic combinations. Statistical analysis was conducted with a Kruskal Wallis test when more than 2 combinations were analysed. A Mann-Whitney U test was used when two combinations were analysed.

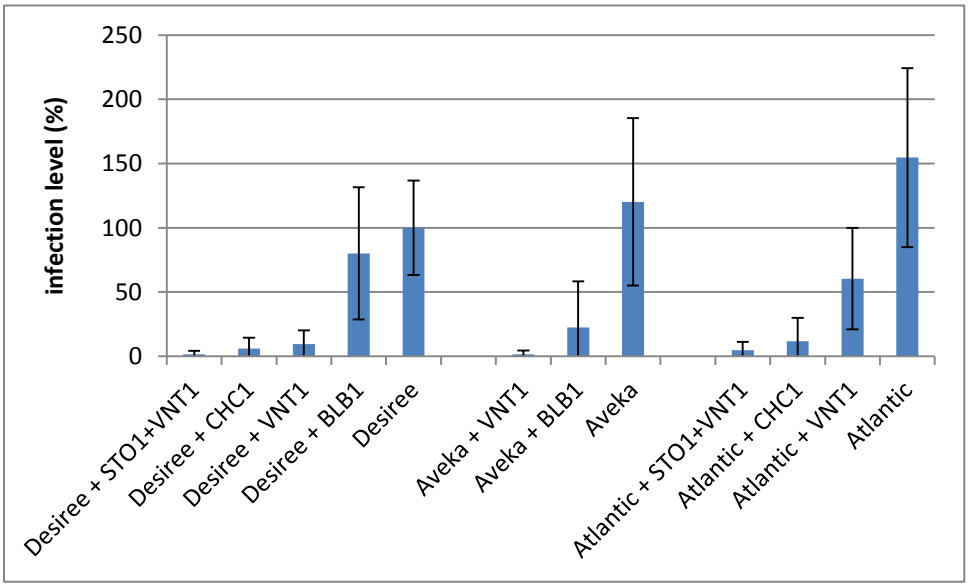


Figure 10. Results from the tuber slice assay performed with transformants of the cultivars Atlantic, Aveka and Desiree containing one or more late blight *R* genes. The mean tuber infection level per genotype relative to cv. Desiree is presented for the isolates Katshaar and IPO C.

Atlantic showed a higher infection level compared to Aveka and Desiree, but no significance was found (p=0.172). Aveka with *VNT1* (2%) and Desiree with *VNT1* (9%) showed a significant lower infection level (p=0.000) compared to Atlantic with *VNT1* (60%). Aveka with *BLB1* (22%) showed a significant lower infection level (p<0.001) compared to Desiree with *BLB1* (80%). The infection level of Desiree with *STO1* +*VNT1* (1.5%) was found to be a little smaller compared to Atlantic with *STO1*+*VNT1* (4.7%), but no significance was found (p=0.192). No significant difference (p=0.662) was found between Desiree with *CHC1* (6%) and Atlantic with *CHC1* (11.7%).

Both Aveka transformants with *VNT1* and *BLB1* scored a significantly lower (p <0.001) infection level compared to Aveka (one outlier was discarded from the *BLB1* data). Significant difference was also found within Atlantic and Atlantic transformants (p<0.001). Atlantic tubers with resistance genes *STO1*+*VNT1* (4.7%), and *CHC1* (11.7%) showed significant lower infection compared to Atlantic. Atlantic with *STO1*+*VNT1* was found to have a significantly lower infection level compared to Atlantic with *VNT1*.

3.1.3 Leaf analysis under field conditions

During 5 weeks the development of late blight infestation was regularly monitored on the transformants. Late blight scoring was performed on a scale from 0 - 10, with 0 = no visible infection and 10 = complete dead due to late blight infection. The differences between the genotypes were best observed at the fourth scoring moment (Figure 11). At this moment some genotypes were heavily infected, while others showed a reduced disease development or had no symptoms. An analysis of variance (ANOVA) on these scores yielded significant variation among the genotypes ($F = 73.39$, $p < .001$). Table 6 shows the results of an ANOVA test with a post hoc Scheffe's test for the different combinations. Combinations that do not have the same letter differ significantly in the level of infection.

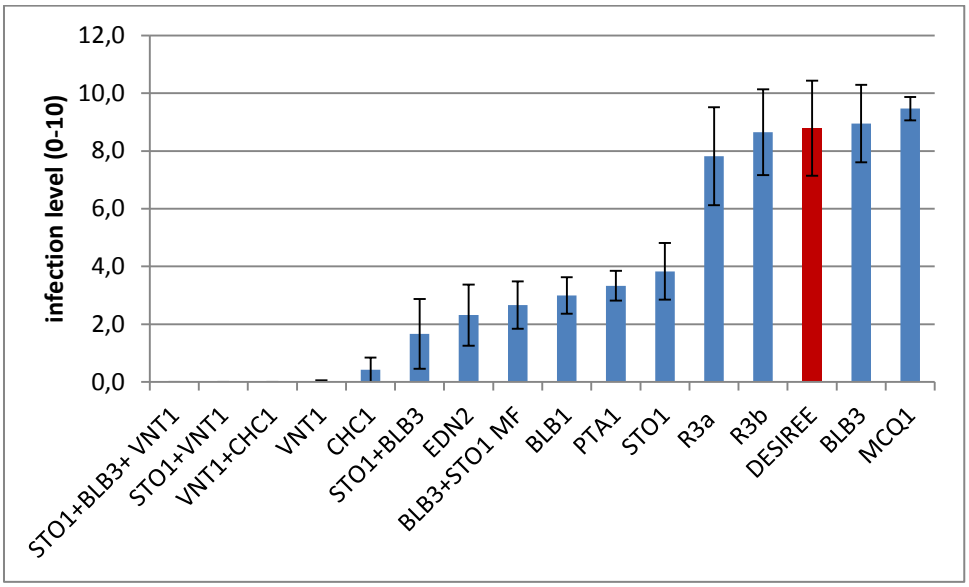


Figure 11. Results from the leaf field analysis performed with Desiree x construct combinations containing one or more late blight *R* genes. The mean leaf infection level per combination was scored from 0 till 10 (score 0 = no infection, score 10 = dead due to infection)

Diversity in susceptibility was observed in the field. The 5 most susceptible combinations scored between 7.8 and 9.5 at the fourth scoring moment. The 5 most resistant combinations were scored between 0 and 0.4. Between these groups there is a group that showed a reduced form of resistance and scored between 1.7 and 3.8. The group of genotypes with the *VNT1* gene were scored with a 0 and *CHC1* with a 0.4. Desiree with *VNT1* differed significantly from *BLB1*, *PTA1*, *STO1*, *R3a*, *R3b*, Desiree, *BLB3* and *MCQ1*. The genotypes *BLB1*, *PTA1* and *STO1* differed significantly from *R3a*, *R3b*, Desiree, *BLB3* and *MCQ1*. The genotypes *BLB3* and *MCQ1* had a higher infection level as cv. Desiree, but no significance was found.

Table 6. Results of the analysis of variance (ANOVA) for the different Desiree combinations containing one or more late blight *R* genes. The score present the mean leaf infection level (score 1-10) per genotype (score 0 = no infection, score 10 = dead due to infection). Combinations that do not have the same letter differ significantly in level of infection.

Genotype	score	Sig.
STO1+BLB3+ VNT1	0.0	a
STO1+VNT1	0.0	a
VNT1+CHC1	0.0	a
VNT1	0.0	a
CHC1	0.4	ab
STO1+BLB3	1.7	abc
EDN2	2.3	abc
***BLB3+STO1* MF**	2.7	abc
BLB1	3.0	bc
PTA1	3.3	bc
STO1	3.8	c
R3a	7.8	d
R3b	8.7	d
DESIREE	8.8	d
BLB3	9.0	d
MCQ1	9.5	d

Figure 12 shows the disease development in the field for the different groups of Desiree transformants. On five scoring moments the disease infection levels were scored, with one week between each scoring moment. About three groups can be distinguished when looking at the score at observation moment 5: one group with a score around 10, a group with a score between 2 and 6 and one group with a score lower than two.

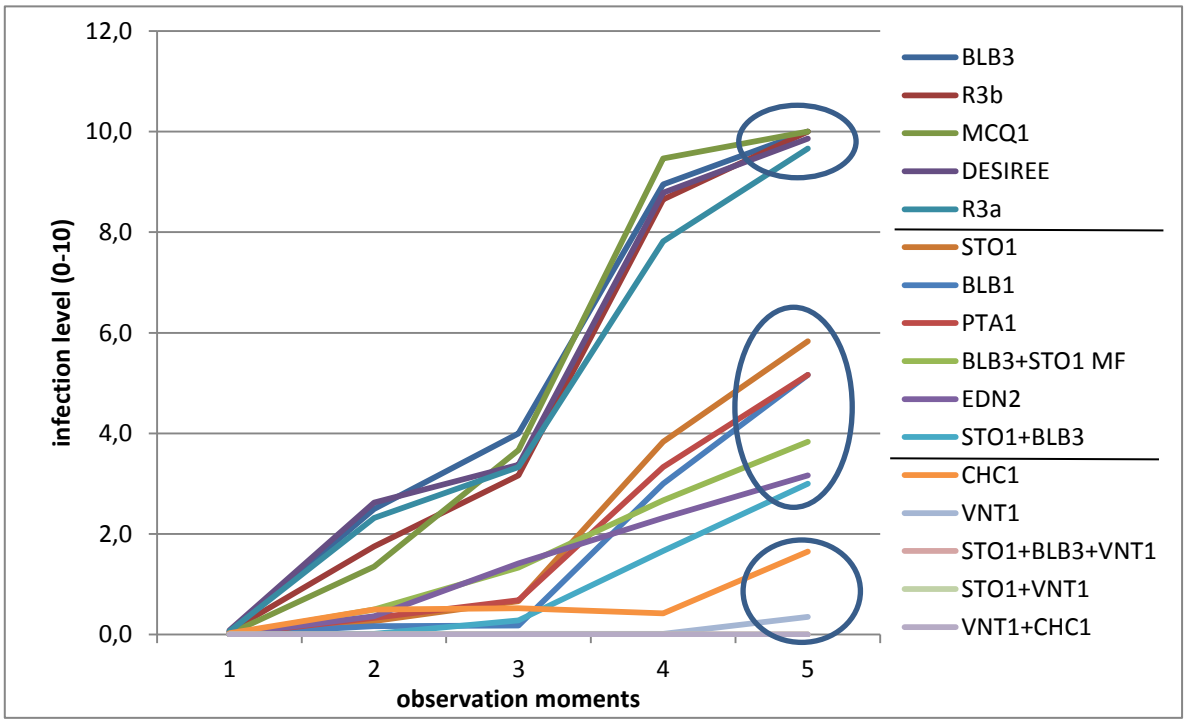


Figure 12. The late blight development in the field for the different groups of Desiree transformants containing one or more late blight *R* genes. Foliar late blight infection was scored during 5 observation moments with one week between each scoring moment (score 0 = no infection, 10 = dead due to infection). The circles represent groups; a group with genotypes that showed high infection levels, a group with genotypes that showed intermediate infection levels and a group that showed low infection levels at the end of the trial.

Premiere, Aveka and Atlantic transformants

Transformants of Premiere, Atlantic and Aveka were included in the leaf analysis in the field to look for the influence of genetic background on the level of resistance for several constructs with resistance genes *BLB1*, *VNT1*, *CHC1* and *STO1* (Figure 13). The results in this figure represent the fourth scoring moment, comparable to the scoring moment of the results of the Desiree transformants.

The results of Desiree and Desiree transformants are included in the figure to compare with the results of the transformants of the other cultivars.

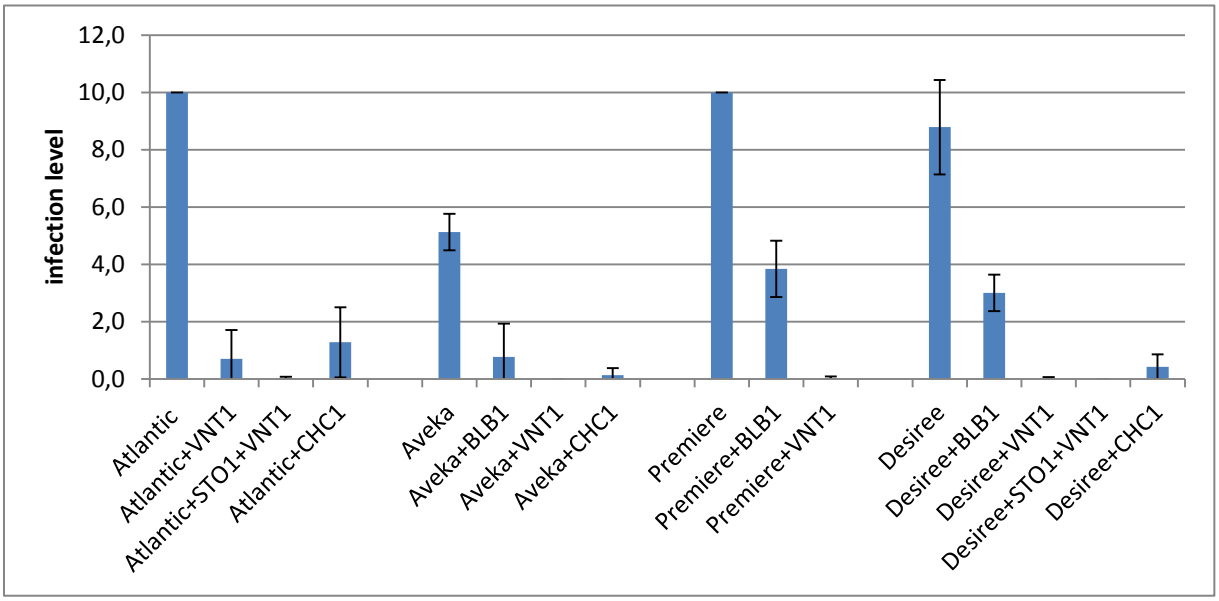


Figure 13. Results from the field leaf analysis performed with transformants of the cultivars Atlantic, Aveka, Desiree and Premiere containing one or more late blight *R* genes. The mean leaf infection level per combination was scored from 0 till 10 (score 0 = no infection, score 10 = dead due to infection).

Difference in infection level was observed between the non-transformed cultivars. Premiere and Atlantic were found to have a significant higher infection level compared to Aveka and Desiree ($p<0.001$).

Low infection and no difference in infection levels was found for the transformed cultivars with *VNT1* ($p=0.056$) and *STO1*+*VNT1* ($p=0.221$). These genotypes scored not above 1. Some difference in infection level was observed for the transformed cultivars with *BLB1*. Premiere+*BLB1* (3.8) and Desiree+*BLB1* (3.0) showed a higher infection level compared to Aveka+*BLB1*, which scored below 1. Significance was only found between Premiere+*BLB1* and Aveka+*BLB1* ($p=0.005$). Transformants with the *CHC1* construct were scored with an infection level of 1.3 (Atlantic) or lower, but no significant difference between the genotypes was found ($p=0.060$).

3.1.4 Tuber analysis under field conditions

The foliage of the potatoes was killed after the leaf analysis was completed. Two weeks after spraying the tubers were harvested from the field and counted for the amount of rotten tubers per plot. The rotten tubers were left in the field and the others were stored for several weeks. After 7 weeks the tubers were scored again. The amount of rotten tubers due to late blight was divided by the total amount of tubers per genotype to end up with a percentage infected tubers per genotype (Figure 14).

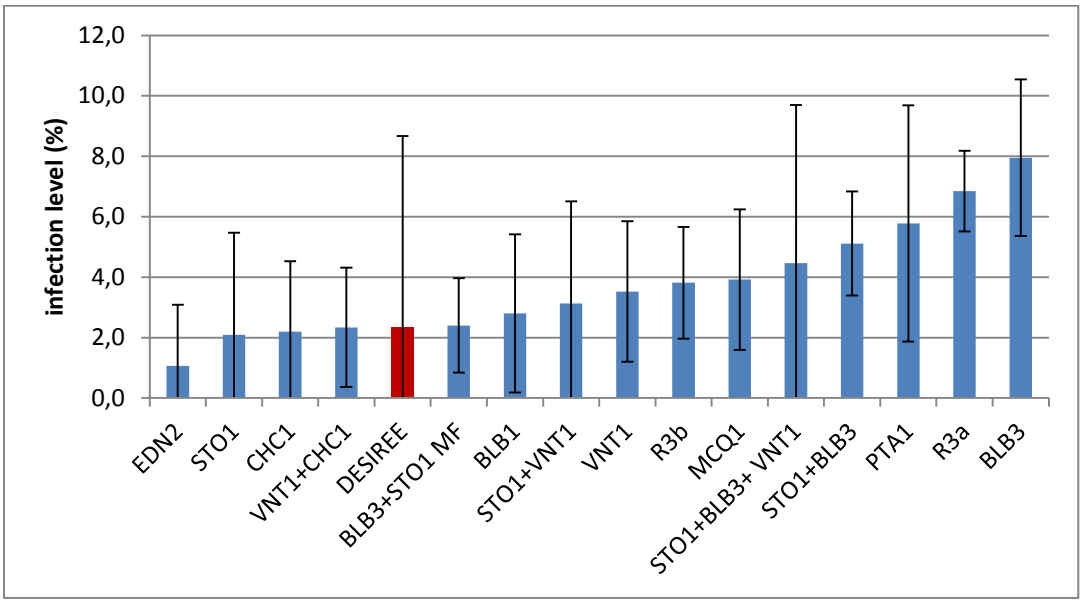


Figure 14. Results from the tuber field analysis performed with Desiree transformants containing one or more late blight *R* genes. The mean tuber infection level per genotype was calculated as a percentage rotten tubers of the total amount of tubers.

The tuber infection level did not exceed 8% of the total amount of tubers harvested per genotype. Most of the genotypes showed a higher infection level as Desiree. Four of the genotypes showed a comparable or lower infection level as Desiree. A Kruskal Wallis test did not show significant difference in infection level among the genotypes (p=0.127).
Next to the Desiree transformants also tubers from transformed cultivars Aveka, Atlantic and Premiere were analysed for the percentage infected tubers (Figure 15). Premiere and Premiere transformants showed the highest infection level. A Kruskal Wallis test showed a significant higher infection level for the cultivar Premiere compared to the other cultivars ($p<0.001$). The Aveka group of transformants with *VNT1* had a significantly lower infection level compared to the transformant groups of Antlantic and Premiere with *VNT1* ($p = 0.001$).

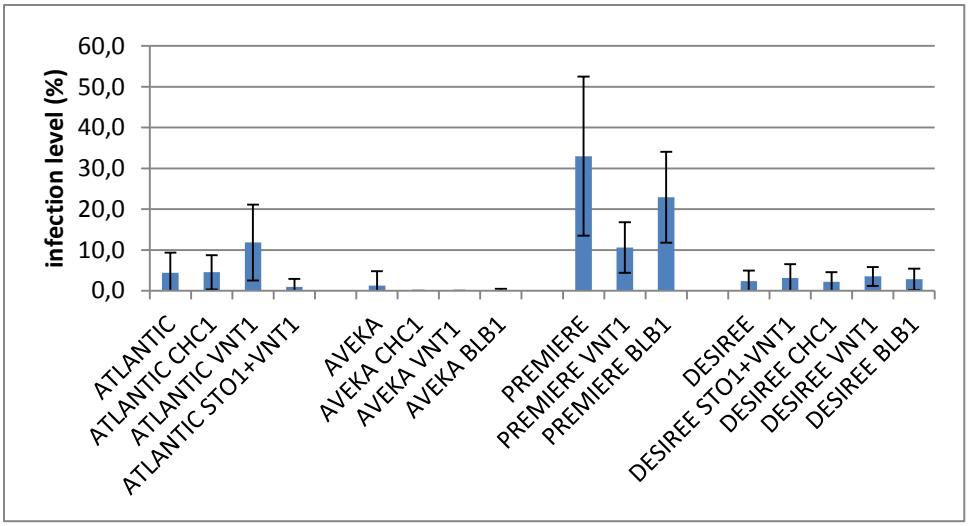


Figure 15. Results from the tuber field analysis performed with transformants of the cultivars Atlantic, Aveka, Premiere and Desiree containing one or more late blight *R* genes. The mean tuber infection level per group of transformants was calculated as a percentage rotten tubers of the total amount of tubers.

Premiere with *BLB1* was found to have a significantly higher infection level (p =0.001) compared to Aveka with *BLB1*. Aveka with *CHC1* scored significantly lower compared to Atlantic with *CHC1*. No significance was found between the cultivars with *STO1*+*VNT1*.

Results within cultivars and their transformants.

Premiere with *VNT1* showed a significant lower infection level compared to Premiere (p = 0.029). No significant differences in infection level were found within the Atlantic (p=0.058) and Aveka genotypes (p=0.676).

3.2 Correlation between the leaf infection level and the tuber infection level.

Leaf infection level vs. tuber infection level in the laboratory

The correlation between tuber late blight resistance and leaf late blight resistance can be analysed by doing a correlation test. A Kendall's tau-b correlation test was used to analyse the association between the data obtained by doing leaf and tuber assays in the laboratory.

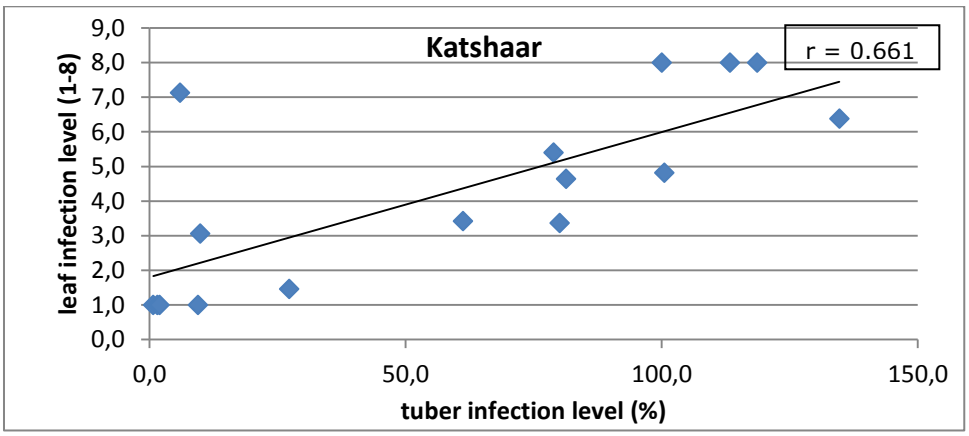


Figure 16. Correlation between the leaf and tuber infection levels scored in the laboratory for all groups off Desiree transformants. Every dot represents one group of Desiree transformants containing one or more late blight resistance genes.

Linear relation was observed between leaf infection level and tuber infection level for the isolate Katshaar, Kendall's tau-b = 0.661, p = 0.001 (2-sided). One outlier is seen at the top left of Figure 16. This data point refers to the genotype *CHC1*. This genotype scored about a 7 for leaf infection level, while the tuber infection level was scored with only 6%. A stronger correlation can be found when this genotype is discarded from the dataset, Kendall's tau-b = 0.791, p = 0.000 (2-sided).
Weaker correlation was found for the dataset with isolate IPO C, Kendall's tau-b = 0.589, p = 0.003 (2-sided). The genotype *CHC1* was again found as outlier, and the correlation coefficient increased when this data point was discarded, Kendall's tau-b = 0.671, p = 0.001 (2-sided).

Leaf infection level vs. tuber infection level in the field

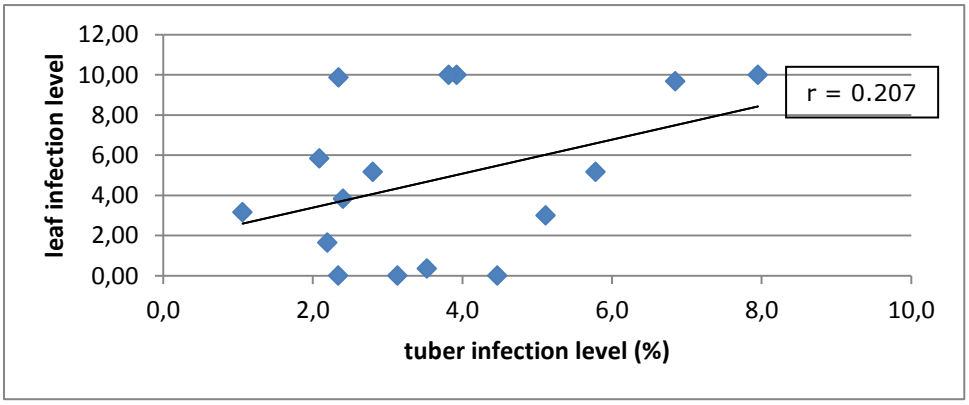


Figure 17. Correlation between the leaf and tuber infection levels scored in the field for all Desiree transformants. Every dot represents one Desiree transformant containing one or more late blight resistance genes.

No significant correlation was found between the leaf infection level in the field and the tuber infection level in the field, Kendall's tau-b = 0.207, p = 0.275 (2-sided) (Figure 17).

3.3 Cultivars and breeding lines containing *Rpi-BLB2, R8* or *Rpi-EDN2*

Fifteen conventional cultivars, three organic cultivars (Bionica (*BLB2*), Sarpo Mira (*R8*) and Toluca (*BLB2*)) and breeding lines containing *Rpi-BLB2* (8 genotypes), *R8* (6 genotypes) and *Rpi-EDN2* (4 genotypes) were screened for leaf and tuber late blight resistance in the laboratory. These breeding lines are originated from the conventional and organic breeding program of Wageningen UR. The cultivars Toluca and Bionica were accommodated in the group containing *Rpi-BLB2*. However, the results of Bionica inoculated with Katshaar were separated from this group, because this genotype contains, next to *Rpi-BLB2*, also *R2* that confers resistant to isolate Katshaar (virulent to *R* genes 1,3,4,7,8,10,11). The results of the *BLB2* group would be disturbed when the results of Bionica were included for isolate Katshaar. The cultivar Sarpo Mira (*R3a, R3b, R4, R8*) was accommodated in the group containing *R8*. The individual results of the breeding lines and cultivars can be found in respectively Appendix 3 and 4.

Figure 18 shows the average performance for the breeding lines (containing *BLB2*, *R8* and *EDN2*), the cultivars and the control Desiree.

The results were statistically analysed by doing a Kruskal Wallis test. This was done due to the fact that the data were not normally distributed and the data lack homogeneity of variances.

3.3.1 Detached leaf assay in the laboratory

Leaves were cut from plants in the field and inoculated with the isolates Katshaar and IPO C. After one week the detached leaves were scored for the level of infection on a scale from 1 - 8 (Figure 18).

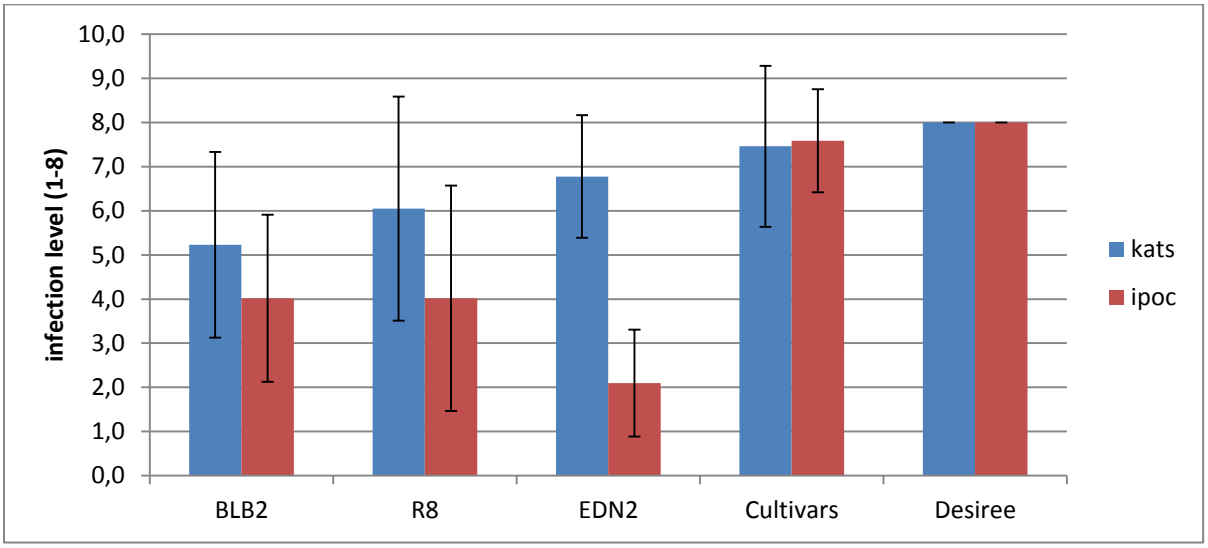


Figure 18. Results from the detached leaf assay performed with 16 cultivars and three groups of breeding lines containing the late blight *R* genes *BLB2, EDN2* or *R8*. The mean leaf infection level per group is presented for the isolates Katshaar and IPO C (score 1 = no infection, score 8 = complete infection).

No much variation was found between the infection levels of the different groups, analysing the results for isolate Katshaar. Only the group of breeding lines containing *BLB2* showed on average a significant lower infection compared to the group of cultivars and cv. Desiree ($F = 6.2$, $p < 0.001$) (Table 7). Analysing the results with IPO C, it is seen that lower infection levels were scored for the breeding line groups with *BLB2, R8* and *EDN2* compared to the cultivar group and cv. Desiree. An analysis of variance (ANOVA) on these scores yielded significant variation among the different groups ($F = 51.35$, $p < .001$).

Table 7 a and b. Results of the detached leaf assay with an analysis of variance (ANOVA) for the groups of breeding lines containing *EDN2*, *R8* and *BLB2*, the control Desiree and the group of cultivars inoculated with Katshaar (a) and IPO C (b). The score present the mean percentage infection per group relative to Desiree. Groups that do not have the same letter differ significantly in level of infection.

Katshaar		
group	**score**	**sig**
BLB2	5.2	a
R8	6.0	ab
EDN2	6.8	ab
Cultivars	7.5	b
Desiree	8.0	b

IPO C		
group	**score**	**sig**
EDN2	2.1	a
R8	4.0	b
BLB2	4.0	b
Cultivars	7.6	c
Desiree	8.0	c

Significant differences between the infection levels of the isolates Katshaar and IPO C per group were analysed by doing a two sample t test. Because the data were not normal distributed it was chosen to use a non-parametric two sample t test, the Man Whitney U test. Significant influence of the isolates was found for the breeding lines containing *BLB2* ($p = 0.038$), *EDN2* ($p < 0.001$) and *R8* ($p = 0.005$). Significant higher infection levels were found for this group when inoculated with isolate Katshaar (Figure 19).

Figure 19. Results from the detached leaf assay performed with the breeding lines BIM10-132-08 containing *EDN2* (nr. 105) and RH4X-841-1 containing *R8* (nr. 97). Both breeding lines showed susceptibility for isolate Katshaar (inoculated at the right site of the leaves) and resistance for isolate IPO C (inoculated at the left site of the leaves).

3.3.2 Tuber slice assay in the laboratory

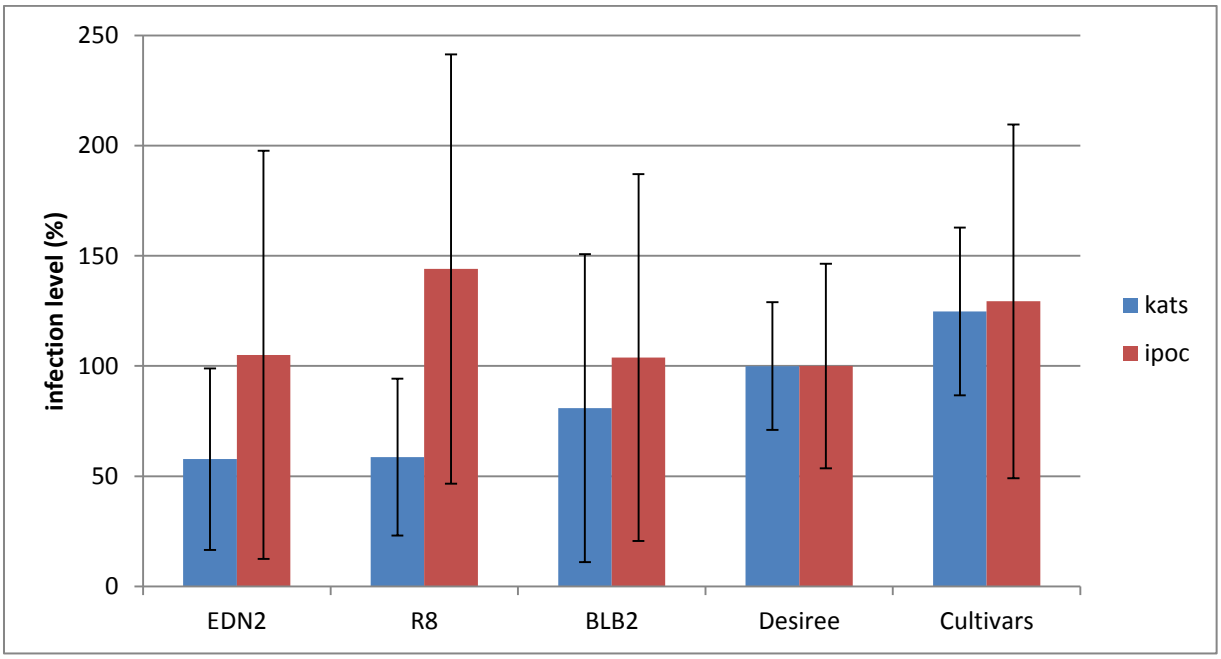


Figure 20. Results from the tuber slice assay performed with 17 cultivars and three groups of breeding lines containing the late blight *R* genes *BLB2*, *EDN2* or *R8*. The mean tuber infection level per group relative to Desiree is presented for the isolates Katshaar and IPO C.

The breeding lines containing *BLB2* and *EDN2* scored comparable infection levels, when inoculated with IPO C, compared to the susceptible control Desiree (Figure 20). The breeding lines containing *R8* and the group of cultivars showed higher infection levels compared to Desiree, but no significance was found. More variation and lower infection levels were found for the different groups when inoculated with Katshaar. An analysis of variance (ANOVA) on these scores yielded significant variation among the different groups ($F = 5.05$, $p < .001$). The breeding lines containing *EDN2* and *R8* scored a significant lower infection level compared to the cultivar group (table 8).

Table 8. Results of the tuber slice assay with an analysis of variance (ANOVA) for the groups of breeding lines containing *EDN2*, *R8* and *BLB2*, the control Desiree and the group of cultivars inoculated with Katshaar. The score present the mean percentage infection per group relative to Desiree. Groups that do not have the same letter differ significantly in level of infection.

Katshaar		
Group	**score**	**sig**
EDN2	58	a
R8	59	a
BLB2	81	ab
Desiree	100	ab
Cultivars	125	b

Significant differences between the infection levels of the isolates Katshaar and IPO C per group were analysed by doing a two sample t test. Because the data were not normal distributed it was chosen to use a non-parametric two sample t test, the Man Whitney U test. Significant influence of the isolates was found for the breeding lines containing *R8* ($p < 0.001$). For this group a significant higher infection level was found when inoculated with isolate IPO C.

Combined results for leaf and tuber infection level in the laboratory.

High variation between the tuber infection levels was found for the group of cultivars. Figure 21 shows the variation in tuber infection level for the cultivars that were found totally susceptible in the detached leaf test for isolate Katshaar. All cultivars were susceptible for the leaves (score 8) when inoculated with Katshaar, while the tuber infection level scored in the laboratory varies from 3% to 187% compared to Desiree. The cultivars Obama, Marabel, Endeavour, Leandra, Divaa and Cronos showed a higher infection level compared to Desiree. The cultivars Canberra, Masai, Laura, Lucinda and Lusa showed lower infection levels compared to Desiree. Canberra showed the lowest infection level with an infection level lower than 5% compared to Desiree. Statistical analysis between the results of the individual cultivars was not conducted, due to the low amount of repetitions per cultivar.

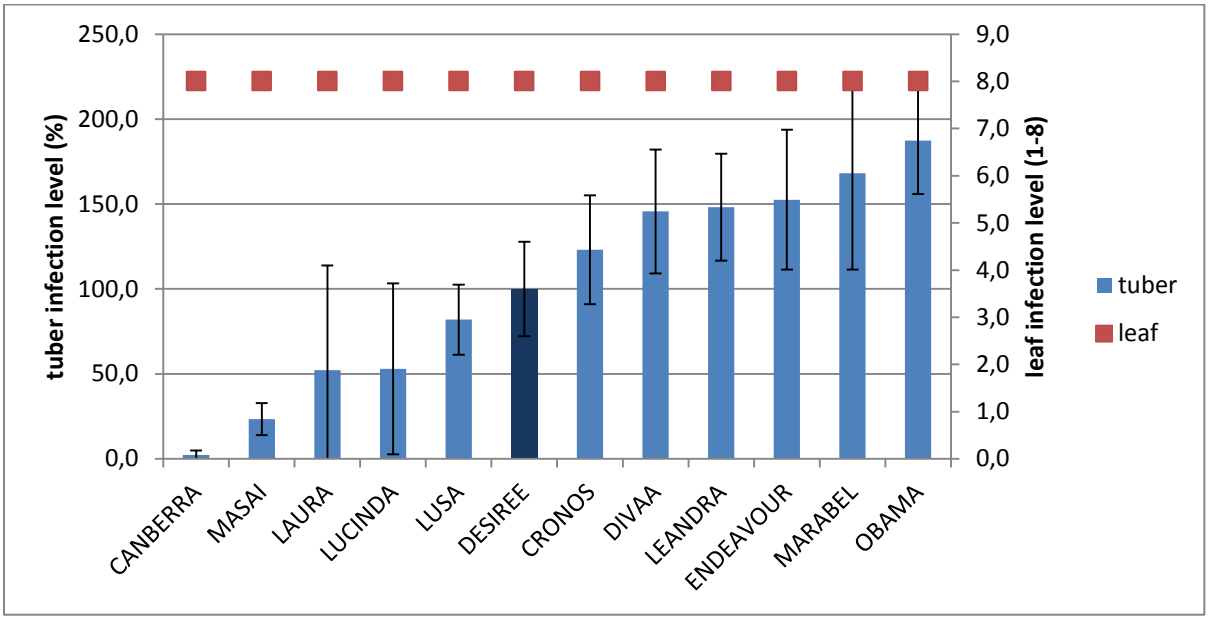


Figure 21. Combined leaf (right vertical axis) and tuber (left vertical axis) infection level for cultivars that showed complete leaf infection in the detached leaf assays. Leaf infection level: score 1 = no infection, score 8 = complete infection. Tuber infection level: in percentage relative to cv. Desiree.

3.3.3 Leaf analysis under field conditions

During 5 weeks the cultivars were observed in the field and scored for late blight infection. The differences between the genotypes were best observed at the fourth scoring moment (Figure 22). At this moment some genotypes were heavily infected, while others showed a reduced disease development or had no symptoms.

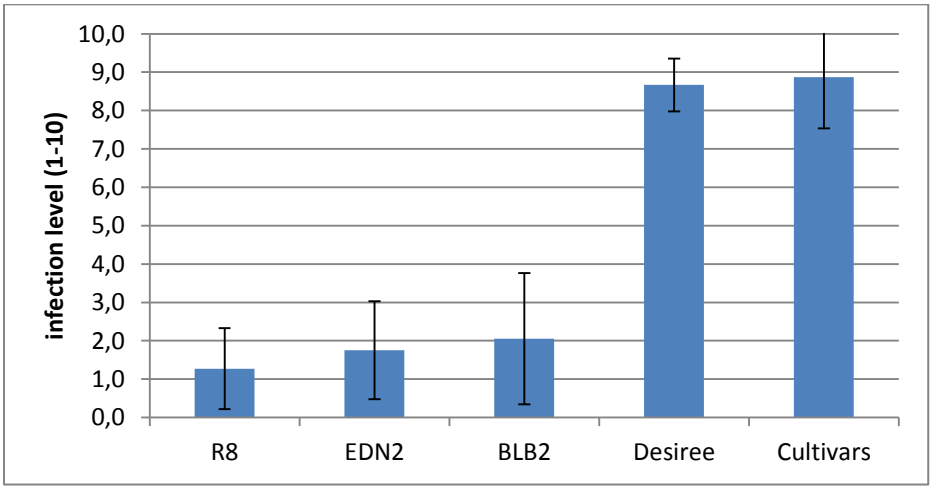


Figure 22. Results from the leaf field analysis performed with 19 cultivars and three groups op genotypes containing the late blight *R* genes *BLB2*, *EDN2* or *R8*. The mean leaf infection level per genotype was scored from 0 till 10 (score 0 = no infection, score 10 = dead due to infection)

Breeding lines containing *R8*, *EDN2* and *BLB2* scored lower infection levels compared to the cultivar group and the control Desiree. These breeding line groups scored around or below a score of 2 and were found to be significant different from the control cv. Desiree and the cultivar group ($F = 205.81$, $p < 0.001$).

3.3.4 Tuber analysis under field conditions

The tubers were harvested when the leaf analysis in the field was accomplished. The tubers were harvested and counted for the number of rotten tubers by late blight. The rest of the tubers were scored after 4 weeks of storage. The total amount of tubers was counted and a percentage infected tubers per genotype was calculated (Figure 23).

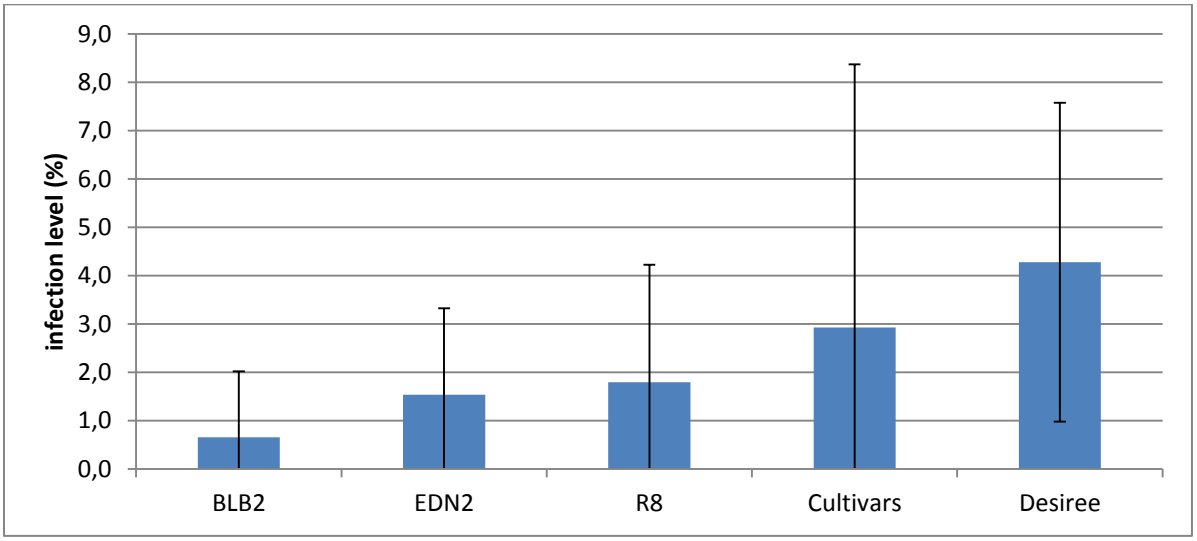


Figure 23. Results from the tuber field analysis performed with 19 cultivars and three groups of genotypes containing the late blight *R* genes *BLB2*, *EDN2* or *R8*. The mean tuber infection level per genotype was calculated as a percentage rotten tubers of the total amount of tubers.

Low infection levels were found for the different groups with only marginal variation between the groups. Desiree showed the highest infection level compared to the rest of the groups, but no significance was found ($F = 2.17$, $p = 0.078$).

3.4 Correlation

3.4.1 Correlation between the detached leaf assay in the laboratory and the leaf field analysis.

A Kendall's tau-b correlation test was done to analyse the association between the detached leaf assays in the laboratory with IPO C and the late blight scoring outdoors in the field (Figure 24 and 25). It was chosen to do the correlation test with the results from the DLA with IPO C, because in the field a comparable infection pattern was found (virulence to *R2*). In Figure 24 the results of the Desiree x construct combinations, the breeding line groups and the cultivar group were used for the correlation test. Every dot in this figure represents the average performance of these groups in the foliage lab and field tests. In Figure 25 the individual results were used of all individual genotypes tested in this study for foliage late blight resistance in the lab and field.
Linear relation was observed between leaf infection level in the field and leaf infection level in the laboratory test in Figure 24, Kendall's tau-b = 0.677, p = 0.000 (2-sided). One main outlier was found and represents the average performance of Desiree with the *CHC1* construct. This *R* gene scored in the laboratory test high infection levels, but low infection levels in the field.

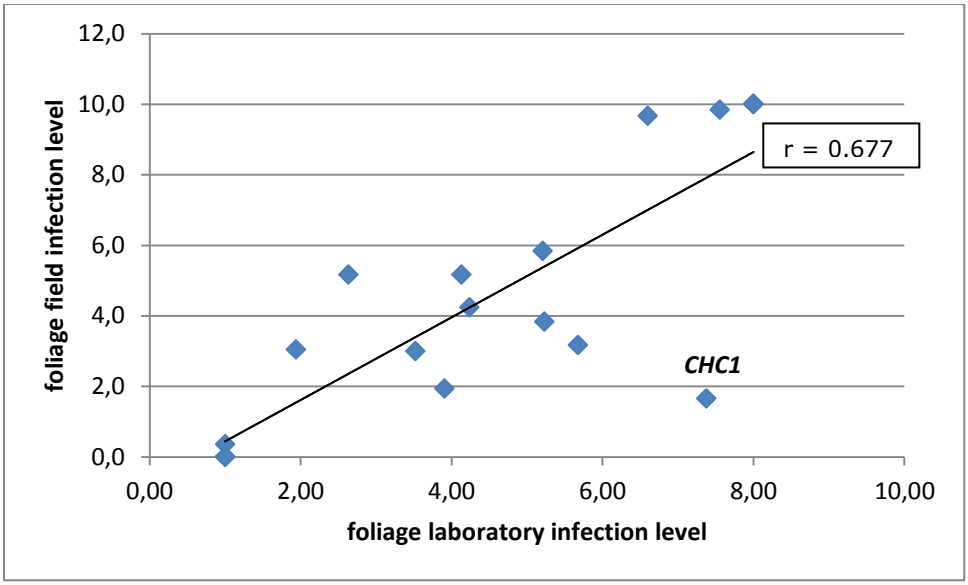


Figure 24. Correlation among the leaf field infection level (score 1-10) and leaf laboratory infection level (score 1-8) for the Desiree x construct combinations, the breeding line groups and the group of cultivars.

Linear relation was either observed between leaf infection level in the field and leaf infection level in the laboratory test when the individual genotype results were analysed, Kendall's tau-b = 0.613, p = 0.000 (2-sided). However, high dispersion was found for the individual data points. Genotypes were found that scored low infection levels in the laboratory, but relative high infection levels in the field and the other way around. Outliers are marked by coloured circles (Figure 25) and linked to the corresponding genotypes in Table 9.

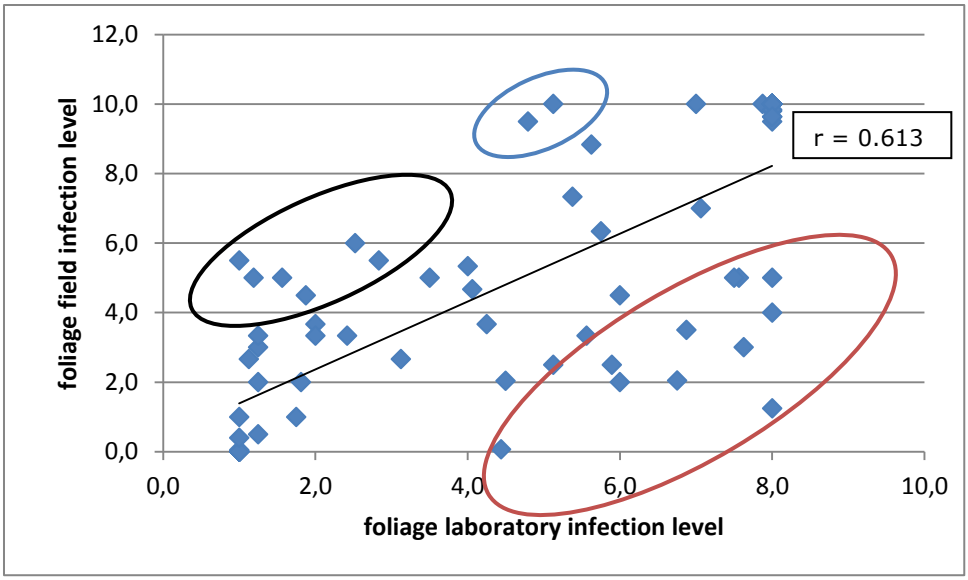


Figure 25. Correlation among the leaf field infection level (score 1-10) and leaf laboratory infection level (score 1-8) for all the results of the individual genotypes tested in this study with isolate IPO C. The coloured circles represent groups of outliers. The red circle represent genotypes that were scored with a high infection level in the laboratory, but a low infection level in the field. The blue and black circles represent genotypes that scored a relatively high infection level in the field, but a relatively low infection level in the laboratory.

	genotype	genes	lab	field
*	SARPO MIRA	*R3a, R3b, R4, R8, R-Smira*	4.4	0.1
*	A17-30	*CHC1*	8.0	1.3
*	A17-27	*CHC1*	6.8	2.1
*	IVP4X-223-13	*R8, R3a, R3b*	6.0	2.0
*	A73.1-36	*EDN2*	5.9	2.5
*	A73.1-52	*EDN2*	5.1	2.5
*	A26-1735	*BLB3+STO1 MF*	7.6	3.0
*	A26-1554	*BLB3+STO1 MF*	6.9	3.5
*	RH4X-694-05	*R8*	8.0	4.0
*	A01-105	*BLB1*	8.0	5.0
*	A09-1	*STO 1*	7.6	5.0
*	A10-67	*STO1+BLB3*	7.5	5.0
*	A01-20	*BLB1*	1.9	4.5
*	A23-21	*PTA1*	1.6	5.0
*	A26-1679	*BLB3+STO1 MF*	1.2	5.0
*	A23-68	*PTA1*	2.8	5.5
*	A09-30	*STO1*	1.0	5.5
*	A01-51	*BLB1*	2.5	6.0
*	A08-50	*R3*	4.8	9.5
*	LAURA		5.1	10.0

Table 9. Outliers of the results of the individual genotypes with colours that correspond with the data points in the circles of figure 25. The red colour represent genotypes that were scored with a high infection level in the laboratory, but a low infection level in the field. The blue and black colours represent genotypes that scored a relatively high infection level in the field, but a relatively low infection level in the laboratory.

3.4.2 Correlation between the tuber slice assay in the laboratory and the tuber field analysis.

A Pearson correlation test was done to analyse the association between the tuber slice assay in the laboratory with IPO C and the tuber late blight scoring with tubers from the field (Figure 26). It was chosen to do the correlation test with the results from the tuber slice assay with IPO C, because in the field a comparable virulence spectrum was found. Every dot represents the average infection level for a unique genotype found in the field and laboratory (including all tested genotypes). No correlation was expected, due to the fact that no significant differences were found between the tuber infection levels from the field, while significant differences were found between the results from the laboratory. No linear relation was found between the tuber infection level scored in the laboratory and the field, Pearson = 0.079, p = 0.591 (2-sided).

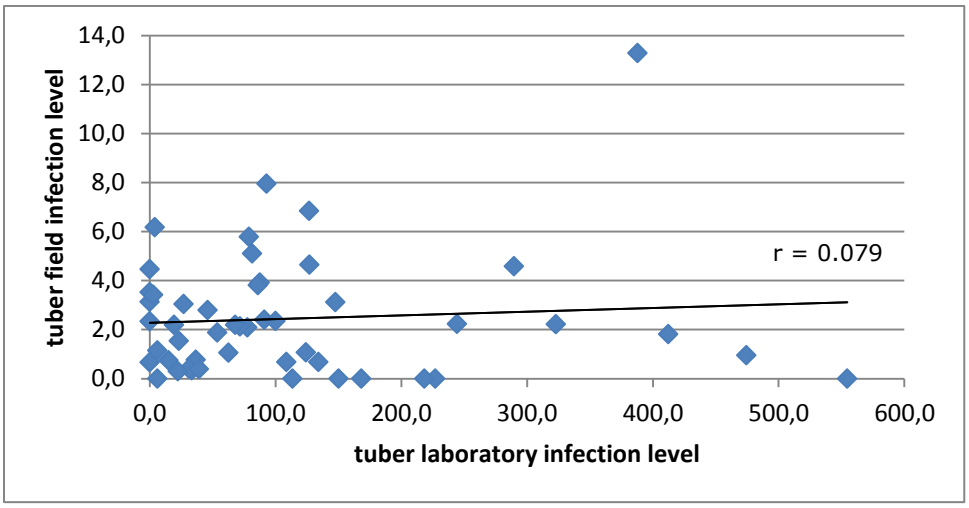


Figure 26. Association among the tuber field infection level (% rotten tubers from the total amount of tubers per genotype) and tuber laboratory infection level (% relative to Desiree) for all genotypes that were tested in this study (including the transformants).

4 Discussion and conclusion

The aim of this study was to analyse the performance of several late blight resistance genes in potato leaves and tubers and investigate the association between foliar and tuber resistance to late blight. These *R* genes were cloned before from wild potatoes and introgressed in cv. Desiree and other cultivars. This genetic modified Desiree material, harbouring single or multiple *R* genes in an identical genetic background, provided accurate characterisation under various testing methods for tuber and late blight resistance. The resistance genes were characterised in four different tests. Next to the transformants also different cultivars and breeding lines were tested. The experiences with these testing methods will be discussed later on. The results of the performance of the resistance genes will be discussed in this part and should give answer to the research questions that were stated in the introduction:

- Which genes confer resistance to both tuber and foliage late blight?
- Which genes confer foliage specific resistance?
- What is the influence of the background in which the foreign gene is introduced on the level of resistance in tuber and foliage?
- What is the influence of stacking genes on the working of the resistance genes in tuber and foliage?
- How do the field and laboratory late blight trials correlate?
- What is the influence of different isolates to the level of resistance of the material that is tested?

The resistance genes will be summarised and discussed in the following part using the results found with the detached leaf assays, tuber slice assays and the late blight analysis in the field. A low level of infection and no significance was found for the tubers from the field. That's why these results are discussed at the next part.

4.1 Transformants

4.1.1 Individual performance of the different cultivar x construct combinations

Rpi-VNT1 (*Solanum venturii*) showed a good resistance level in all testing methods as well in the foliage as in the tubers. All transformants with *VNT1* (*STO1*+*BLB3*+*VNT1*, *STO1*+*VNT1*, *VNT1*+*CHC1*, *VNT1*) showed a high level of resistance in the DLA. These genotypes were the only genotypes that scored a 1 in the DLA and no infection was found when inoculated with both isolates. It was not possible to analyse the influence of stacking *VNT1* with *STO1*, *BLB3* and *CHC1*, because *VNT1* on its own gave already the highest level of resistance. High resistance was also found for cv. Premiere, Aveka and Atlantic with the *VNT1* gene or combinations with *VNT1*, so no influence of genetic background was found. Foliage blight resistance for this *R* gene was also investigated by Foster et al. (2009). Transgenic potato carrying *Rpi-VNT1.1* were found to be late blight resistant for a broad virulence spectrum. Only one isolate from Ecuador, of the in total 11 isolates that were tested, was able to overcome *Rpi-VNT1.1.*

High resistance was also found in the tuber slice assay. All transformants with *VNT1* (*STO1*+*BLB3*+*VNT1*, *STO1*+*VNT1*, *VNT1*+*CHC1*, *VNT1*) showed a high level of resistance. These genotypes scored the lowest percentage infection of all genotypes. Total resistance was found when

the genotypes were inoculated with IPO C and only a few sporangia were found when inoculated with Katshaar. The highest infection was found with only *VNT1*. No significance was found, but it seemed that stacking *VNT1* with other *R* genes (*STO1*, *BLB3*, *CHC1*) provides a higher level of resistance. Stacking with the resistant gene *BLB3* could only have a beneficial effect when the isolate does not overcome the *R2*-gene family, such as Katshaar in this study.
Significant difference in infection level was found between the transformants of Atlantic with *VNT1* and *STO1*+*VNT1*. Atlantic with *VNT1* showed an infection level of 60%, while Atlantic with *STO1*+*VNT1* showed an infection level of 5% relative to Desiree. These results could indicate that stacking *VNT1* and *STO1* resulted in a higher level of resistance. Remarkably, Desiree with *STO1* was found to be infected with 80% compared to cv. Desiree, so the beneficial effect of *STO1* stacked with *VNT1* should be questioned.
Aveka and Desiree with *VNT1* showed an significant lower level of infection compared to Atlantic with *VNT1*, which may indicated the influence of the genetic background on the level of resistance. Differences in resistance level between transformants with the same construct, but introgressed in different cultivars could be caused by the resistance level of the cultivar. This was also observed in the results. Atlantic showed a higher infection level compared to the other cultivars, however no significance was found.
Significant higher infection levels with Katshaar were found for the genotypes with *VNT1* and *VNT1*+*CHC1* compared to IPO C in the tuber slice assay, which could indicate influence of the isolates on the infection level. Although both isolates have to express the Avr*VNT1*, difference in infection level was observed between both isolates. Probably this is caused by the aggressiveness of the isolates.
The foliage analysis in the field showed comparable results as the tuber and leaf test in the laboratory. All transformants with *VNT1* (*STO1*+*BLB3*+*VNT1*, *STO1*+*VNT1*, *VNT1*+*CHC1*, *VNT1*)showed a high level of resistance and did not differ from each other with a score of 0. Same results were found for the other transformed cultivars with *VNT1* and *STO1*+*VNT1*, however Atlantic+*VNT1* showed a slightly increased susceptibility, but was not significantly different from the others.
The experiments showed high tuber and foliage late blight resistance in the field and laboratory for the Desiree transformants with *VNT1*. The results in the field correlated well with the results found in the laboratory.

Rpi-CHC1 (*Solanum chacoense*) showed variable levels of resistance for the different tests.
High susceptibility was found when leaves of Desiree with *CHC1* were inoculated with IPO C and Katshaar. The infection level was on average scored with a 7 and was not found to be different from cv. Desiree. Stacking with *VNT1* resulted in high resistance caused by *VNT1*. Desiree with *CHC1* showed a significant higher infection level compared to Atlantic and Aveka with *CHC1*, which could indicate the influence of the genetic background.
In contrast to the DLA, high resistance was found for Desiree with *CHC1* in the tuber slice assay. A significant higher infection level was found when the tubers were inoculated with Katshaar.
The leaf analysis in the field showed like the tuber slice assay high resistance levels for the Desiree plants with *CHC1* gene. The results of the tests are conflicting with each other. The tuber slice assay and the leaf analysis in the field correlated well with each other, while the DLA did not correlate. The high concentration of spores inoculated on the detached leaves in the DLA could be an explanation for the high susceptibility found in the laboratory. However, the same or even higher spore concentration were used for the inoculation of the tubers. Apparently the tubers were resistant

against such a high concentration of spores, in contrast to the leaves. The foliage resistance of Desiree with *CHC1* in the field could be explained by a initially lower concentration of spores with a more gradual growth of the spore concentration over time.

Rpi-BLB3 (*Solanum bulbocastanum*) showed different infection levels for Katshaar and IPO C. It was expected that Desiree transformants with *BLB3* were susceptible for isolate IPO C, due to the fact that this isolate is virulent to the *R2* gene family where *BLB3* belongs to. *BLB3* is also called a functional homolog of *R2*. The results confirmed this hypothesis. Detached leaves from Desiree with *BLB3* were found to be susceptible (score 8) when inoculated with IPO C, while strong reduced susceptibility (score 3) was found when the leaves were inoculated with Katshaar. Stacking *BLB3* with *STO1* results in a considerable lower infection level for both isolates. Although no significance was found, it seems that stacking with *STO1* gives a beneficial effect on the infection level which was halved with the presence of *STO1*. The beneficial effect of *STO1* is remarkable, because Desiree with *STO1* resulted in a relatively high infection level. Apparently the combination of both *STO1* and *BLB3* resulted in a better performance compared to a single introgression of one of these genes. Desiree with *STO1* and *BLB3* resulted in a lower infection compared with the marker free form of this transformant. The marker free form is characterised by missing the Kanamycin selectable marker, which makes the potatoes cisgenic instead of transgenic. In previous *Avr*-assays it was found that *BLB3* did not work in marker free transformants, or only partially (personal communication, Jack Vossen, 2014), which can explain the higher infection levels of the marker free transformants.
The same infection pattern was found in the tuber slice assays. Desiree with *BLB3* showed a comparable infection level as cv. Desiree when inoculated with IPO C, while only 10% when inoculated with Katshaar. Desiree with *BLB3* showed a relative lower infection level in the tuber slice assay compared to the DLA. A higher infection level was observed for *STO1*+*BLB3* (27%) and *STO1*+*BLB3*MF (61%) compared to *BLB3* (10%), when the tubers were inoculated with Katshaar. Although no significance was found, it seems from these results that stacking with *STO1* had no beneficial effect on the infection level.
Significant influence on the level of infection was found for the isolates. This was expected due the virulence of IPO C to the *R2* gene family and the avirulence of Katshaar to the *R2*-gene family. Significant positive influence was found in the leaf field analysis for Desiree stacked with *BLB3* and *STO1* compared to Desiree with *BLB3*. Desiree with *STO1* and *BLB3* scored an infection level below 2, while Desiree with *BLB3* scored comparable to Desiree. This also indicates that the isolates that were present in the field were virulent to *R2*. Despite of the high susceptibility of Desiree with *BLB3*, it was found that Desiree with *STO1* and *BLB3* performed better compared to Desiree with *BLB3* or Desiree with *STO1*.

Rpi-PTA1 (*Solanum papita*), ***Rpi-STO1*** (*Solanum stoloniferum*) and ***Rpi-BLB1*** (*Solanum bulbocastanum*) will be discussed as a group, because *PTA1* and *STO1* were detected as functional homologs of *BLB1* (Vleeshouwers et al., 2008). *Rpi-BLB1* is described in literature as a broad spectrum *R* gene with race-nonspecific resistance and virulent races seem to be rare.
Desiree with *PTA1*, *STO1* or *BLB1* showed susceptibility in the DLA. However, a reduced form of susceptibility was observed compared to Desiree (a score between 2.6 and 5.4). Differences between the *R* genes were observed, but no significance was found. Desiree+*BLB1* showed significant higher infection compared to Premiere and Aveka with *BLB1*. Comparable results were found in the tuber slice assay. Desiree with *BLB1* (80%) showed significant higher infection compared to Aveka with *BLB1* (25%). These results indicated the influence of the genetic background where a *R* gene is

introgressed in.
Relative high infection levels were found for *PTA1*, *STO1* and *BLB1* inoculated with isolate Katshaar (79-81%) in the tuber slice assay. No significant difference was found between the infection levels of these *R* genes and cv. Desiree. Significant influence of the isolates was found for *BLB1* which showed a significant higher infection level when inoculated with Katshaar.
Comparable infection levels were found for the three *R* genes in the leaf analysis in the field. However, relative low infection levels were found compared to the tuber slice assay and significant difference was found with cv. Desiree. These results could indicate that *Rpi-BLB1* is a foliage specific *R* gene, confirming the results of Halterman et al. (2008).
High resistance was not found for the *R* genes *PTA1*, *STO1* and *BLB1* in the three testing methods. High infection levels were found in the tuber slice assay, while more reduced susceptibility was found in the DLA and the field, which confirmed for these *R* gene homologs that they are foliage specific. *Rpi-BLB1* was originally described as a *R* gene with resistance to a broad virulence spectrum. The resistance against a broad spectrum of isolates could be explained by the presence of *ipiO* in most late blight isolates collected worldwide. The in planta induced gene *ipiO* was found to be identical to *Avr-BLB1*, the *BLB1* avirulence protein which confers hypersensitive responsive when matching with *Rpi-BLB1*. Therefore it was concluded that *Rpi-BLB1* is a promising *R* gene for agriculture. However, it is found that introgression of *Rpi-BLB1* in a cultivated potato will influence the resistance level, the gene confers only partial resistance to aggressive isolates (Vleeshouwers et al., 2011). This is according to the results that were found in this study. IPO C and Katshaar are both observed as aggressive isolates (compared to IPO 0 and Dinteloord), which resulted in partial resistance in the DLA. The isolates that were present in the field caused also partial resistance for these genes. However, the infection levels in the tuber slice assay were relatively high and showed only a small reduced form of susceptibility compared to cv. Desiree.
These results suggest that introgression of *BLB1* or other functional homologs of this *R* gene are not sufficient to provide a proper resistance level in potato. Stacking with other *R* genes could be a solutions, which seems to be confirmed by the results of Desiree with *BLB3* stacked with *STO1*.

Rpi-MCQ1 (*Solanum mochiquense*) was mapped to a similar genetic location as *Rpi-VNT1*. Although they are homologs, it was found that *Rpi-MCQ1* and *Rpi-VNT1* have distinct recognition specificities (Smilde et al., 2005). This is according to the results found in this study. Desiree with *Rpi-MCQ1* showed comparable infection levels to cv. Desiree, while cv. Desiree with *Rpi-VNT1* showed high levels of resistance.
Significant higher infection was found when Desiree with *MCQ1* was inoculated with IPO C in the DLA. However, the results with IPO C showed high variability with big error bars. This influence of the isolate was not observed in the tuber slice assay. The tuber slice assay as well the leaf field analysis showed high susceptibility for Desiree with *MCQ1* comparable to cv. Desiree. Neither resistance for this *R* gene was found by Jones et al. (2014). *Rpi-MCQ1* did not confer resistance in the field as well as in the laboratory and showed similar results as non-transgenic control plants.

Rpi-EDN2 (*Solanum edinense*) showed for both the DLA and the tuber slice assay high susceptibility for isolate Katshaar and a reduced form of susceptibility for isolate IPO C. However, in the field *Rpi-EDN2* showed a good form of resistance with a score just above 2. The susceptibility in a laboratory test is more often experienced, while an intermediate form of resistance is often found in the field. Probably *Rpi-EDN2* cannot withstand the high spore concentration of the inoculum, which is much higher compared to the natural situation in the field. These results indicate that the detached leaf

assay in the laboratory may be not a good testing method for predicting the performance of *Rpi-EDN2* in the field. Not much information about the resistance level of *Rpi-EDN2* was found in literature, because this gene has only recently been cloned (Vetten et al., 2011)

R3a and ***R3b*** (*Solanum demissum*) performed comparable to Desiree with high susceptibility in all experiments. Sometimes even higher infection levels were found compared to cv. Desiree, but no significant difference was found. These results are in line with the description of these genes in literature (Vleeshouwers et al., 2008). *R3* has been widely used in breeding programmes, but most of the isolates are virulent to *R3a* in potato growing areas. It can be concluded that *R3a* and *R3b* on their own did not contribute to late blight resistance in this study. However, both isolates were virulent to *R3a* and *R3b*, so it is difficult to conclude about the performance of these genes with regard to tuber resistance.

4.1.2 Stacking resistance genes

Several resistance genes were characterized in this study by using different methods for testing late blight resistance in the foliage and tuber. High resistance, different levels of intermediate resistance and high susceptibility were found in this study. Rpi-*VNT1* introgressed in Desiree was the only resistance gene that showed complete resistance in all the testing methods. However, it is found that all resistance genes could be broken by isolates in the Netherlands. Rpi-*VNT1*, that showed high resistance in this study was broken down in a field trial in the Netherlands in 2014 (Geert Kessel, personal communication, 2014). However, no susceptibility was found in the field trial in Wageningen, which showed the difference in virulence spectrum per area.
The rapid break down of single resistance genes increased the importance of stacking multiple broad spectrum *R* genes(Jo et al., 2014). *P. infestans* isolates which are virulent to a new introduced *R* gene could easily grow in population size, especially in the absence of chemical control. In various crops it is proven that stacking multiple *R* genes, especially *R* genes originating from different gene-clusters with different HR reactions, is important to obtain sufficient and durable resistance (Que et al., 2010; Zhu et al., 2012). *R* gene stacking can be achieved by genetic crossing, but is in potato difficult due to the high level of heterozygosity, linkage drag and the different ploidy levels. Sarpo Mira is an example of a conventional bred cultivar with five stacked genes (*R3a,R3b,R4,R8* and Rpi-*Smira*) that has retained resistance in the field for several years (Rietman et al., 2012). Beneficial effects of stacking were found with cv. Desiree introgressed with Rpi-*BLB3* and Rpi-*STO1*, which showed clearly higher foliar resistance in the field compared to cv. Desiree with Rpi-*BLB3* or Rpi-*STO1*. These results indicate that stacked resistance genes could perform better compared to the average performance of the single resistance genes. The positive influence of stacking was less visible in the tuber slice assays, where Sarpo Mira showed a comparable infection level as Desiree when inoculated with Katshaar and comparable to *R8* when inoculated with IPO C.
Partial or field resistance to late blight can also be used for breeders as a durable form of resistance. This intermediate form of resistance, evident in many current *S. tuberosum* cultivars and relatives, is proven to be stable over long periods of cultivation (Collins et al., 1999). Partial resistance is thought to be controlled by multiple genetic loci instead of one genetic loci, which is the case for *R* genes The multi genic character avoids that new virulent late blight races can easily overcome the resistance and is called a quantitative trait loci (QTL). It has been observed that partial

resistance in potato is often associated with late plant maturity. However, late maturity is not always desired by most breeders and growers, who aim at early maturing potato plants to shorten the time of potato production (Collins et al., 1999).

4.1.3 Relation between tuber and foliage late blight resistance

The relationship between tuber and foliage late blight resistance is not clear and often and results in literature are often contradicting. Variable results were found in several studies investigating the association between foliar and tuber resistance. Expression for tuber or foliage late blight resistance depends on the *R* genes. The resistance genes *Rpi-BLB1*, *R2*, *R3a* and *R4* were found to be foliage specific (Lapwood and McKee 1961, Roer and Toxopeus 1961, Park et al., 2005, Halterman et al., 2008). The resistance genes *R1* or *R1* like, *R3b*, *Rpi*-phu1, and *Rpi*-ber worked in both the foliage and tuber (Lapwood and Mckee 1961, Park et al., 2005, Sliwka et al., 2006, Mayton et al., 2011).
A graph was made with the results of the laboratory tests and the foliage analysis of the field trial combined to get a good understanding of the relation between tuber and late blight resistance for the resistance genes in this trial (Figure 27).

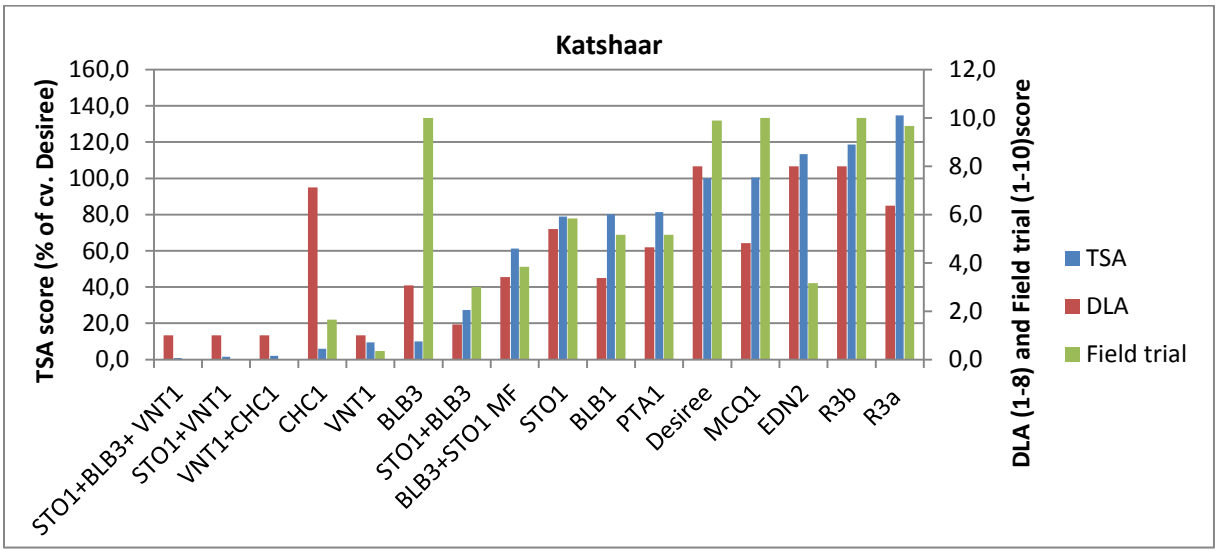


Figure 27. The relation between tuber and foliage late blight resistance for cv. Desiree introgressed with different resistance genes for isolate Katshaar. The left y-axis represent the score for the tuber slice assay (TSA). The right y-axis represent the score for the detached leaf test (DLA) and the foliage analysis of the field trial.

Rpi-VNT1 showed to be effective in both foliage and tubers under laboratory and field conditions. ***Rpi-CHC1*** showed resistance in both foliage and tubers. However, high susceptibility was found in the detached leaf test. It can be concluded that the detached leaf test is not a good method for testing foliage resistance for this gene. Up to now no information in literature was available about the relationship between foliage and tuber resistance of this gene.
Rpi-BLB3 showed a good level of tuber and foliage resistance in the laboratory tests. High susceptibility was found in the field and after inoculation with Katshaar, caused by the virulence to *R2* (homolog of *Rpi-BLB3*) in the field. From these results it can be concluded that *Rpi-BLB3* confers resistance to both tubers and foliage, dependant on the virulence spectrum of the *P. infestans* isolate. *R2* has already been used in agriculture, but has been defeated by late blight in the field. Although *R2* has been overcome, resistance has been found for local *P. infestans* population in various regions (G. Kessel, unpublished data). Probably *R2* and *BLB3* could be valuable *R* genes in the

future by stacking them with other *R* genes.

Rpi-BLB1 showed intermediate resistance for all testing methods for isolate Katshaar. However, the tuber infection levels were quite high and showed only small reduction in susceptibility. Intermediate resistance was found when the tubers were infected with IPO C. These results are more or less in line with the literature, that appointed *Rpi-BLB1* as a foliage specific gene (Halterman et al., 2008). However, *Rpi-BLB1* did not show high foliage resistance in this study.

Rpi-STO1 and ***Rpi-PTA1*** showed the same relation between foliar and tuber resistance as *Rpi-BLB1*, as expected, because they are functional homologs of *Rpi-BLB1*. Both *Rpi-STO1* and *Rpi-PTA1* showed susceptibility in the tuber slice assay, while intermediate resistance in the foliage tests in the lab and in the field. Up to now no information in literature was available about the relationship between foliage and tuber resistance of these gene.

Rpi-MCQ1 was found to be susceptible in the field and in the tuber slice assay. However, this *R* gene showed a reduced form of susceptibility for the DLA. Inoculated with IPO C showed *Rpi-MCQ1* for all tests high susceptibility. It can be concluded that *Rpi-MCQ1* does not work in the tuber nor in the foliage. Up to now no information in literature was available about the relationship between foliage and tuber resistance of this gene.

Rpi-EDN2 showed high susceptibility for the laboratory tests, but scored a good level of intermediate resistance for the foliage in the field. It is difficult to conclude something about the relationship between tuber and foliage late blight for this R gene with these results, because no reliable data is available of the tuber resistance in the field. What can be concluded is that the laboratory tests are not suitable for testing late blight resistance for *Rpi-EDN2*, because the foliage results of the lab do not correlate with the foliage results of the field. Up to now no information in literature was available about the relationship between foliage and tuber resistance of this gene.

R3a and ***R3b*** were highly susceptible in all the tests for both isolates. From literature it is known that *R3a* confers leaf specific resistance, while *R3b* confers resistance to both foliage and tubers (Park, 2005). However, it was not possible in this study to investigate the relation between tuber an leaf late blight resistance for these genes, because the isolates (IPO C and Katshaar) were both virulent to *R3*. The intention was to use also the isolates IPO 0 (virulant to *R3b*) and Dinteloord (virulent to *R1*, *R2* and *R4*), but it was chosen to stop the experiments with these isolates due to low and variable infection levels.

4.2 Cultivars and breeding lines containing *Rpi-BLB2*, *R8* or *Rpi-EDN2*

The results of all the testing methods (except for the tuber field analysis) of the breeding lines containing *BLB2*, *EDN2* and *R8*, the control cv. Desiree and the group of cultivars are summarised in Figure 28 and 29.

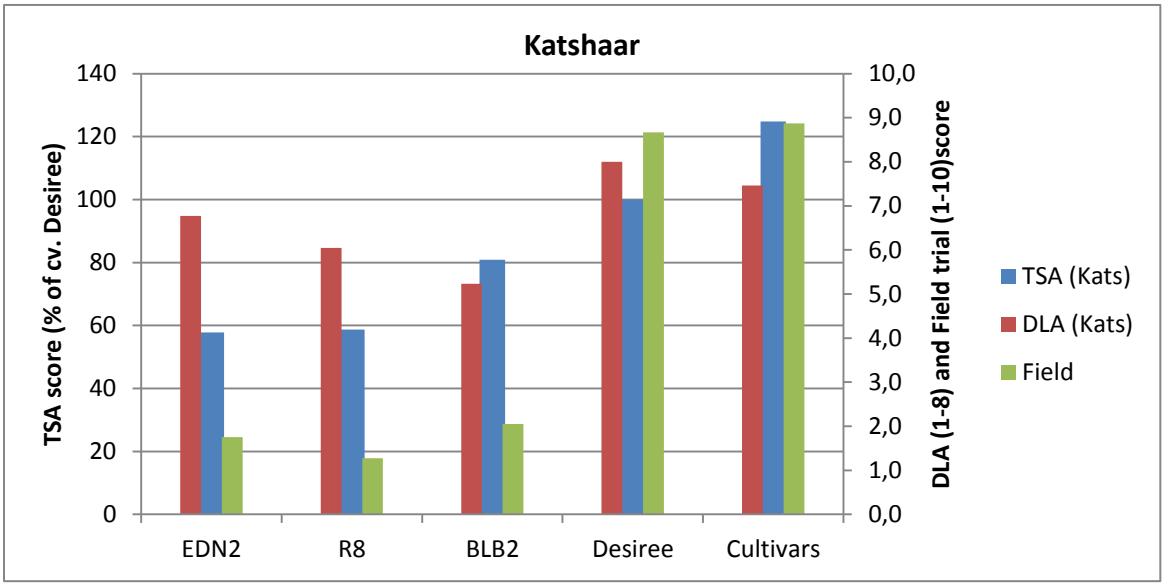


Figure 28. The relation between tuber and foliage late blight resistance for the breeding lines containing *BLB2*, *EDN2* and *R8*, the control cv. Desiree and the group of cultivars inoculated with Katshaar (Kats). The left y-axis represent the score for the tuber slice assay (TSA). The right y-axis represent the score for the detached leaf test (DLA) and the foliage analysis of the field trial.

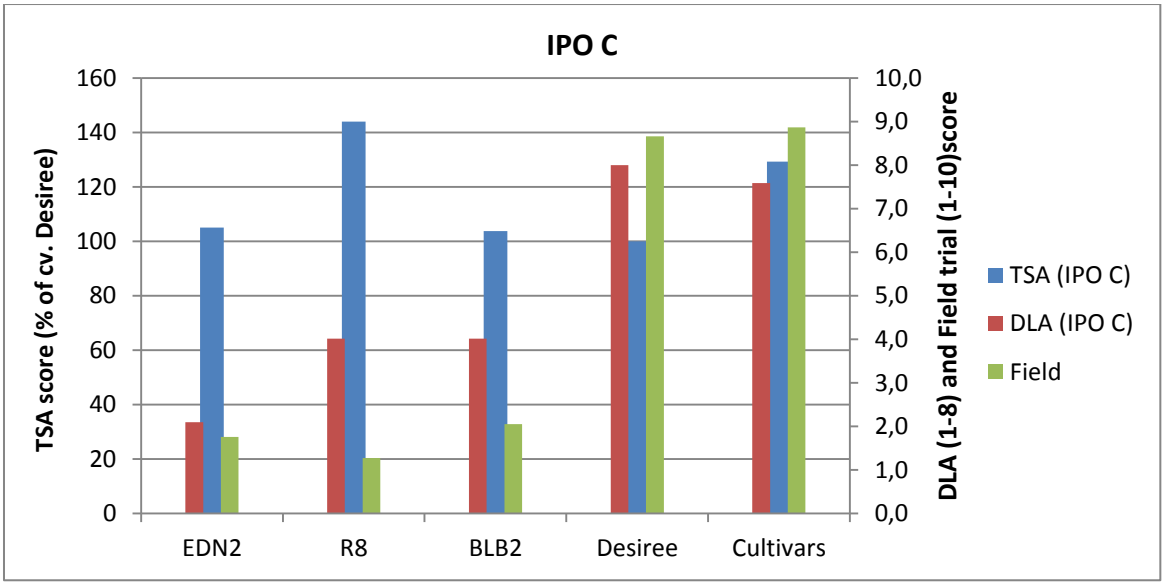


Figure 29. The relation between tuber and foliage late blight resistance for the breeding lines containing *BLB2*, *EDN2* and *R8*, the control cv. Desiree and the group of cultivars inoculated with IPO C. The left y-axis represent the score for the tuber slice assay (TSA). The right y-axis represent the score for the detached leaf test (DLA) and the foliage analysis of the field trial.

The susceptible control cv. Desiree was found to be susceptible in the laboratory assays and in the field, even as the group of cultivars. Susceptibility of the cultivar group was expected, because these cultivars contain *R* genes, such as *R1*, *R3a* and *R3b*, that do not confer resistance to the isolates used in this study; Katshaar and IPO C. However, high variation was found among the different cultivars in

the tuber slice assay. Canberra, Masai, Laura, Lucinda, and Lusa showed a reduced tuber infection in the laboratory, but were all find to be susceptible on the leaves in the laboratory and the field (Figure 21). Canberra showed an infection level of 2% compared to Desiree and Masai 23%. Low tuber infection levels (<10%) were also found when the tubers were inoculated with IPO C for the cultivars Lusa, Masai, Canberra and Laura (Appendix 4)
The reason behind the tuber resistance of these cultivars is hard to explain. The cultivars do not contain resistance genes that confer high resistance, as far is known. It is not possible that the periderm and cortical tissue cause the resistance in the tubers in the laboratory test, because the isolates were directly inoculated on the tuber slices. This tuber slice method is an ideal method for testing resistance to colonization and *R* gene recognition (Dorrance and Inglis, 1989). Although no tuber late blight specific *R* genes are known from literature, it is a possibility that unknown tuber late blight specific genes initiated the HR. An option to reveal the genetics behind the tuber resistance could be NBS profiling, where conserved motifs are used for tagging NBS-LRR type of *R* genes (Van der Linden et al., 2004). Testing the tubers with more isolates with other virulence spectra could be used for better characterisation of the genetics behind the tuber resistance.

The breeding lines containing *EDN2*, *R8* and *BLB2* performed comparable to each other when inoculated with isolate Katshaar. They showed slightly reduced susceptibility in the tubers and foliage in the laboratory, but a good level of resistance under field conditions. Comparable results were found when inoculated with IPO C, only the infection level was higher for the tuber assay, and the infection lower for the detached leaf assay compared to the results of isolate Katshaar.
It can be concluded for *EDN2, R8* and *BLB2,* introgressed in different breeding lines, that they are foliage specific, especially looking at the results of isolate IPO C. In general it was observed that these genes conferred better resistance under field conditions compared to the laboratory assays.

The genotype group containing ***EDN2*** showed susceptibility in the tuber slice assay for both isolates, but showed good foliage resistance in the field. High susceptibility was found for the DLA inoculated with isolate Katshaar, but not for isolate IPO C. Resistance to IPO C was also found by Verzaux (2010). Probably caused the higher aggressiveness of Katshaar (compared to IPO C) combined with a high spore concentration the foliage susceptibility in the laboratory. Another possibility could be that *EDN2* is a homolog to *R8*, which is defeated by isolate Katshaar. This could be an explanation, because *Rpi-EDN2* and *R8* were both mapped to the long arm of chromosome IX, even as *Rpi-VNT1*, *Rpi-MCQ1* and *Rpi-PHU1* (Sliwka et al., 2006). It is not yet clear whether *Rpi-EDN2* and *R8* represent different alleles of the same gene or whether they are different genes and have to be further investigated (Jo et al., 2011). The susceptibility of *EDN2* to isolate Katshaar can also be observed when looking at the results of the individual genotypes harbouring *EDN2* in Appendix 3.
Remarkable is that genotypes where *EDN2* was introgressed by classical breeding were found to be significantly ($p=0.000$) less infected in the DLA inoculated with IPO C, compared to the Desiree combinations with *EDN2*. It could be that the transformation events influenced the working of the *EDN2* gene, which was also found for *BLB1* (Vleeshouwers et al., 2011). The infection levels scored at the field were also found to be higher for the Desiree transformants with *EDN2* compared to the breeding lines with *EDN2*, but no significance was found ($p = 0.378$).
Based on the results with *EDN2*, it is may be better to test the functionality of *EDN2* in the field, especially for the transformants, to get reliable information for agricultural purposes.

Remarkable is the higher infection levels that were found in the tuber assay for the breeding lines containing ***R8***, when inoculated with IPO C. Isolate IPO C is not virulent to *R8* in contrast to isolate Katshaar that is virulent to *R8*. Looking at these virulence spectra it was expected that the breeding lines containing *R8* showed higher infection levels when inoculated with Katshaar. However, the opposite was found for the tubers. *R8* did not confer resistance in the tubers when inoculated with IPO C and showed infection levels higher than 100% compared to cv. Desiree. However, intermediate resistance was found in the DLA and only marginal infection in the field. Apparently, *R8* was less functional in the tuber as the foliage. The field resistance could be caused by *R8*, because it was not known which virulence spectra was present at the field. The results of the detached leaf assay were more in line with the expectations. Breeding lines with *R8* showed significantly higher infection levels when inoculated with Katshaar compared to inoculation with IPO C, caused by the virulence of Katshaar to *R8*.

BLB2 showed even as *EDN2* and *R8* a good level of resistance under field conditions. However, *BLB2* did not confer tuber resistance in this study and reduced susceptibility was found in the detached leaf assay in the laboratory. *BLB2* is a valuable gene for breeding according to the literature, because this *R* gene confers resistance to most of the *P. infestans* strains (Van der Vossen, 2005). The organic cultivars Bionica, and Toluca are a result of 46 years of breeding efforts were *Rpi-BLB2* was introgressed by classical breeding (Vleeshouwers et al., 2011). The breeding process of introgressing valuable characters of wild Solanum species is time consuming, because most of the wild Solanum species are diploid (2n = 2x) and cannot be hybridised directly with *S. tuberosum* cultivars that are tetraploid (2n = 4x). The resistance of *S. bulbocastanum* has been transferred to *S. tuberosum* through several bridge crosses with *S. acaule* (2n = 4x), *S. phureja* (2n = 2x), *S. bulbocastanum* and *S. tuberosum* (Hermsen & Ramanna, 1973; Lammerts van Bueren et al., 2008). Several backcrossings were performed to diminish linkage drag. In general it takes three to four back-cross generations of four to five years to create parent lines for that can be used in a breeding program (Tiemens et al., 2013). Another possibility is by using unreduced pollen. Some diploid wild species produce double haploid pollen which can be observed under a microscope. By using these unreduced pollen, crosses can be made with tetraploid *S. tuberosum* species. In this way the bridgecrosses can be left and time is saved.

4.3 Correlation analysis

Significant correlation was found between the data of the leaf analysis from the field and the detached leaf test inoculated with IPO C. High association means that the results of a detached leaf test in the laboratory are predicting for the performance under field conditions. However, several outliers were found, especially for the correlation rank test with the results of the individual genotypes. One outlier was found in the correlation test with the grouped results for the Desiree x construct combinations, breeding lines containing *BLB2*, *EDN2* or *R8*, and cultivars. The transformed Desiree plants with *CHC1* showed high infection levels in the laboratory, while low infection levels in the field. It can be concluded from these results that the detached leaf test is not the ideal testing method for analysing leaf resistance for transformants with *CHC1*.

High dispersion of the data points was observed when looking at all individual genotype results. However, this dispersion did not influence the correlation coefficient, compared to the correlation test with the averaged data points. Outliers were observed that showed low infection levels in the field, but high infection levels in the laboratory, such as *CHC1*, *EDN2* and *R8*. From these results it could be concluded that testing genotypes harbouring one of these genes in the laboratory is probably not predicting for the performance under field conditions. Next to these genes, also genotypes were found containing other *R* genes, such as *BLB1, BLB3+STO1* MF and *STO1*. However, these genotypes were either found in the group of outliers that scored low infection in the laboratory, but relatively high infection under field conditions, indicating the variability between genotypes harbouring the same *R* gene(s) in this study.

Cultivar Laura showed low infection levels in the laboratory, while high infection levels were found in the field. These results are hard to explain, because it can be assumed that the spore concentration in the field are lower compared to the spore concentrations used in the laboratory.

Next to these extreme outliers also a bunch of data points were found that showed a comparable field infection level, but great variation in the laboratory. These data points scored around 3 for the field infection level, but varied between 1 and 6 for the laboratory infection level.

The results of the correlation rank tests implies that the results from this study in the laboratory are predictive for a certain level for the results in the field, but not for all genotypes and testing in the field remains important to provide a reliable *R* gene characterisation for agricultural use, especially for some specific *R* genes.

4.4 Discussion material and methods

4.4.1 Detached leaf assay in the laboratory

The detached leaf assay was completed in a relatively short time with good results. Young fresh leaves were cut from the field and inoculated with the isolates IPO C and Katshaar in the laboratory. The leaves were screened one week after inoculation. Clear infection was observed for Desiree and resistance was observed by clear HR spots. Correlation was found between the results of the DLA and the analysis of foliar late blight resistance in the field. This indicates that the resistance level found in a DLA gives a proper prediction for the resistance level in the field. However, multiple outliers were found that showed good resistance in the field, but only marginal resistance in the detached leaf assay, like *EDN2* (especially for the transgenics) *, R8, CHC1* and to a lesser extent for *BLB2*. It seems important to test the material on leaf late blight resistance also under field conditions to give a proper validation for agricultural use.

4.4.2 Tuber slice assay in the laboratory

Different methods are described in literature for testing tuber late blight resistance. Park et al. (2005) tested three different methods to find an optimal method for the determination of tuber blight resistance. They compared the results of a wounded tuber assay, described by Roer and Toxopeus (1961), with the tuber slice methods, described by Dorrance and Inglis (1998). The wounded tuber assay was performed by making a hole in an intact tuber and the inoculum was applied in the wound. No difference in resistance outcome was found between these methods. For this study it was chosen to use the tuber slice assay. With this method slices of about 1 cm were made of the middle of whole tubers. The spores were inoculated on the middle of the slices on the flesh of the tuber. *R* gene recognition can be perfectly determined with the tuber slice method; whereas a whole-tuber assay is more suitable for the evaluation of infection of the periderm and cortical tissues (Swiezynski, 1991; Pathak and Clarke, 1987; Davila, 1964)

A protocol was used based on tuber experiments from Wageningen UR in 2013 (unpublished data). Different problems were faced in the beginning of the experiments, but also later on. The biggest problem was failure of infection of the tuber slices and especially the susceptible control Desiree. No infection, or only a few spores were detected when the first trays were analysed. This continued for a couple of trays and it was decided to adjust the protocol with the goal to get a successful infection. A small experiment was conducted with multiple susceptible cultivars and multiple spore concentrations. The goal was to find the ideal spore concentration for successful infection and to find a susceptible control which was really susceptible. Although not much infection was found in this trial, it was chosen to adjust the protocol at some points.
The amount of water in the tray was enlarged from 70 ml to 400 ml to provide a higher relative humidity in the trays. The blotting paper was folded on an artificial piece of grass which prevented the tubers from rotting, that could be caused by the higher water level. The spore concentration in the inoculum was doubled. Also the amount of days after inoculation for analysing the infection level was enlarged to stimulate a higher infection level. Analysis of the infection level was prescribed for seven days after inoculation in the protocol. The amount of days after inoculation was increased to nine days after inoculation for the isolate IPO C and eight days after inoculation for the isolate Katshaar. This was done due to the difference in aggressiveness between the isolates. It was experienced that isolate Katshaar showed a higher aggressiveness and was more vulnerable for

rotting compared to IPO C. A couple of times it was found that the time between inoculation and analysing was too long for isolate Katshaar and rotting was already started. As a result of the rotten tubers the counting of the sporangia became more difficult, because the starch cells were washed of together with the spores. It was then difficult to differentiate between sporangia and starch cells. It seemed that also the appearance of the inoculum had influence on the infection level. It was experienced that the isolates increased a bit in aggressiveness after being transferred on rye agar for a couple of times.

Higher infection levels were observed, due to the adjustments in the testing method and the increase in aggressiveness. However, large variation was observed within the infection levels of Desiree (Figure 30), but also in other genotypes. Because it was chosen to relate the results of the genotypes to the results of cv. Desiree of the corresponding tray, it was possible to compare genotypes from different trays with each other. However, trays with a too low infection level or with huge variances within Desiree were discarded and these tests were repeated. After a while it was decided to continue with the isolates Katshaar and IPO C and quit the experiments with IPO 0 and Dinteldoord, due to too low and too variable infection levels for Desiree with these isolates. It can be concluded from these experiences that not all isolates are suitable to perform tuber slice assays with, because of differences in virulence and aggressiveness to tubers. The difference between aggressiveness among Katshaar and IPO C is visible in Figure 30, where IPO C produced considerable lower amounts of sporangia compared to Katshaar. High variation is visible for the amount of sporangia counted on Desiree slices between the trays inoculated with the same isolate.

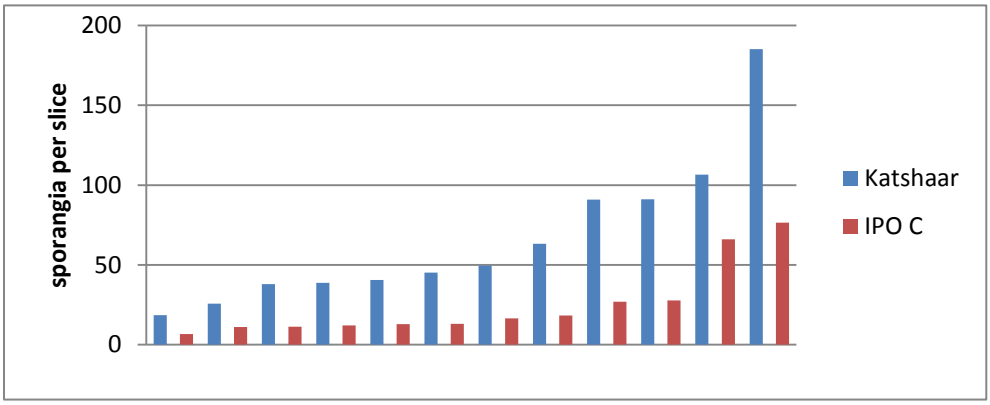


Figure 30. The mean amount of sporangia (per 4 squares of a counting glass) on the tuber slice of Desiree for 24 trays that were tested with IPO C or Katshaar. Each column represents one tray.

One could concluded at the end of this study that perhaps cv. Desiree is not the optimal susceptible control for a tuber slice assay. Several cultivars in this study showed a higher infection level compared to Desiree as is visible in Appendix 4. Because most of the transformants were made with Desiree, it was chosen to continue with Desiree in this study as a control.
It is hard to detect the cause of the high variability between the infection levels of the same genotype in one tray, but also between trays. More studies were confronted with the problem to obtain reliable data for tuber blight in the laboratory and the field (Darsow, 1987; Doster et al., 1990; Dorrance and Inglis, 1998). Darsow (1987) studied tuber late blight for 12 years using the tuber slice method. The test results showed variations of up to six scores in a 9-score scheme, which illustrates the high variability. It is suggested that the tubers should be tested for multiple years to come up with reliable results.

Lower variation within genotypes was detected for the detached leaf assay. An explanation could be that the tuber slice assay is analysed in a quantitative manner, while the detached leaf assay is analysed in a visual manner. Perhaps either high variation is found for the infection levels in a DLA when the infection levels are analysed in a quantitative way, thus by counting sporangia.
Remarkable is that this study is different in performing tuber analysis by doing a tuber slice assay compared to other studies. Several studies are reported that made use of the tuber slice assay for the investigation of tuber blight resistance (Dorrance and Inglis, 1998; Mayton, 2010; Mayton, 2011). However, the tuber slices were all rated visually for percentage of surface area that was infected or on a scale from 1-9. It is experienced in this study that visible mycelium not always is associated with high amounts of sporangia. That's why analysis on a quantitative manner is probably a more reliable and accurate way to determine tuber infection. However, the results of the tuber slice assays performed by Darsow (1987) were either variable and not reliable. It may can be concluded at the end of this study that there is not jet an optimal method for testing tubers on late blight resistance.
Variable results were also found between different clone numbers with the same construct, which was also found with the correlation rank test. Examples can be detected from Table 10. Clone number A01-105 showed for example high susceptibility for IPO C, while clone numbers A01-20 and A01-51 showed a good level of resistance. The same pattern was observed for isolate Katshaar and to a lesser extent for the tuber scores. Variation between clone numbers was observed for more constructs, such as *STO1*, *STO1+BLB3* and *MCQ1*. Sometimes the results of the laboratory tests correlated well with the field score, like for the clone numbers with *STO1+BLB3*. Sometimes no correlation is found with the field scores, such as the clone numbers with *STO1*.
Difference in infection levels between genotypes of the same cultivar x construct combination could be caused by different expression levels of the *R* genes. By marker analysis it can be checked whether or not an *R* gene is present. With expression analysis it is possible to analyse the expression level of a gene and these results could be linked to resistance levels of a clone. The expression results could help a breeder with the selection of clones. Analysis of the expression levels of the *R* genes in the constructs is not used in this study, because the expression data of the transformants with variable data were not present.

Table 10. The results of the foliage and tuber laboratory assays for some Desiree clone numbers. Variation was found between the infection levels of different clone numbers harbouring the same resistance gene. The scores of the tuber assays (TSA) are relative to cv. Desiree. The leaves (DLA) were scored from 1-8 (1 = no infection, 8 = highly infected). The leaves were scored from 0-10 in the field (1 = no infection, 10 = highly infected).

clone nr	gene	Katshaar TSA (%)	IPO C TSA (%)	Katshaar DLA leaf	IPO C DLA leaf	Field leaf score
A01-105	*BLB1*	98	71	5.4	8.0	5
A01-20	*BLB1*	69	29	2.1	1.9	4.5
A01-51	*BLB1*	74	38	2.5	2.5	6
A09-1	*STO1*	78	51	7.6	7.6	5
A09-30	*STO1*	76	60	1.0	1.0	5.5
A09-7	*STO1*	83	122	7.6	7.1	7
A10-43	*STO1+BLB3*	0	39	1.0	1.3	2
A10-48	*STO1+BLB3*	7	114	1.1	1.8	2
A10-67	*STO1+BLB3*	75	91	2.3	7.5	5
A31-1	*MCQ1*	104	84	1.0	8.0	10
A31-46	*MCQ1*	98	93	5.4	8.0	10
A31-5	*MCQ1*	99	85	8.0	8.0	10

The high variation between transformants with the same construct, but also within the results of one clone number resulted in large LSD (Least Significant Difference) values. Due to these large LSD's it is possible that no significant difference between Desiree x construct combinations was found, while large differences in mean values were observed. The number of replications also plays a role in the LSD values. More replications in a study would lower the LSD values in general.
At the end of this study it could be concluded that the tuber slice assay is a time consuming and hard method to investigate tuber blight resistance. Variable data were obtained for the isolates Katshaar and IPO C. However, by testing multiple times in multiple years it is possible to obtain sufficient data to make reliable conclusions about tuber blight resistance.
Last year (2013) several Desiree x construct combinations were either tested for tuber late blight resistance, by performing tuber slice assays with isolate IPO C. That makes it possible to compare the results of 2013 with the results of this study, for Desiree combination that were tested in both years with IPO C (Figure 31). Significant correlation was found between tuber infection data of 2013 and the infection data from this study for several Desiree transformants inoculated with IPO C, Kendall's tau-b = 0.662, p = 0.000 (2-sided). The *VNT1* transformants show good resistance in both years. Comparable infection levels were found for *CHC1*, *PTA1* and *BLB3*. Desiree with *STO1*, *R3b* and *STO1+BLB3* showed higher infection in 2013, but in both years susceptibility was found. The results of *BLB1* in 2013 are more in line with the results of *PTA1* and *STO1* with infection levels around 80% (also found for isolate Katshaar in this study). Probably a too low infection level for *BLB1* was found in this study. The higher infection level of *BLB1* is also more in line with the statement in literature that *BLB1* is a foliage specific *R* gene, and does not confer resistance to the tuber. The difference in results for *BLB1* between 2013 and 2014 indicates the importance for testing multiple time over multiple years.

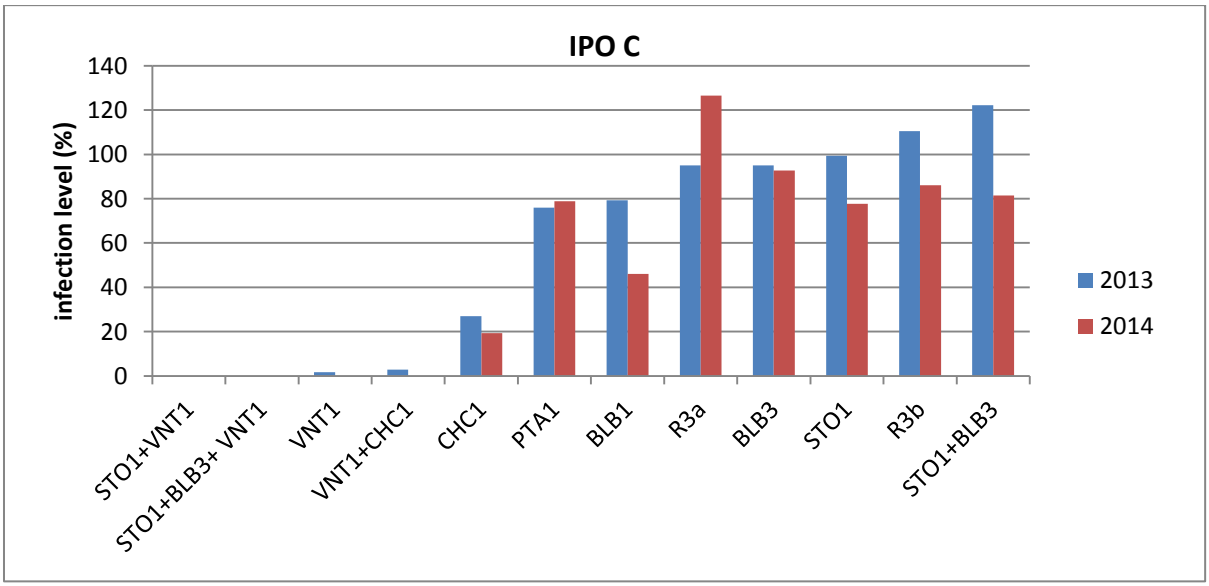


Figure 31. Tuber infection data for Desiree transformants with one or multiple genes obtained from tuber slice assays in the laboratory for 2013 and 2014. The infection level (%) is relative to cv. Desiree.

4.4.3 Foliar analysis in the field

Formally the late blight trial field had to be infected at the first of July. Due to the high late blight pressure in 2014, naturally infection was already noticed before the first of July and it was chosen to skip the inoculation with IPO C. Inoculation of the already infected field with IPO C would probably not have influenced the infection of the field, because most actual isolates are more aggressive than

IPO C and would displace IPO C quite soon (Geert Kessel, personal communication, 2014).
At the beginning of July the border rows of Bintje showed first late blight infection, and from that moment onwards started the weekly evaluation. Later on infection was also found on genotypes with *Rpi-BLB3*, which indicated that the late blight isolates were virulent to *R2* (just as IPO C). Almost all isolates that are currently found in the Netherlands break through *R2* or genes of this family such as *Rpi-BLB3* (Geert Kessel, personal communication, 2014).
The late blight infection went well and after six weeks it was decided to stop the analysis. Several genotypes were completely dead at that time, due to late blight infection. The field was sprayed to kill the foliage before the potatoes were harvested for the tuber analysis.

4.4.4 Tuber analysis in the field

The tubers were harvested two weeks after spraying. All tubers of one plot (6 plants)were harvested and the number of rotten tubers was counted. Rotten tubers caused by late blight infection were clearly visible, especially for the highly susceptible cultivars Bintje and Premiere. The tubers were stored to stimulate rotting of already infected tubers without visible symptoms. The cultivars and breeding lines were analysed four weeks after harvest. The transformants were analysed about seven weeks after harvest.

Not much rotten tubers were found, even for the tubers that were stored for seven weeks. The transformants as well as cv. Desiree showed a low infection level (<8%) and no significant difference was found between the results of the transformants. Only cv. Bintje (20% infected tubers) and cv. Premiere (30% infected tubers) showed higher infection levels. Correlation analysis showed no correlation between the results of the tuber slice assay in the laboratory and tuber late blight analysis with tubers from the field. Also no correlation was found for the results between foliage and tuber results of the field. These results indicated that more factors, than only the presence of *R* genes, have an influence on the tuber infection levels in the field (Dorrance and Inglis, 1998). The tubers were directly inoculated on the flesh in the laboratory, while tubers in the field are protected by the periderm and cortical tissue, which could influence the infection level of the tubers. Next to that, tubers in the field are protected by the ridge and the location in the ridge influences the chance to become infected. Tubers that are located deeper in the ridge are less vulnerable for late blight infection compared to tubers that are more located at the surface of the ridge. Tubers from susceptible genotypes could in this way escape from infection. The susceptibility of the leaves of a genotypes has also influence on the amount of spores that can reach the tubers. It is known that tubers can become infected when sporangia and spores are washed from the leaves into the soil. Another possibility is that the disease is growing down to the lower stem, through the stolon's to the tubers. Tubers of susceptible genotypes are in this way exposed to much higher spore concentrations compared to tubers of resistant genotypes and a proper comparison between genotypes becomes difficult. Looking at those factors that prevent tubers from infection by late blight in the field, it can be wondered whether *R* genes play a major role in tuber late blight resistance. It was observed that Desiree combinations, breeding lines and cultivars containing one or more *R* genes showed low tuber infection in the field, in contrast to highly susceptible cultivars without *R* genes, such as Premiere and Bintje. However, the cultivar Desiree, that contains no *R* genes and was used as susceptible control in the laboratory assays, did neither show high infection levels and no significant difference was found between Desiree and Desiree x construct combinations.
Perhaps it can be concluded that *R* genes, conferring tuber resistance in the laboratory, are also

functional in the field. However, several other discussed factors probably play a bigger role in preventing tubers to become infected by late blight in the field.

In general it can be wondered if this method of testing for tuber blight resistance under field conditions could be successful in the future. Very low infection levels with marginal differences between genotypes were found. No correlation was found with the other testing methods, such as the tuber slice assay and the leaf field analysis. However, Dorrance and Inglis (1998) reported several studies from before 1996 that found some degree of correlation between tuber lab and field results. Mayton et al. (2009) found, even as in this study, little or no tuber blight infection in the field and no correlation was found between foliar and tuber late blight infection. However, they found difference in blight severity between years of testing. This indicates that field trials have to be done multiple times to make proper conclusions about the results and the method to test for tuber resistance.
It can be wondered, based on the results of the tuber field analysis, whether it is necessary to store the tubers in following field experiments. Harvesting the tubers in boxes and store them is time consuming. In total 373 (3% of total harvested tubers) rotten tubers were found in the field for the cultivars and breeding lines. The infection after storage was found to be lower, and only 133 (1% of total harvested tubers) rotten tubers were found in the second scoring moment. Relatively more rotten potatoes were counted for the transformants. 496 (3.3% of total harvested tubers) rotten tubers were counted in the field, while 255 (1.7% of total harvested tubers) rotten tubers were scored after storage. These results show that relatively more rotten tubers after storage are observed when the tubers were stored for seven weeks, instead of four weeks. It may can be concluded that storage of the tubers is useful when the time of storage is used of at least 7 weeks. It can be tested in further research whether a longer storage time is benefitting the tuber blight infection in the boxes.

4.5 Summary

At the end of this study some conclusions can be made about the performance of the different *R* genes tested in this study and the testing methods that were used.
High variation in resistance was observed between the different transformants:
- *VNT1* showed high tuber and foliage resistance in the laboratory tests and under field conditions.
- *CHC1* showed tuber resistance, but susceptibility in the foliage in the laboratory tests. However, foliage resistance was observed in the field.
- *BLB3* showed isolate-specific resistance for both the tubers and foliage.
- *BLB1, STO1* and *PTA1* showed foliage specific resistance, with intermediate resistance in the foliage and almost complete susceptibility in the tubers.
- *EDN2* showed a good level of resistance in the field, but was susceptible in the tuber and foliage in the laboratory tests.
- *MCQ1, R3a* and *R3b* were susceptible in both foliage and tubers.
Influence of stacking *R* genes was observed for the combination of *BLB3+STO1*, which performed better compared to *BLB3* or *STO1.* Influence of the genetic background for the transgenic potatoes was observed by comparing several cultivar x construct combinations containing the same *R* gene.

Next to the transformants also a set of cultivars and breeding lines were screened for late blight resistance in the foliage and tubers. Although most of the cultivars were susceptible in both foliage and tubers, some cultivars were found with tuber specific resistance. Breeding lines with *BLB2* showed foliage resistance in the field, but tuber susceptibility and partial foliage resistance in the laboratory. *R8* and *EDN2* showed either a good level of foliage resistance in the field, but showed susceptibility in the tubers and isolate-specific foliage resistance in the laboratory.
In general the tuber and foliage late blight analysis went well. However, only marginal variation in tuber infection was found between the genotypes in the field. No correlation was found between the results of the tuber tests in the laboratory and the field analysis. However, significant correlation was found between the foliage test in the laboratory and the foliage analysis in the field. Testing under field conditions remains important to differentiate genotypes that are susceptible in the laboratory, but resistant in the field, such as *EDN2*, *R8* and *CHC1*.
Although good infection levels were observed during the experiments, it is suggested to repeat the experiments, because variation was observed in the different tests within the results of genotypes.

5 Appendix

Appendix 1. The transformants, cultivars and breeding lines used in this study.

Transformants

CLONE	GENES		CLONE	GENES
DESIREE (A)	geen		PREMIERE (B)	*R10*
A01-20	*BLB1*		B01-16	*BLB1*
A01-51	*BLB1*		B01-126	*BLB1*
A01-105	*BLB1*		B01-129	*BLB1*
A03-142	*BLB3*		B13-2	*VNT1*
A04-33	*R3a*		B13-16	*VNT1*
A08-41	*R3a* MF		B13-33	*VNT1*
A08-50	*R3a* MF			
A09-1	*STO1*		AVEKA (C)	*R3*
A09-7	*STO1*		C01-6	*BLB1*
A09-30	*STO1*		C01-10	*BLB1*
A10-43	*STO1+BLB3*		C01-19	*BLB1*
A10-48	*STO1+BLB3*		C13-1	*VNT1*
A10-67	*STO1+BLB3*		C13-11	*VNT1*
A13-13	*VNT1*		C13-12	*VNT1*
A13-17	*VNT1*		C17-4	*CHC1*
A13-28	*VNT1*		C17-5	*CHC1*
A14-81	*STO1+BLB3+ VNT1*			
A14-83	*STO1+BLB3+ VNT1*		ATLANTIC (H)	
A14-97	*STO1+BLB3+ VNT1*		H15-2K	*VNT1* MF
A16-2	*STO1+VNT1*		H15-5K	*VNT1* MF
A16-38	*STO1+VNT1*		H15-7K	*VNT1* MF
A16-95	*STO1+VNT1*		H43-2K	*STO1+VNT1* MF
A17-27	*CHC1*		H43-4K	*STO1+VNT1* MF
A17-30	*CHC1*		H49-3P	*CHC1* MF
A19-67	*VNT1+CHC1*		H49-13P	*CHC1* MF
A19-96	*VNT1+CHC1*			
A19-99	*VNT1+CHC1*			
A23-11	*PTA1*			
A23-21	*PTA1*			
A23-68	*PTA1*			
A25-11	*R3b*			
A25-12	*R3b*			
A25-14	*R3b*			
A26-1554*	*BLB3+STO1* MF			
A26-1679*	*BLB3+STO1* MF			
A26-1735*	*BLB3+STO1* MF			
A31-1	*MCQ1*			
A31-5	*MCQ1*			
A31-46	*MCQ1*			
A73.1-5	*EDN2*			
A73.1-36	*EDN2*			
A73.1-52	*EDN2*			

Cultivars and other genotypes

CLONE	GENES
LEANDRA	*R3a R3b*
ANUSCHKA	*R3a*
VOYAGER	*R2+R3a+R3b*
LUCINDA	*R3b*
LADY ANNA	*R3a*
CRONOS	*R1+R3a+R3b*
DIVAA	*R1*
ENDEAVOUR	*R1*
OBAMA	*R3a*
MARABEL	*R3a+R3b*
LUSA	*R3b*
LAURA	
CANBERRA	*R3b R1*
MASAI	
EVOLUTION	*R2*
BIONICA	*BLB2+R2+R3a+R3b*
TOLUCA	*BLB2+R3a*
BIM09-001-01	*BLB2+R2*
BIM09-043-02	*BLB2*
IVP4X-116-14	*BLB2+R2*
IVP4X-271-09	*BLB2+R2+R3a+R3b*
BIM10-050-09	*BLB2*
BIM10-067-08	*BLB2*
BIM10-072-04	*BLB2*
BIM10-082-07	*BLB2*
RH4X-667-01	*R8*
RH4X-694-05	*R8*
RH4X-715-06	*R8 R3a*
RH4X-839-03	*R8*
RH4X-841-01	*R8*
RH4X-855-06	*R8*
SARPO MIRA	*R3a R3b R4 R8 R-Smira*
IVP4X-223-13	*R8 R3a R3b*
BIM09-012-01	*R8 R3a*
BIM09-018-01	*R8 R2*
BIM10-109-01	*EDN2*
BIM10-128-05	*EDN2*
BIM10-132-08	*EDN2*
BIM10-133-03	*EDN2*
BIM10-134-01	*EDN2*
BIM10-135-02	*EDN2*

Appendix 2. Infection data for all tested material.

TSA = tuber slice assay (% infection of Desiree), DLA = detached leaf assay (1-8) with 1 =no infection and 8 = heavy infection, FIELD LEAF = leaf late blight analysis in the field (1-10) with 1 = no infection and 10 = heavy infection.

		TSA	DLA	FIELD LEAF	TSA	DLA	
clone	**genes**	KATS	KATS		IPO C	IPO C	**clone**
A01-105	*BLB1*	98	5.4	5.0	71	8.0	A01-105
A01-20	*BLB1*	69	2.1	4.5	29	1.9	A01-20
A01-51	*BLB1*	74	2.5	6.0	38	2.5	A01-51
A03-142	*BLB3*	10	3.1	10.0	93	8.0	A03-142
A04-33	*R3a*	100	8.0	10.0	115	7.0	A04-33
A08-41	*R3a* MF	136	8.0	9.5	196	8.0	A08-41
A08-50	*R3a* MF	169	3.1	9.5	68	4.8	A08-50
A09-1	*STO1*	78	7.6	5.0	51	7.6	A09-1
A09-30	*STO1*	76	1.0	5.5	60	1.0	A09-30
A09-7	*STO1*	83	7.6	7.0	122	7.1	A09-7
A10-43	*STO1+BLB3*	0	1.0	2.0	39	1.3	A10-43
A10-48	*STO1+BLB3*	7	1.1	2.0	114	1.8	A10-48
A10-67	*STO1+BLB3*	75	2.3	5.0	91	7.5	A10-67
A13-13	*VNT1*	10	1.0	1.0	0	1.0	A13-13
A13-17	*VNT1*	13	1.0	0.1	0	1.0	A13-17
A13-28	*VNT1*	6	1.0	0.0	0	1.0	A13-28
A14-81	*STO1+BLB3+ VNT1*	2	1.0	0.0	0	1.0	A14-81
A14-83	*STO1+BLB3+ VNT1*	0	1.0	0.0	0	1.0	A14-83
A14-97	*STO1+BLB3+ VNT1*	0	1.0	0.0	0	1.0	A14-97
A16-2	*STO1+VNT1*	0	1.0	0.0	0	1.0	A16-2
A16-38	*STO1+VNT1*	4	1.0	0.0	0	1.0	A16-38
A16-95	*STO1+VNT1*	1	1.0	0.0	0	1.0	A16-95
A17-27	*CHC1*	10	6.3	2.1	17	6.8	A17-27
A17-30	*CHC1*	2	8.0	1.3	22	8.0	A17-30
A19-67	*VNT1+CHC1*	3	1.0	0.0	0	1.0	A19-67

		TSA	DLA	FIELD LEAF	TSA	DLA	
clone	**genes**	KATS	KATS		IPO C	IPO C	**clone**
A19-96	*VNT1 CHC1*	1	1.0	0.0	0	1.0	A19-96
A19-99	*VNT1 CHC1*	2	1.0	0.0	0	1.0	A19-99
A23-11	*PTA1*	31	6.2	5.0	81	3.5	A23-11
A23-21	*PTA1*	105	4.0	5.0	37	1.6	A23-21
A23-68	*PTA1*	108	3.8	5.5	119	2.8	A23-68
A25-11	*R3b*	141	8.0	10.0	111	8.0	A25-11
A25-12	*R3b*	105	8.0	10.0	76	8.0	A25-12
A25-14	*R3b*	111	8.0	10.0	72	8.0	A25-14
A26-1554	*BLB3 STO1* MF	46	4.6	3.5	112	6.9	A26-1554
A26-1679	*BLB3 STO1* MF	67	1.3	5.0	80	1.2	A26-1679
A26-1735	*BLB3 STO1* MF	71	4.3	3.0	81	7.6	A26-1735
A31-1	*MCQ1*	104	1.0	10.0	84	8.0	A31-1
A31-46	*MCQ1*	98	5.4	10.0	93	8.0	A31-46
A31-5	*MCQ1*	99	8.0	10.0	85	8.0	A31-5
A73.1-36	*EDN2*	115	8.0	2.5	120	5.9	A73.1-36
A73.1-5	*EDN2*	138	8.0	4.5	29	6.0	A73.1-5
A73.1-52	*EDN2*	87	8.0	2.5	39	5.1	A73.1-52
PREMIERE (B)	*R10*	n/a	8.0	10.0	n/a	8.0	PREMIERE (B)
B01-16	*BLB1*	n/a	1.0	6.0	n/a	1.0	B01-16
B01-126	*BLB1*	n/a	1.5	7.5	n/a	1.4	B01-126
B01-129	*BLB1*	n/a	1.0	5.5	n/a	1.9	B01-129
B13-2	*VNT1*	n/a	1.3	2.5	n/a	1.2	B13-2
B13-16	*VNT1*	n/a	1.0	0.5	n/a	1.0	B13-16
B13-33	*VNT1*	n/a	1.1	1.5	n/a	1.1	B13-33

		TSA	DLA	FIELD LEAF	TSA	DLA	
clone	**genes**	KATS	KATS		IPO C	IPO C	**clone**
AVEKA	*R3*	120	8.0	7.5	175	8.0	AVEKA
C01-10	*BLB1*	16	1.0	0.1	13	1.0	C01-10
C01-19	*BLB1*	49	3.5	2.0	67	4.2	C01-19
C01-6	*BLB1*	1	1.0	0.1	0	1.0	C01-6
C13-1	*VNT1*	2	1.0	0.0	0	1.0	C13-1
C13-11	*VNT1*	2	1.0	0.5	0	1.0	C13-11
C13-12	*VNT1*	1	1.0	0.0	0	1.0	C13-12
ATLANTIC		202	8.0	10.0	138	8.0	ATLANTIC
H15-2k	*VNT1* MF	80	1.3	0.1	42	1.0	H15-2k
H15-5k	*VNT1* MF	126	1.0	3.0	18	1.0	H15-5k
H15-7k	*VNT1* MF	32	1.0	0.5	11	1.0	H15-7k
H43-2k	*STO1 VNT1* MF	11	1.0	0.0	0	1.0	H43-2k
H43-4k	*STO1 VNT1* MF	1	1.0	0.1	4	1.0	H43-4k
H49-3p	*CHC1* MF	15	6.9	2.5	18	5.1	H49-3p
ANUSCHKA	*R3a*	463	n/a	n/a	213	n/a	ANUSCHKA
CANBERRA		2	8.0	10.0	2	7.9	CANBERRA
CRONOS	*R3a*	123	8.0	10.0	201	8.0	CRONOS
DIVAA		146	8.0	10.0	474	8.0	DIVAA
ENDEAV		153	8.0	10.0	191	8.0	ENDEAV
LADY ANNA	*R3a*	69	n/a	n/a	63	n/a	LADY ANNA
LAURA		52	8.0	10.0	9	5.1	LAURA
LEANDRA		148	8.0	10.0	244	8.0	LEANDRA
LUCINDA		53	8.0	9.8	0	8.0	LUCINDA
LUSA		82	8.0	10.0	67	8.0	LUSA

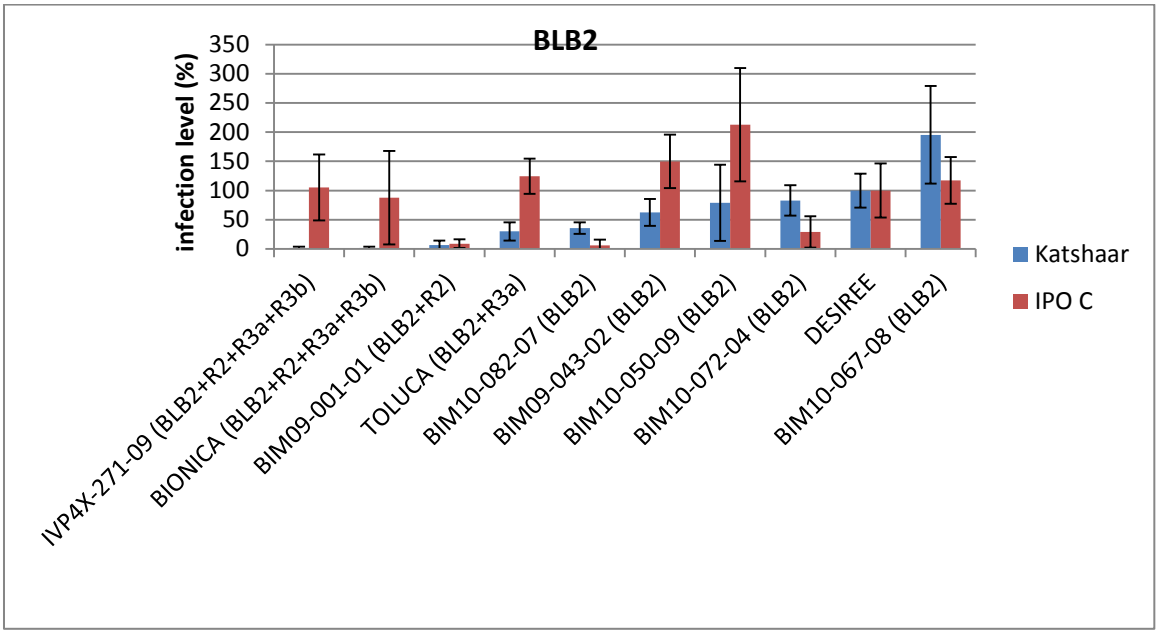
		TSA	DLA	FIELD LEAF	TSA	DLA	
clone	**genes**	KATS	KATS		IPO C	IPO C	**clone**
MARABEL	*R3a*	168	8.0	9.8	75	8.0	MARABEL
MASAI		23	8.0	9.6	2	8.0	MASAI
OBAMA	*R3a*	287	8.0	10.0	134	8.0	OBAMA
VOYAGER	*R2 R3a*	77	1.5	8.8	134	5.6	VOYAGER
SARPO MIRA	*R3a R3b R4 R8*	48	1.5	0.1	118	4.4	SARPO MIRA
IVP4X-223-13	*R8 R3a R3b*	104	7.0	2.0	191	6.0	IVP4X-223-13
TOLUCA	*BLB2 R3a*	30	2.5	2.7	124	1.1	TOLUCA
BIONICA	*BLB2 R2 R3a R3b*	2	1.0	3.7	88	4.3	BIONICA
BIM09-001-01	*BLB2 R2*	7	1.5	3.7	9	2.0	BIM09-001-01
BIM09-012-01	*R8 R3a*	48	8.0	2.0	72	4.5	BIM09-012-01
BIM09-018-01	*R8 R2*	3	1.6	2.7	42	3.1	BIM09-018-01
IVP4X-271-09	*BLB2 R2*	2	2.0	9.6	105	8.0	IVP4X-271-09
BIM09-043-02	*BLB2*	62	3.0	3.3	150	2.0	BIM09-043-02
BIM10-050-09	*BLB2*	79	7.5	6.3	213	5.8	BIM10-050-09
BIM10-067-08	*BLB2*	195	7.0	7.3	117	5.4	BIM10-067-08
BIM10-072-04	*BLB2*	83	6.4	3.3	29	5.6	BIM10-072-04
BIM10-082-07	*BLB2*	36	5.0	4.7	6	4.1	BIM10-082-07
BIM10-109-01	*EDN2*	93	8.0	5.3	210	4.0	BIM10-109-01
BIM10-132-08	*EDN2*	14	5.5	3.0	31	1.3	BIM10-132-08
BIM10-133-03	*EDN2*	30	6.4	3.3	68	1.3	BIM10-133-03
BIM10-134-01	*EDN2*	94	8.0	0.5	111	1.3	BIM10-134-01
RH4X-667-01	*R8*	8	8.0	3.3	22	2.4	RH4X-667-01
RH4X-694-05	*R8*	73	8.0	4.0	290	8.0	RH4X-694-05

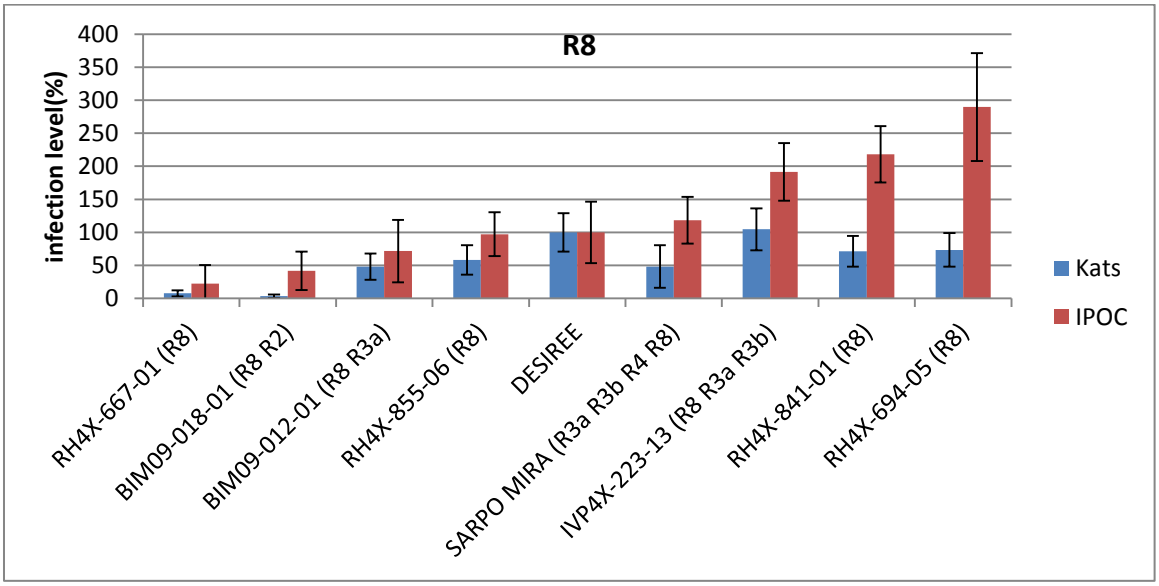
		TSA	DLA	FIELD LEAF	TSA	DLA	
clone	**genes**	KATS	KATS		IPO C	IPO C	**clone**
RH4X-841-01	*R8*	71	5.8	1.0	218	1.8	RH4X-841-01
RH4X-855-06	*R8*	58	4.1	0.4	97	1.0	RH4X-855-06

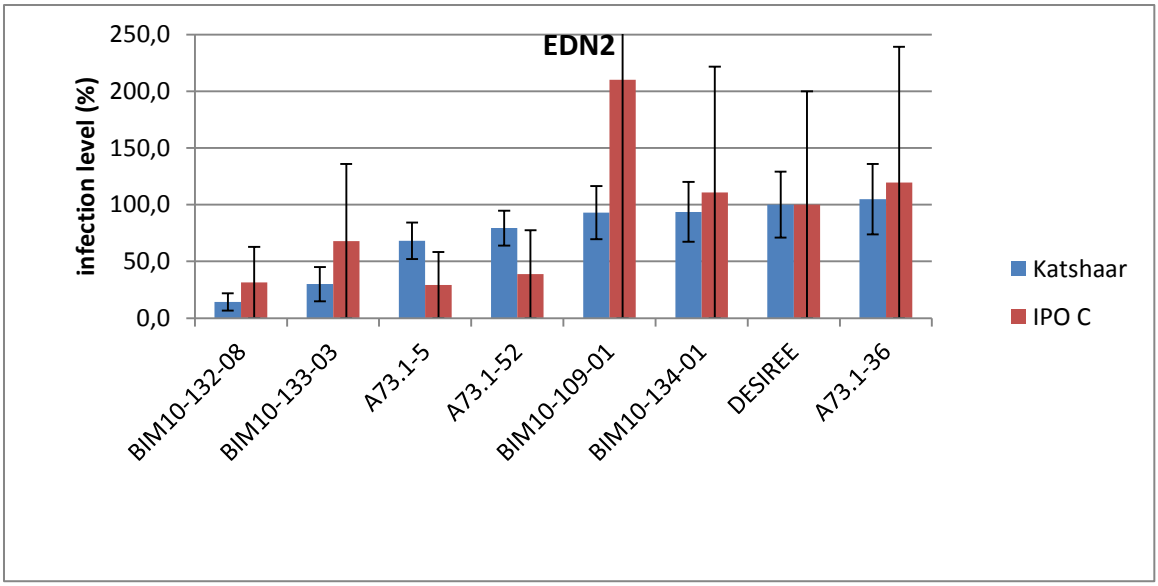
Appendix 3. The results of the individual breeding lines containing *BLB2, R8* and *EDN2*

Results for the tuber slice assay, detached leaf assay and the leaf analysis in the field. Genotypes with a *BIM* abbreviation are derived from the Bioimpulse program. Genotypes with a I*VP4X* or *RH4X* are derived from the conventional breeding program of Wageningen UR. The genotypes starting with *A73* represent Desiree transformants with *EDN2*.

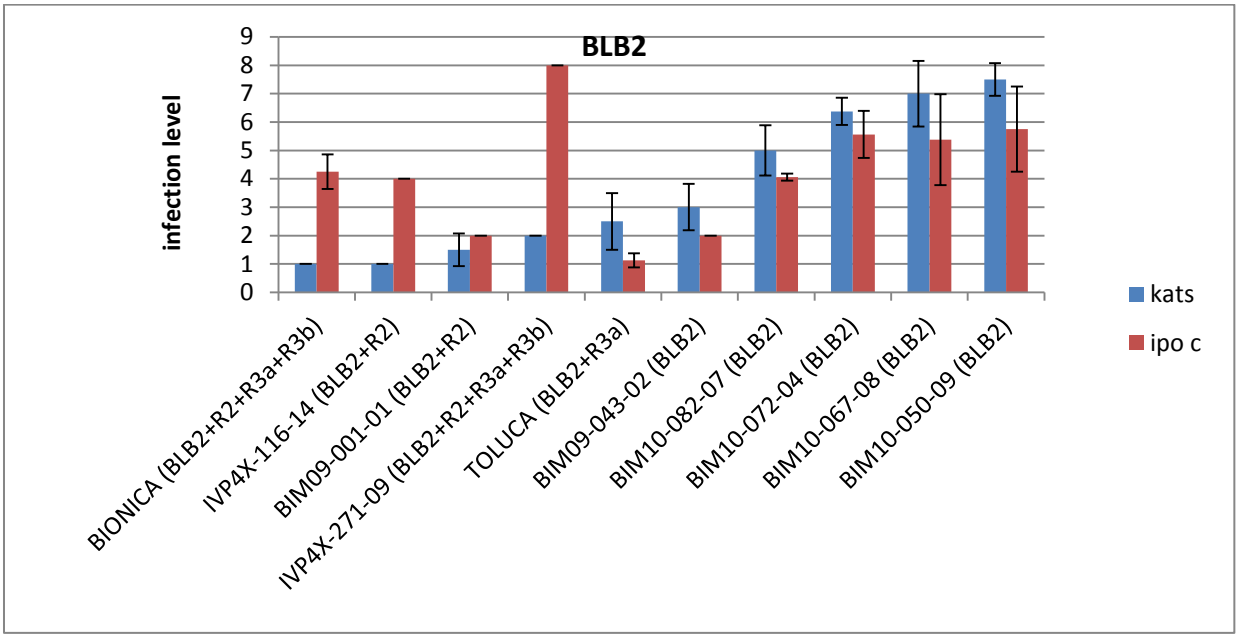
Tuber slice assay

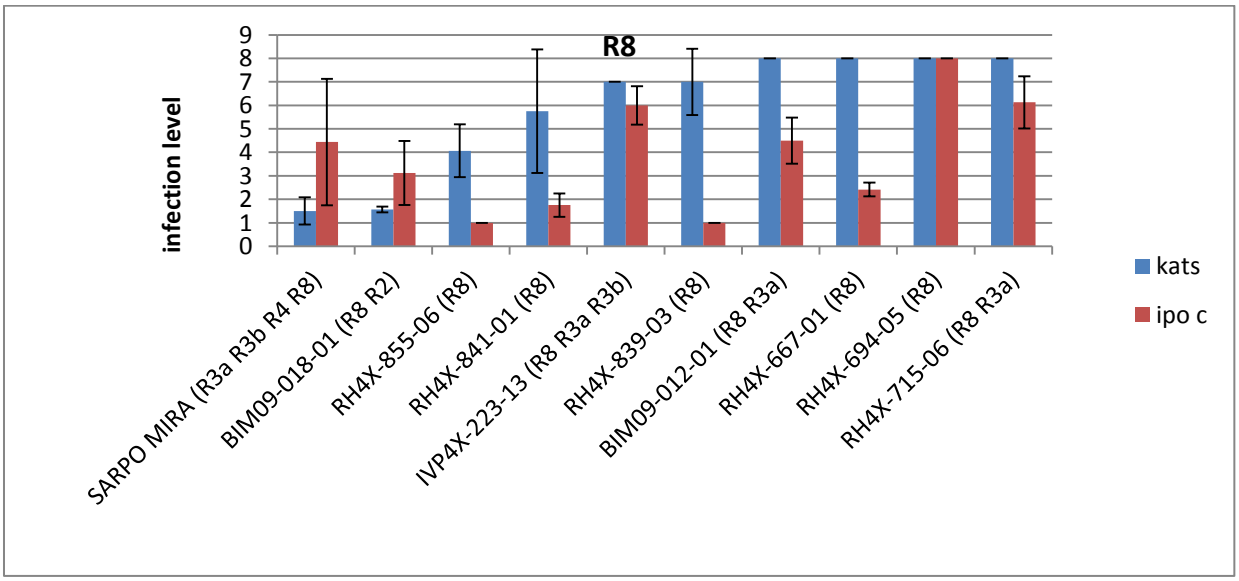


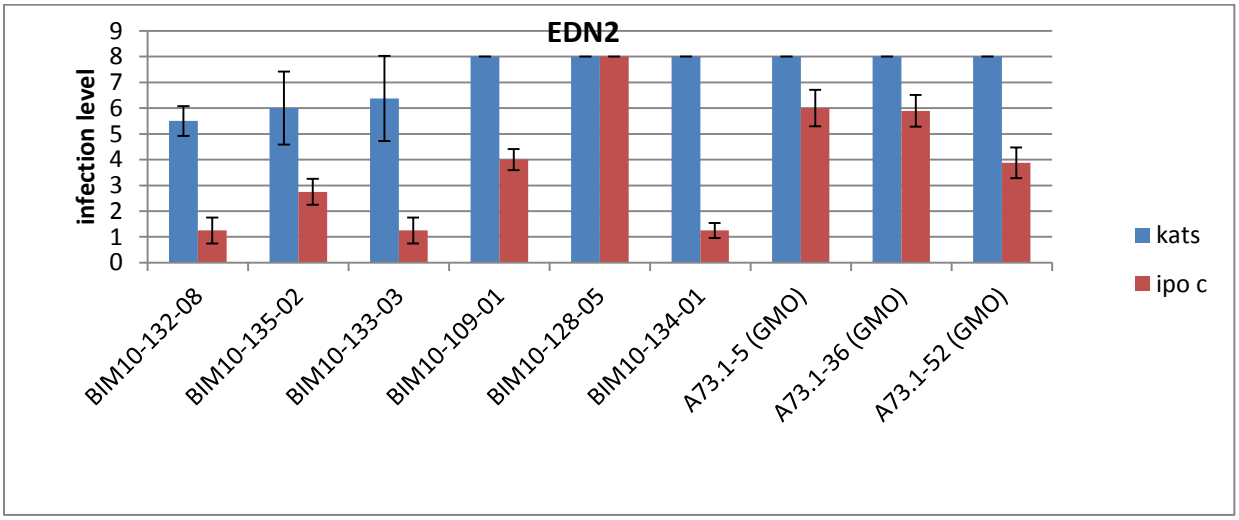



EDN2
infection level (%)
250,0
200,0
150,0
100,0
50,0
0,0
BIM10-132-08
BIM10-133-03
A73.1-5
A73.1-52
BIM10-109-01
BIM10-134-01
DESIREE
A73.1-36
Katshaar
IPO C

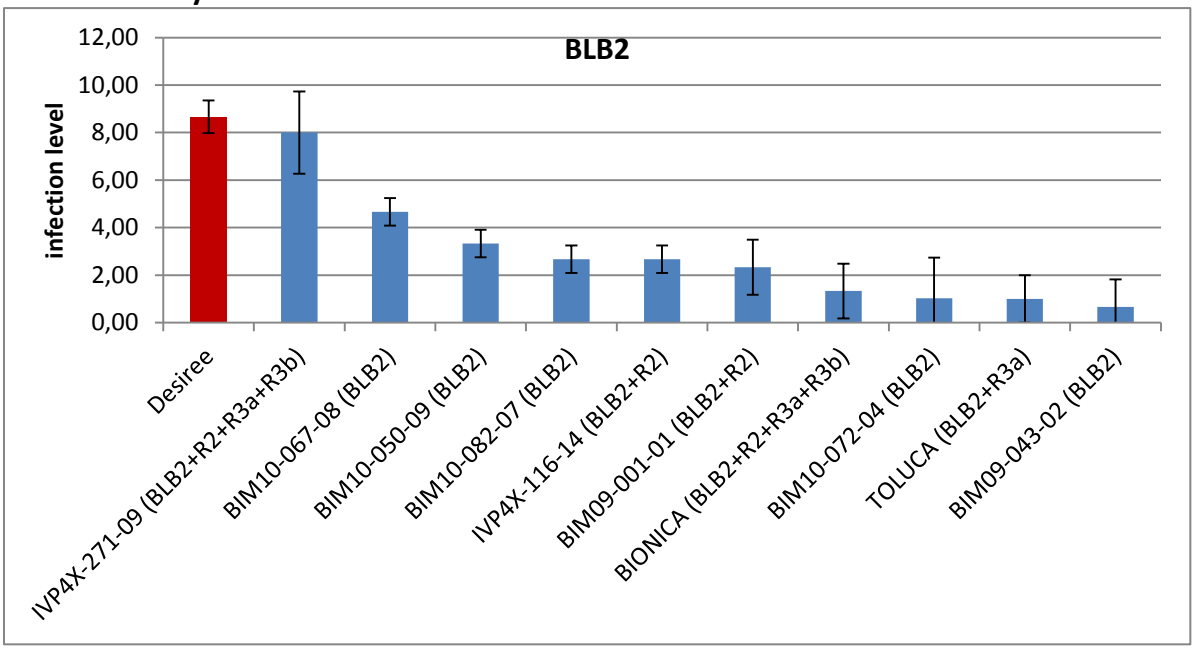
Detached leaf assay

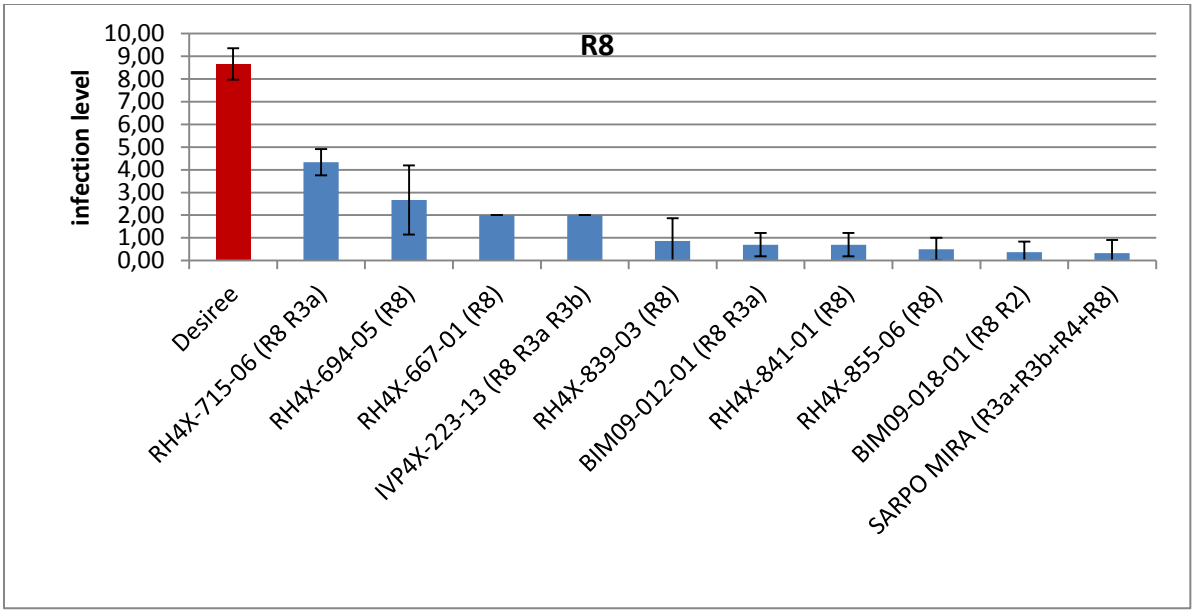


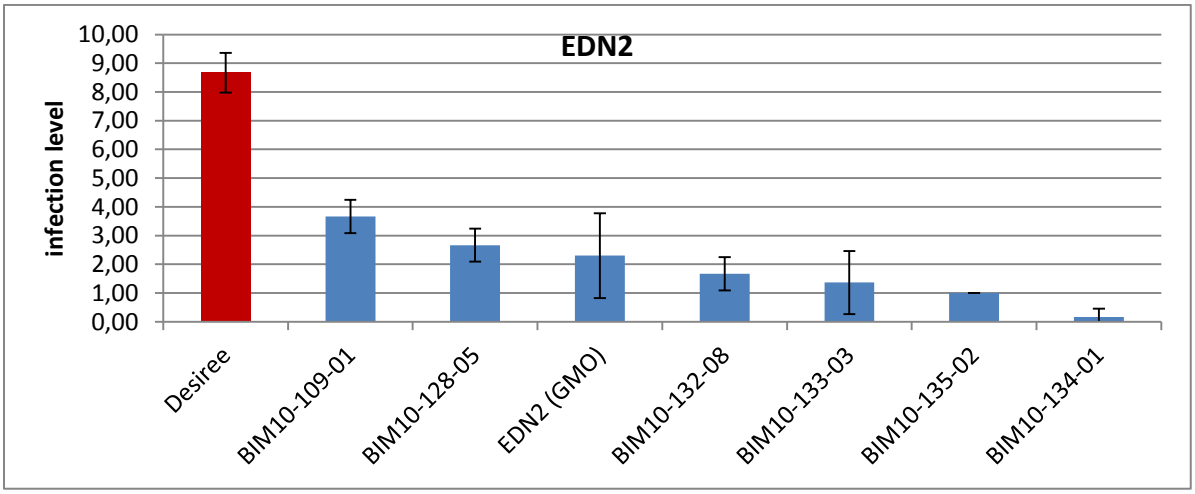




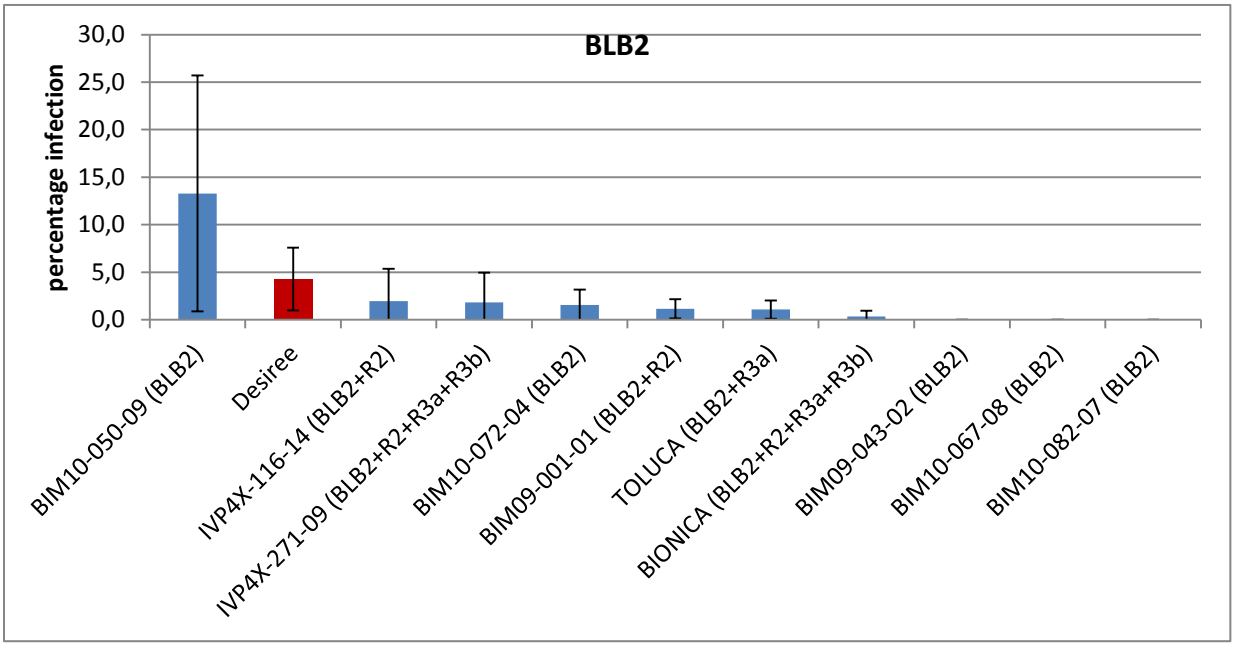
Leaf field analysis

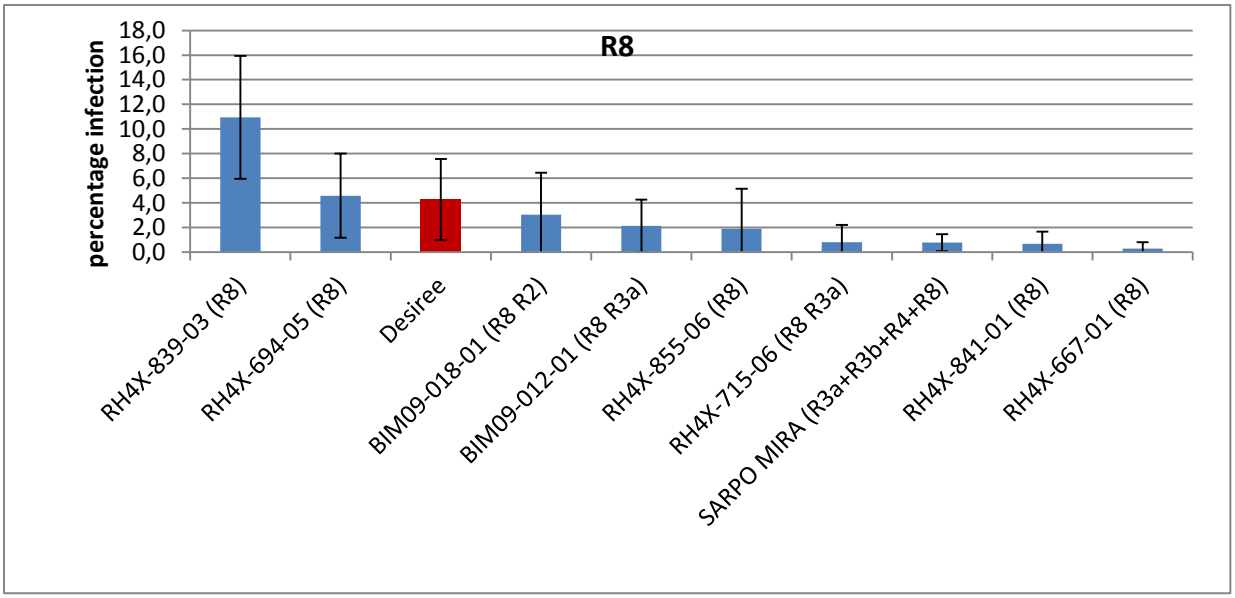


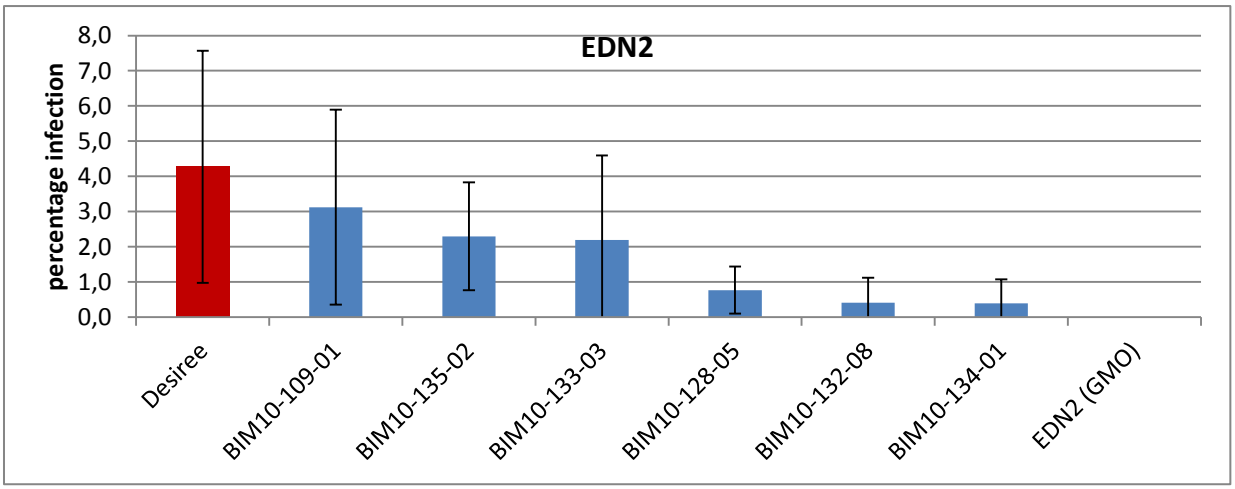




Tuber field analysis

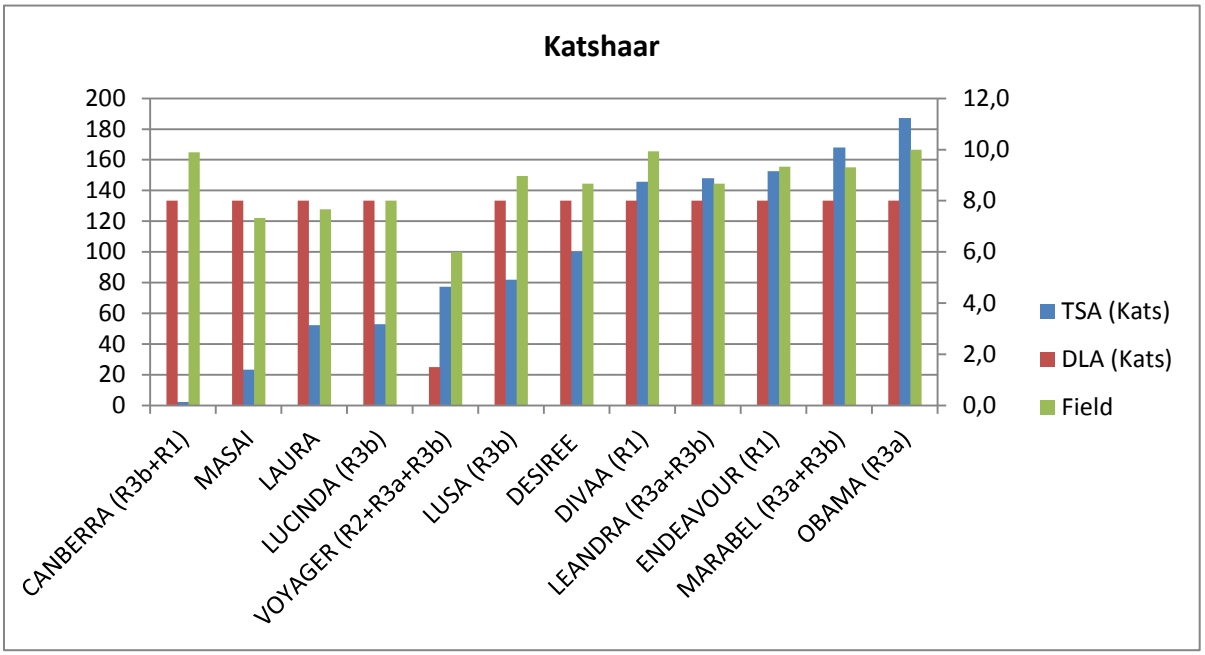


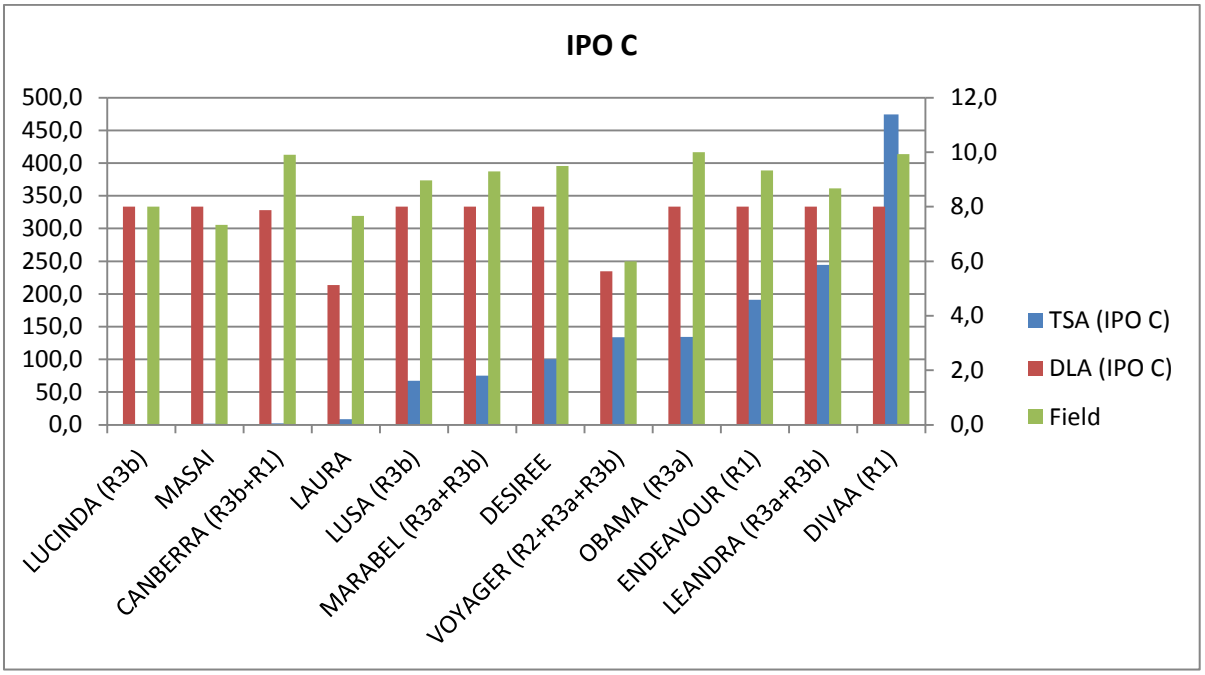




Appendix 4. The results of the individual cultivars

Results for the tuber slice assay (TSA), detached leaf assay (DLA) and the leaf analysis in the field.





Appendix 5. Photos of the late blight trials in the laboratory and in the field.

Detached leaf essay

Photos of genotypes that showed resistance to Katshaar, but susceptibility to IPO C (nr. 82 = Evolution (*R2*)) and genotypes that showed resistance to IPO C and susceptibility to Katshaar (nr. 106 = BIM10-133-03 (*EDN2*) and nr 97. = RH4X-841-01 (*R8*)).

Desiree showed high susceptibility (right side of the tray) and the Premiere transformant B13-16 with *VNT1* showed high resistance with visible HR spots (left side of the tray).

Tuber slice assay

An example of a perfect infected tuber slice assay with Desiree transformants with one or more genes inoculated with Katshaar (photo above). The abbreviation DES refers to the control cv. Desiree. Desiree with *VNT1* showed clearly resistance with visible HR sport on the tuber slices. The genotypes on the photo below were distributed on the same way as the photo above, but inoculated with IPO C. It is visible that IPO C was less aggressive and produced less mycelium compared to isolate Katshaar.

Leaf analysis on the late blight field

A photo of the late blight trial field near Wageningen UR. Clear difference between resistant and susceptible genotypes was observed at the end of the leaf analysis.

A heavily infected potato leaf from the late blight trial field.

Tuber analysis on the late blight field and after storage

Photos from the tuber analysis in the field and after storage. The upper left photo shows the rotten tubers that were counted in the field. The rest of the tubers was stored in plastic boxes (upper right). After several weeks the boxes were analysed on amount of rotten tubers on the boxes (the photo below).

6 References

Black, W., Mastenbroek, C., Mills, W. R., & Peterson, L. C. (1953). A proposal for an international nomenclature of races of Phytophthora infestans and of genes controlling immunity in Solanum demissum derivatives. *Euphytica*, *2*(3), 173-179.

Collins, A., Milbourne, D., Ramsay, L., Meyer, R., Chatot-Balandras, C., Oberhagemann, P. & Waugh, R. (1999). QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. *Molecular breeding*, *5*(5), 387-398.

Davila, E. (1964). Late blight infection of potato tubers. *American Potato Journal*, *41*(4), 103-112.

Dorrance, A. E., & Inglis, D. A. (1998). Assessment of laboratory methods for evaluating potato tubers for resistance to late blight. *Plant disease*, *82*(4), 442-446.

Doster, M. A., Milgroom, M. G., & Fry, W. E. (1990). Quantification of factors influencing potato late blight suppression and selection for metalaxyl resistance in Phytophthora infestans: A simulation approach. *Phytopathology*, *80*(11), 1190-1198.

Foster, S. J., Park, T. H., Pel, M., Brigneti, G., Sliwka, J., Jagger, L., ... & Jones, J. D. (2009). *Rpi-VNT1*. 1, a Tm-22 homolog from Solanum venturii, confers resistance to potato late blight. *Molecular plant-microbe interactions*, *22*(5), 589-600.

Frinking, H. D., & Van der Stoel, M. C. (1987). Production of conidia by Peronospora farinosa f. sp. spinaciae. *Netherlands Journal of Plant Pathology*, *93*(4), 189-194.

Goodwin, S. B., Sujkowski, L. S., & Fry, W. E. (1996). Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of Phytophthora infestans in the United States and western Canada. *Phytopathology*, *86*(7), 793-799.

Halterman, D. A., Kramer, L. C., Wielgus, S., & Jiang, J. (2008). Performance of transgenic potato containing the late blight resistance gene RB. *Plant Disease*, *92*(3), 339-343.

Hardham, A. R. (2007). Cell biology of plant–oomycete interactions. *Cellular microbiology*, *9*(1), 31-39.

Hawkes, J. G., & Francisco-Ortega, J. (1993). The early history of the potato in Europe. *Euphytica*, *70*(1-2), 1-7.

Hermsen, J. T., & Ramanna, M. S. (1973). Double-bridge hybrids of Solanum bulbocastanum and cultivars of Solanum tuberosum. *Euphytica*, *22*(3), 457-466.

Huang, S., Van Der Vossen, E. A., Kuang, H., Vleeshouwers, V. G., Zhang, N., Borm, T. J., ... & Visser, R. G. (2005). Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *The Plant Journal*, *42*(2), 251-261.

Jacobs, M. M., van den Berg, R. G., Vleeshouwers, V. G., Visser, M., Mank, R., Sengers, M., ... & Vosman, B. (2008). AFLP analysis reveals a lack of phylogenetic structure within Solanum section Petota. *BMC evolutionary biology*, *8*(1), 145.

Jo, K. R., Kim, C. J., Kim, S. J., Kim, T. Y., Bergervoet, M., Jongsma, M. A., ... & Vossen, J. H. (2014). Development of late blight resistant potatoes by cisgene stacking. *BMC biotechnology*, *14*(1), 50.

Jones, J. D., Witek, K., Verweij, W., Jupe, F., Cooke, D., Dorling, S., ... & Foster, S. (2014). Elevating crop disease resistance with cloned genes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1639), 20130087.

Judelson, Howard S., and Flavio A. Blanco. "The spores of Phytophthora: weapons of the plant destroyer." *Nature Reviews Microbiology* 3.1 (2005): 47-58.

Kamoun, S., Huitema, E., & Vleeshouwers, V. G. (1999). Resistance to oomycetes: a general role for the hypersensitive response?. *Trends in Plant Science*, *4*(5), 196-200.

Kolasa, K. M. (1993). The potato and human nutrition. *American Potato Journal*, *70*(5), 375-384.

Lammerts van Bueren, E. L., Tiemens-Hulscher, M., & Struik, P. C. (2008). Cisgenesis does not solve the late blight problem of organic potato production: alternative breeding strategies. *Potato Research*, *51*(1), 89-99.

Lapwood, D. H., & Kee, R. K. (1961). Reaction of tubers of *R* gene potato clones to inoculation with specialized races of Phytophthora infestans. *European Potato Journal*, *4*(1), 3-13.

Li, G., Huang, S., Guo, X., Li, Y., Yang, Y., Guo, Z., ... & Vossen, J. H. (2011). Cloning and characterization of *R3b*; members of the *R3* superfamily of late blight resistance genes show sequence and functional divergence. *Molecular Plant-Microbe Interactions*, *24*(10), 1132-1142.

Lokossou, A. A., Park, T. H., van Arkel, G., Arens, M., Ruyter-Spira, C., Morales, J., ... & van der Vossen, E. A. (2009). Exploiting knowledge of R/Avr genes to rapidly clone a new LZ-NBS-LRR family of late blight resistance genes from potato linkage group IV. *Molecular Plant-Microbe Interactions*, *22*(6), 630-641.

Mayton, H., Griffiths, H., Simko, I., Cheng, S., Lorenzen, J., De Jong, W., & Fry, W. E. (2010). Foliar and tuber late blight resistance in a Solanum tuberosum breeding population. *Plant breeding*, *129*(2), 197-201.

Mayton, H., Rauscher, G., Simko, I., & Fry, W. E. (2011). Evaluation of the RPi-ber late blight resistance gene for tuber resistance in the field and laboratory. *Plant Breeding*, *130*(4), 464-468.

Niks, R. E., Parlevliet, J. E., Lindhout, P., & Bai, Y. (2011). *Breeding crops with resistance to diseases and pests*. Wageningen Academic Publishers.

Oberhagemann, P., Chatot-Balandras, C., Schäfer-Pregl, R., Wegener, D., Palomino, C., Salamini, F., ... & Gebhardt, C. (1999). A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. *Molecular Breeding*, *5*(5), 399-415.

Park, T. H., Vleeshouwers, V. G., Kim, J. B., Hutten, R. C., & Visser, R. G. (2005). Dissection of foliage and tuber late blight resistance in mapping populations of potato. *Euphytica*, *143*(1-2), 75-83.

Pathak, N., & Clarke, D. D. (1987). Studies on the resistance of the outer cortical tissues of the tubers of some potato cultivars to< i> Phytophthora infestans</i>. *Physiological and molecular plant pathology*, *31*(1), 123-132.

Pel, M. A., Foster, S. J., Park, T. H., Rietman, H., van Arkel, G., Jones, J. D., ... & Van der Vossen, E. A. (2009). Mapping and cloning of late blight resistance genes from Solanum venturii using an interspecific candidate gene approach. *Molecular plant-microbe interactions*, *22*(5), 601-615.

Pel, M. (2010). Mapping, isolation and characterization of genes responsible for late blight resistance in potato.

Perfect, S. E., & Green, J. R. (2001). Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. Molecular Plant Pathology, 2(2), 101-108.

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., & Nelson, R. J. (2009). Shades of gray: the world of quantitative disease resistance. *Trends in plant science*, *14*(1), 21-29.

Que, Q., Chilton, M. D. M., de Fontes, C. M., He, C., Nuccio, M., Zhu, T., ... & Shi, L. (2010). Trait stacking in transgenic crops. *pat*, *24236*, 5.

Rietman, H., Bijsterbosch, G., Cano, L. M., Lee, H. R., Vossen, J. H., Jacobsen, E., ... & Vleeshouwers, V. G. (2012). Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors. *Molecular Plant-Microbe Interactions*, 25(7), 910-919.

Roer, L., & Toxopeus, H. J. (1961). The effect of *R* genes for hypersensitivity in potato-leaves on tuber resistance to Phytophthora infestans. *Euphytica*, *10*(1), 35-42.

Simko, I., Costanzo, S., Ramanjulu, V., Christ, B. J., & Haynes, K. G. (2006). Mapping polygenes for tuber resistance to late blight in a diploid Solanum phureja× S. stenotomum hybrid population. *Plant breeding*, *125*(4), 385-389.

Śliwka, J., Jakuczun, H., Lebecka, R., Marczewski, W., Gebhardt, C., & Zimnoch-Guzowska, E. (2006). The novel, major locus *Rpi*-phu1 for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period. *Theoretical and applied genetics*, *113*(4), 685-695.

Smilde, W. D., Brigneti, G., Jagger, L., Perkins, S., & Jones, J. D. G. (2005). Solanum mochiquense chromosome IX carries a novel late blight resistance gene *Rpi*-moc1. *Theoretical and Applied Genetics*, *110*(2), 252-258.

Spooner, D. M., McLean, K., Ramsay, G., Waugh, R., & Bryan, G. J. (2005). A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(41), 14694-14699.

Stewart, H. E., Bradshaw, J. E., & Wastie, R. L. (1994). Correlation between resistance to late blight in foliage and tubers in potato clones from parents of contrasting resistance. *Potato Research*, *37*(4), 429-434.

Swiezynski, K. M., Sieczka, M. T., Sujkowski, L. S., Zarzycka, H., & Zimnoch-Guzowska, E. (1991). Resistance to Phytophthora infestans in potato genotypes originating from wild species. *Plant breeding*, *107*(1), 28-38.

Tai, G. (1998). Relationship between resistance to late blight in potato foliage and tubers of cultivars and breeding selections with different resistance levels. *American Journal of Potato Research*, *75*(4), 173-178.

Tiemens-Hulscher, M.; Delleman, J.; Eisinger, E.; Lammerts Van Bueren, E. (2013) Aardappelkweekboek : praktijkhandboek voor de aardappelketen.

van der Linden, C. G., Wouters, D. C., Mihalka, V., Kochieva, E. Z., Smulders, M. J., & Vosman, B. (2004). Efficient targeting of plant disease resistance loci using NBS profiling. *Theoretical and Applied Genetics*, *109*(2), 384-393.

van der Vossen, E., Sikkema, A., Hekkert, B. T. L., Gros, J., Stevens, P., Muskens, M., ... & Allefs, S. (2003). An ancient R gene from the wild potato species Solanum bulbocastanum confers broad-spectrum resistance to Phytophthora infestans in cultivated potato and tomato. *The Plant Journal*, *36*(6), 867-882.

van der Vossen, E. A., Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., ... & Allefs, S. (2005). The Rpi-*BLB2* gene from Solanum bulbocastanum is an Mi-1 gene homolog conferring broad-spectrum late blight resistance in potato. *The Plant Journal*, *44*(2), 208-222.

Vetten, N. C. M. H., Verzaux, E. C., Vossen, J. H., Rietman, H., Vleeshouwers, V. G. A. A., Jacobsen, E., & Visser, R. G. F. (2011). U.S. Patent Application 13/699,334.

Verzaux E (2010) Resistance and susceptibility to late blight in Solanum: gene mapping, cloning and stacking. PhD Thesis, Wageningen University, The Netherlands

Vossen, J. H., Nijenhuis, M., Arens-De Reuver, M. J. B., Van Der Vossen, E. A. G., Jacobsen, E., & Visser, R. G. F. (2010). U.S. Patent Application 13/496,845.

Visker, M. H. P. W., Keizer, L., Van Eck, H., Jacobsen, E., Colon, L., & Struik, P. (2003). Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type?. *Theoretical and Applied Genetics*, *106*(2), 317-325.

Vleeshouwers, V. G., Rietman, H., Krenek, P., Champouret, N., Young, C., Oh, S. K., ... & Van der Vossen, E. A. (2008). Effector genomics accelerates discovery and functional profiling of potato disease resistance and Phytophthora infestans avirulence genes. *PLoS One*, *3*(8), e2875.

Vleeshouwers, V. G., Raffaele, S., Vossen, J. H., Champouret, N., Oliva, R., Segretin, M. E., ... & Kamoun, S. (2011). Understanding and exploiting late blight resistance in the age of effectors. *Annual review of phytopathology*, *49*, 507-531.

Zhu, S., Li, Y., Vossen, J. H., Visser, R. G., & Jacobsen, E. (2012). Functional stacking of three resistance genes against Phytophthora infestans in potato. *Transgenic research*, *21*(1), 89-99.