CONFIDENTIAL

Resistance to *Oidium neolycopersici* in modern tomato cultivars is not only mediated by *Ol-1* to *Ol-6*

MSc Thesis

Jeroen Vermuë

WAGENINGEN
UNIVERSITY & RESEARCH

Resistance to *Oidium neolycopersici* in modern tomato cultivars is not only mediated by *Ol-1* to *Ol-6*

MSc Thesis

Submitted to the Plant Breeding department of the Wageningen University & Research in fulfillment for the requirements for the degree of Master in Plant Sciences

With specialization

Breeding for resistance in Solanaceae

PBR-80436

Jeroen Vermuë

Student number: 821120-880-060

E-mail: jpvermue@hotmail.com

Supervision by:

Miguel Isauro Santillán Martínez

prof. dr. Yuling Bai

dr. Anne-marie Wolters

Plant Breeding

Wageningen University & Research

August, 2018

Abstract

Oidium neolycopersici (powdery mildew) is the causual agent of a widespread fungus and a well-known threat to the tomato production in moderate climates. The disease pressure is expected to increase for high-tech greenhouse production, as the production must take place under more dark and humid conditions and with less input of chemical agents. To overcome this threat, there are six resistance genes known, named *Ol-1* to *Ol-6.* Although the sources and markers of these genes are well described, it is unknown whether this information is currently used to breed for modern cultivars. This study gives more insight in the level of resistance of modern cultivars and the allele frequency of the known resistance genes. Information is gathered from Dutch authorities and compared with the cultivars offered on the websites of the seed companies. Ten cultivars are selected and grown together with the *Ol-1* to *Ol-6* controls in a disease test. DNA is isolated from the plants and analyzed on presence of resistance genes. Results from the disease test and marker analysis identified the source of resistance in variety "Rebelski" as being *Ol-1*. The variety "Merlice" carries a resistance gene that is assumed to be an allelic variant of *Ol-4*, which might be *Ol-6.* The source of resistance of the other varieties could not be identified. These other varieties, although claimed as intermediate resistant, were heavily infected up to the level of the susceptible control "Moneymaker". We therefore concluded that this resistance might be a form of adult plant resistance coming from an unknown source. In a second experiment, DNA of 69 plants of the population PV033045, segregating for *On* resistance, was analyzed to determine the relationship between *Ol-1* and *Ol-5.* This relationship however, could not be further identified, as a third gene *Ol-4* was involved. Isolating the *Ol-1* / *Ol-5* region from *Ol-4,* with new markers, would make it possible to further identify this region.

List of abbreviations

a: homozygous for the *esculentum* allele

APR: adult plant resistance

b: homozygous for the donor allele

bp: base pair

CAPS: cleaved amplified polymorphic sequence

cM: centimorgan

cv: cultivar

DNA: deoxyribonucleic acid

Dpi: days post inoculation

h: heterozygous

ha: hectare

HR: hypersensitive response

Kg: Kilogram

MAS: marker assisted selection

MM: *Solanum lycopersicum* cv. 'Moneymaker'

NIL: Near Isogenic Line

Nr.: number

NRR: Dutch variety registration list (Nederlands Rassenregister)

O.: *Oidium*

On: *Oidium neolycopersici*

PCR: polymerase chain reaction

QTL: quantitative trait loci

R-gene: resistance gene

S-gene: susceptability gene

S.: *Solanaceae*

SCAR: sequence characterized amplified region

SNP: single nucleotide polymorphism

WUR: Wageningen University & Research

Table of Contents

1. Introduction

1.1 Tomato

With a global production of 177 million tons, Tomato (*Solanum lycopersicum*) is considered as one of the most widely grown food crops in the world ("Food and Agriculture Organisation (FAO)," 2018). In the Netherlands, tomatoes are produced under 1775 ha high tech greenhouses. In 2016, 900 million kg tomatoes were produced ("Centraal Bureau voor de Statistiek," 2017). Yields of 60 kg /m^2 are normal even under low light conditions. In Spain, where no high-tech greenhouses are used, even with high light levels, the yield does not come above 30 kg /m^2 (Peet & Welles, 2005). The growth system therefore, has a significant influence on the yield/ha.

The modern cultivars of *S. lycopersicum* belongs to the Asterid family *Solanaceae,* which contains a great diversity of wild tomatoes. As part of a group of 13 closely related species, it is one of the few which is domesticated. Together, there are more than 3000 wild relatives, which all originate from the Andean region on the west coast of South America (Causse, Giovannoni, Mondher, & Zouine, 2016). Although much variation is available, modern cultivars, have a narrow genetic basis, as domestication took place with a limited gene pool (Heuvelink, Costa, & Lindhout, 2005).

New traits are often derived from wild species. When these wild species are crossed with modern cultivars, many undesired genes from the wild donors are inherited to the progeny. Marker assisted selection (MAS) is used to overcome this linkage drag. The identification of markers (often SNPs) is supported by genomic information which is available in the SOL genomics network in the form of sequences, maps and markers. The tomato genome itself has been sequenced and consists out of 950 Mb (The Tomato Genome Consortium, 2012). Because of its high quality, this genome is considered a reference for fleshy fruit crops.

Tomato is a diploid with 12 sets of chromosomes (2n=2x=24) and is autogamous, but cross pollination is possible when the flower is emasculated. One fruit can contain many seeds and an annual production of at least two generations is possible. Tomato has therefore a high multiplication factor. Due to this high multiplication factor, new varieties can be introduced between three to four years. Varieties are typically chosen based on yield potential, taste, truss quality, shelf life, labor friendliness and disease resistances (Aquaah, 2012). And with a seed price of €90.000,- per kg (gold has a price of €35.000,- per kg) this is a very interesting business for seed companies to invest in (De Bruine, 2018).

1.1.1 Trends in high tech tomato production

Tomatoes are grown year-round in Dutch greenhouses and can be harvested from mid-April to the end of October. During these months, there is an abundance of tomatoes on the market, which lowers the prices (**Figure 2**) ("Groenten en fruit," 2018). To harvest earlier in the season and sell at higher prices, modern greenhouses are illuminated. An additional benefit of tomato production under illuminated conditions is that the quality is better than the tomatoes produced in Spain and Morocco (*Jaarverslag Kwaliteits Controle Bureau 2017*, 2017). Nevertheless, the lights cannot fully replace the lack of sun during the winter months, resulting in production under relatively dark conditions.

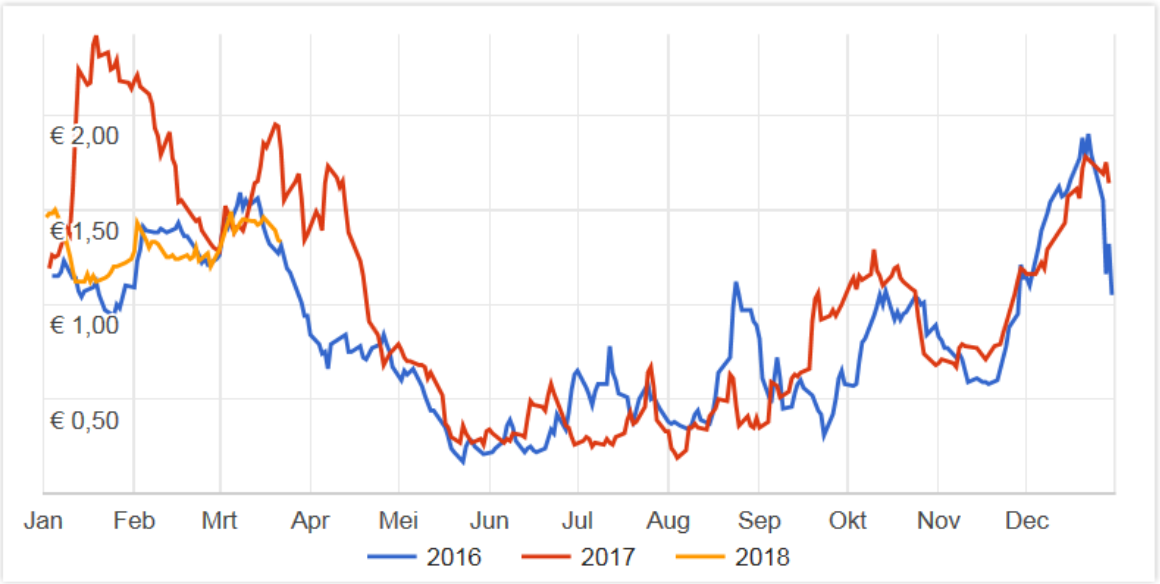


Figure 2. Tomato price for large truss tomatoes on Dutch auctions. The price is in Euro / kg and given for the period January 2016 up to mid April 2018.

A second trend has been initiated by the horticulture sector in the Netherlands: energy-neutral production from 2020 on. Minimizing heat and light emissions will reduce the usage of fossil fuels. To reach this ambition it is expected that tomato production in closed or semi-closed greenhouses will increase ("Saving energy and sustainable energy in greenhouse horticulture," 2018). Therefore, tomato production is expected to take place under more humid conditions (Qian et al., 2011). Adverse effects of these darker and humid conditions are that they increase the pressure of the already present pest and diseases (Peet & Welles, 2005).

At the same time, as a third trend, the intervention with chemical agents decreases (**figure 3**). Almost all Dutch growers, use biological control measurements for their pest management (Boonekamp, 2018). Intervening with chemicals can harm the biological control and can disturb the balance between the pest and the predator. In France, where a similar trend is visible, three grower-cooperation's will introduce a new label for their tomatoes in 2019: "Sans pesticides, 100% nature". They expect that 30% to 40% of their production can carry this label and that all growers are willing to cooperate (Cossardeaux, 2018; "Nieuw Frans predicaat voor residuvrije AGF," 2018). Additionally, the market for organic products increases. In 2015, growth was measured in European countries, by approximately 13%. In 2016, Ireland and France were the countries that registered the biggest growth, their market increased by 22 percent each. In Denmark and Norway, the markets increased by 20 percent each (Willer & Lernoud, 2016).

1.2 Tomato powdery mildew (*Odium neolycopersici*)

The powdery mildew species *Oidium neolycopersici* (*On)* was first reported in Western Europe in 1986 (Paternotte, 1988). After that, the pathogen spread rapidly across the world. The relatively wide host range, combined with a high and fast reproduction, containing several cycles of asexual reproduction (polycyclic) per growth season makes it possible that the spores can be dispersed over great distances by wind (Brown & Hovmøller, 2002; Glawe, 2008; Jones, Whipps, & Gurr, 2001). In 2001, it was recognized as a worldwide emerging pathogen and an important threat to the protected tomato production in the moderate climatic regions of the world (Jones et al., 2001).

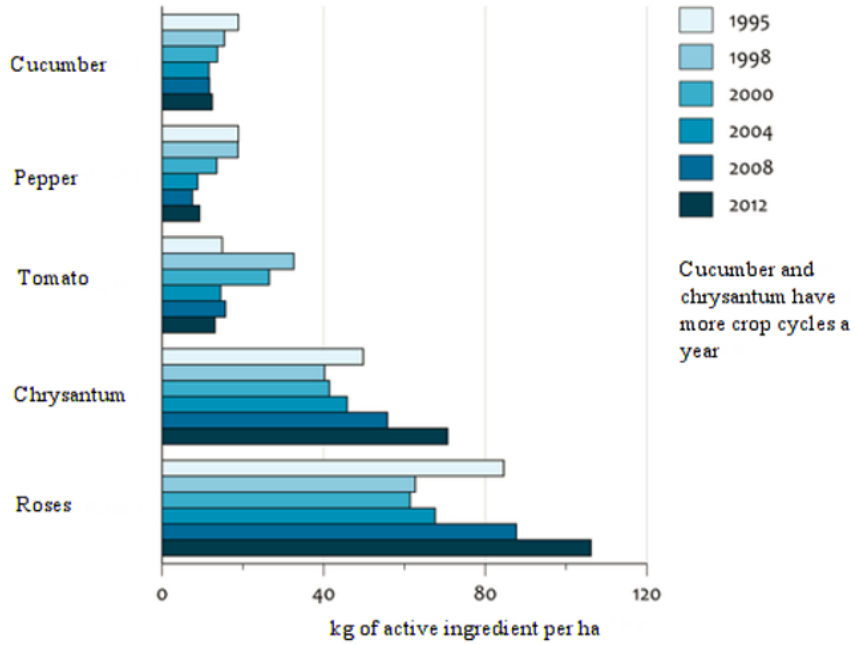


Figure 3. Use of chemical agents. Results shown are in kg / ha active ingredient in the horticulture sector in the Netherlands from 1995 up to 2012. ("Centraal Bureau voor de Statistiek," 2017)

The disease develops very well under low intensity light and temperatures between 20 and 27 C°, accompanied by high relative humidity. The optimal relative humidity for infection is between 80% and 90%, however, infection can occur at a lower relative humidity (50%) as well (Jacob, David, Sztjenberg, & Elad, 2008; J. Whipps & Budge, 2000).

1.2.1 Symptoms

During the growth season the fungus forms a long-lasting relationship with its host by forming haustoria in the plant cells. The disease first appears as small circular areas of whitish fungal growth with sporulation occurring mainly on the upper leaf surface (**Figure 4**). Other plant parts that become infected are the stem, petiole and calyx, but the fruit remains uninfected. As the sporulating lesions grow, the underlying leaf tissue turns yellow, eventually becoming brown and shriveled. Sporulation typically occurs on the upper leaf surface which distinguishes *On* from *Leveillula taurica* which sporulates on the lower surface. The plant can survive mild infection. However when the infection is severe leaf chlorosis, premature senescence and a marked reduction in fruit size and quality can appear(J. M. Whipps, Budge, & Fenlon, 1998).

Figure 4. Leaf of the susceptible cultivated *Solanum lycopersicum cv.* Moneymaker infected with the powdery mildew *Oidium neolycopersici* at 9dpi

1.2.2 Race specificities

No races are officially assigned within *On* (European Seed Association, 2017). However, interactions between near isogenic lines (NILs) and several *On* isolates have been investigated. In 2004 it was reported that resistance to *On* can be race specific as resistant plants carrying gene *Ol-4* were found to be susceptible by a Czech isolate. Because *Ol-4* is a single dominant gene based on hypersensitive response (HR), a gene-for-gene relationship is assumed (Bai, 2004).

Research has been done on two other isolates, KTP-01 and KTP-02, originating from Japan. Kashimoto *et al.* reported in 2003 that isolate KTP-01 was able to infect resistant cultivar Grace, suggesting that this Japanese isolate differs in pathogenicity compared to isolates originating from Europe and America (Kashimoto et al., 2003). In 2012, a second isolate from Japan was reported, KTP-02, harboring another virulence spectrum. Both KTP-01 and KTP-02 were tested on an accession containing the *Ol-4* resistance gene. And while *Ol-4* offers complete resistance against KTP-01, it does not towards isolate KTP-02. Progress of the infection was monitored by a digital microscope and showed that no local acquired resistance was induced. Possibly KTP-02 and the Czech isolate, mentioned before, belong to the same race (Seifi et al., 2012).

1.2.2 Possibilities for control

There are several fungicides available to control *On*, of which sulfur is the most commonly used. Sulfur is one of the oldest known fungicides and is applied to a wide array of crops. Up to the 1980s sulfur was blown naturally in the agricultural fields by industrial pollution and wind. Since then the air pollution decreased and less sulfur is naturally deposited over the fields (Bloem, Haneklaus, & Schnug, 2015). Sulfur is used as a phytoalexin In *Brassica* crops which has led to deficiencies (Williams & Cooper, 2004). Dutch horticulture companies that produce tomatoes mostly evaporate sulfur over the foliage. When this is done in a timely matter, infection can be prevented and damage can be limited.

In the study of Llorens *et al.* (2017) infected plants received different sulfur treatments. In all cases the treated plants showed lower infection levels, better physiological parameters and a higher level of chlorophyll (Llorens et al., 2017). In the Netherlands, the Dutch Board for the Authorization of Plant Protection Products and Biocides (Ctgb) assesses whether plant protection products and biocidal products are safe for humans, animals and the environment before they can be sold. Sulfur is seen as less hazardous and was therefore added to the Pesticides Exemption Scheme (RUB) which allows it to be sold on the Dutch market, without regular authorization. However, because the Dutch regulation needs to be aligned with that of other European countries, this exclusion will be withdrawn. What this means for the future allowance of sulfur is currently unknown (College voor de toelating van gewasbeschermingsmiddelen en biociden, 2018).

Additional possibilities for control are intermediate resistant (IR) tomato varieties, available as modern cultivars. High resistance or immunity is not claimed (European Seed Association, 2017). The disease resistance claims are being harmonized by the European Seed Association, with the goal to provide clear and consistent communication to the vegetable industry. In the harmonized resistance table of tomato, the variety Ducovery of De Ruiter Seeds (Monsanto) is mentioned as example variety for the IR level. The genetic makeup of IR, however, can differ between varieties, resulting in different resistance responses within the level of IR.

1.3 Resistance against *O. neolycopersici* in tomato

In search of resistance against *On*, several genomic regions that offer resistance have been identified in different wild species (**Table 1**). The resistance genes have been named *Ol-1* to *Ol-6*, of which *Ol-1* and *Ol*-3, and *Ol-4* and *Ol-6* are probably allelic (Bai, 2004; Huang, Van De Putte, Haanstra-Van Der

Meer, J.G.; Meijer-Dekens, & Lindhout, 2000). The genes and QTLs are located on chromosome 4, 6 and 12 (**Figure 5**).

Table 1. Description of *Ol* genes and QTL regions conferring resistance to tomato powdery mildew (Bai et al., 2005, 2008; Giovanni et al., 2004; Huang et al., 2000; Seifi et al., 2013)

Gene / region	Origin	Chromosome location[a]	Genetic basis	Defense mechanism
Ol-1	*S. habrochaites* G1.1560	Chr. 6: 34 Mbp	Dominant	Delayed cell death
Ol-3	*S. habrochaites* G1.1290	Chr. 6: 34 Mbp	Dominant	Delayed cell death
Ol-5	*S. habrochaites* PI247087	Chr. 6: 34 Mbp	Dominant	Slow HR
ol-2	*S. lycopersum* LA1230	Chr. 4: 38.7 Mbp	Recessive	Papillae formation
Ol-4	*S. peruvianum* LA2172	Chr. 6: 2.5 Mbp	Dominant	HR
Ol-6	Unkown	Chr. 6: 2.5 Mbp	Dominant	HR
Ol-qtl1	*S. neorickii* G1.1601	Chr. 6: 35–39 Mbp	QTL	Unkown
Ol-qtl2	*S. neorickii* G1.1601	Chr. 12: 3 Mbp	QTL	Unkown
Ol-qtl3	*S. neorickii* G1.1601	Chr. 12: 29–47 Mbp	QTL	Unkown

[a] Position based on *S. lycopersicum* 'Heinz' sequence

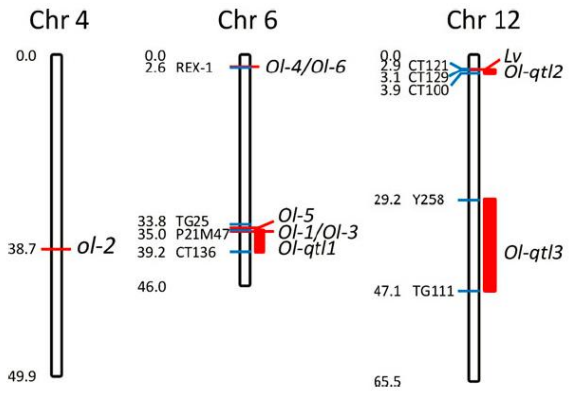


Figure 5. Physical map of the genetic regions harboring *On* resistance. The genetic regions are shown in red on chromosomes 4, 6 and 12, distance is in Mbp (Seifi et al., 2013).

1.3.1 *Ol-1*, *Ol-3* and *Ol-5*

The resistance genes *Ol-1*, *Ol-3* and *Ol-5* are derived from three different sources and are all dominant, closely linked genes on the long arm of chromosome 6. *Ol-qtl1*, found in *S. neorickii* accession G1.1601, has been located in the same region. The donor alleles provide incomplete resistance, facilitated by delayed cell death (Huang et al., 2000; Lindhout et al., 1994). Delayed cell death occurs in epidermal cells invaded by haustoria. The conidia produce functional primary haustoria and elongated secondary hyphae after which epidermal cell death occurs. This leads to moderate but not complete resistance to *On* (Bai, 2004).

Figure 6 shows the genomic region around *Ol-1* and *Ol-5*. *Ol-5* has not been fine mapped yet and still spans the region located between markers tg25 and P21M47 (Bai, 2004; Seifi, 2011). The ALS3 gene, which was shown to play an important role in *Ol-1* mediated resistance, is located as well in this region. Marker 648 is in the middle of the ALS3 gene (Gao, Huibers, Loonen, Visser, & Wolters, 2014). *Ol-1* spans a smaller region. The first markers were reported in a study from Bai et al., 2003, by making use

of the cross *S. lycopersicum* (Moneymaker) x S. *habrochaites* G1.1601. Subsequently, the location of Ol-1 was fine-mapped to a 73 Kb interval by Seifi *et al* in 2011.

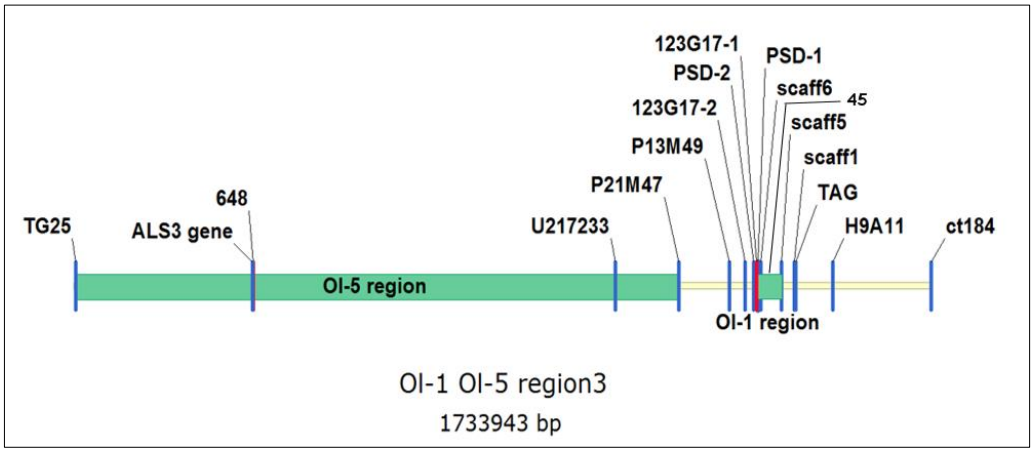
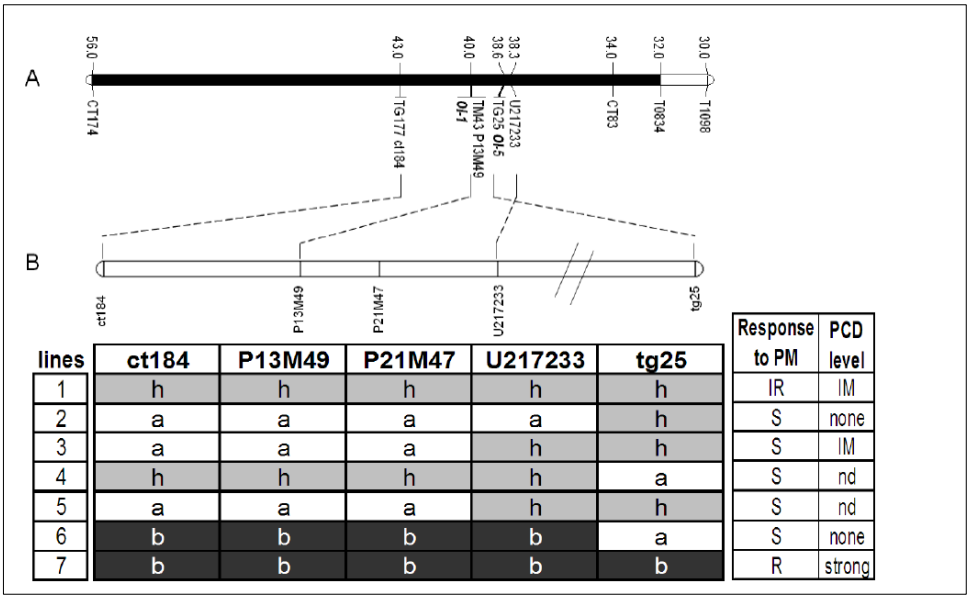


Figure 6. Overview of the *Ol-5 and Ol-1* region. The region is visualized using Vector NTI and includes the ALS3 gene and several marker locations (Wolters, 2018).

In the study of Seifi et al., 2011, it was suggested that *Ol-1* interacts with *Ol-5* to confer resistance. A recombinant inbred line (RIL) was tested which contained the *Ol-1* locus, but not *Ol-5*, and showed completely susceptibility. This indicates that *Ol-1* might be involved in the regulation and timing of *Ol-5* and can therefore be a homeodomain-leucine zipper (HD-Zip) transcription factor. **Figure 7** shows the genetic and physical map, in combination with the results of the disease test and marker assays. Line 6 was found to be susceptible to powdery mildew, but was *Ol-1*-like in the marker assay.



lines	ct184	P13M49	P21M47	U217233	tg25	Response to PM	PCD level
1	h	h	h	h	h	IR	IM
2	a	a	a	a	h	S	none
3	a	a	a	h	h	S	IM
4	h	h	h	h	a	S	nd
5	a	a	a	h	h	S	nd
6	b	b	b	b	a	S	none
7	b	b	b	b	b	R	strong

Figure 7. A. genetic map of a short part of the long arm of chromosome 6, harboring loci Ol-1 and Ol-5. B. Physical map with genotypes and phenotypes of the recombinants reported by Seifi et al 2011. Black indicates that the locus is homozygous for the donor alleleat that location (b), white indicates that the locus is homozygous for the *esculentum* allele at that location (a) and grey means heterozygous (h). The response to powdery mildew (PM): IR = Intermediate resistance, S = susceptible and R = resistant. Programmed cell death (PCD) level: IM = intermediate, nd = not defined, none and strong.

1.3.2 *Ol-2*

Unlike the other resistance genes, which are located on chromosome 6, *ol-2* is located on chromosome 4 and inherits recessively (Ciccarese, Amenduni, Schiavone, & Cirulli, 1998). The resistance is based on the formation of a papilla at the moment of penetration by the fungus (Bai et al. 2005). A 19 bp deletion is responsible for a loss of function in the *SlMlo1* gene, originating from *S. lycopersicum,* accession LA1230. The gene is located in the pericentromeric heterochromatic region of the short arm, close to the centromere (Pavan et al., 2008). This region is characterized by recombination suppression and a high number of repetitive sequences. This *S*-gene is an *mlo* homologue, conferring high sequence relatedness to similar *mlo* genes in barley and Arabidopsis and known for its high level of resistance and durability (Appiano, 2016; Bai et al., 2008; Pavan et al., 2008). *Ol-2* has, as well, a positive effect on resistance against another powdery mildew variant named *Leveillula taurica* (*Lv*) (Zheng et al., 2013). Pleiotropic effects as early senescence-like leaf chlorosis has been reported to occur both in barley and *Arabidopsis mlo* mutants. However, the introgression reported by Bai *et al.* in 2008 lacked these effects. Consonni and colleagues demonstrated that the expression of the pleiotropic effect can considerably dependent on environmental factors (Consonni et al., 2006).

1.3.3 *Ol-4* and *Ol-6*

Ol-4 confers complete resistance to *On* and originates from wild relative *S. peruvianum* LA2172. The resistance is based on fast HR which stops early development of the fungus when the primary haustoria is formed. Therefore, no hyphae or secondary appressoria are developed. The HR affects the single invaded cells and was also observed in some cells adjacent to haustorium-invaded cells (spreading necrosis). *Ol-6* originates from a breeding line with unknown ancestry and is mapped to the same position as *Ol-4* (Bai et al., 2005).

The genes inherit dominantly and are mapped in a cluster of *R*-genes on the short arm of chromosome 6 close to the centromere. The region contains the *Mi-1* gene that offers resistance against root knot nematodes, aphids, whiteflies, as well as multiple genes that offer resistance against *Cladosporium fulvum*. *Ol-4* and *Ol-6* are homologous (joined ancestry) of the *Mi-1* gene and have the advantage that they co-segregate with resistance to nematodes (Seifi et al., 2011). As *Ol-4* is multi-allelic, it is possible that *Ol-4* and *Ol-6* are different copies on the same locus (Bai et al., 2005).

The *Ol-4* region was further fine-mapped in 2011 by Seifi *et al.*, who did not find any recombinants in the area between 32.5Cla and REX-1. Both markers are localized in the middle of the *Mi-1* cluster as illustrated in **figure 8**.

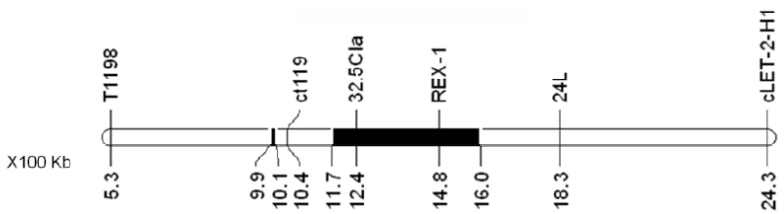


Figure 8. Physical map of the *Mi-1* gene cluster. The Black bar indicates the *Mi-1* gene cluster (11.7 to 16.0 kb), harboring the Ol-4 gene (Seifi et al., 2011)

1.3.4 *Ol-qtls*

Three QTLs were mapped in the study of Bai et al., 2003, originating from parent *S. neorickii* G1.1601. *Ol-qtl1* is mapped in the *Ol-1* region on chromosome 6, while *Ol-qtl2* and *Ol-qtl3* located on chromosome 12. *Ol-qtl2* and *Ol-qtl3* are separated by 25 cM and located in the vicinity of the *Lv* locus, which is the dominant resistance gene against the other powdery mildew species *Leveillula taurica*. The nearest marker to *Ol-qtl2* is P18M51-701c and the region is flanked by markers ct99 and ct129. The one LOD support interval of *Ol-qtl3* is flanked by Y258 and tg111, while the nearest marker is B432u. **Figure 9** shows the map of the region. The three QTLs jointly explain most of the total phenotypic variation found in parent *S. neorickii* G1.1601 and show only additive effects. This accession therefore shows a very high level of resistance, while none of the observed F2 plants, that contain all of the QTLs, was as resistant as the parent. This indicates that some minor QTLs might play a role as well. Which resistance mechanism the *Ol-qtl1, Ol-qtl2* and *Ol-qtl3* independently confer is unclear (Bai et al., 2003; Faino et al., 2012)

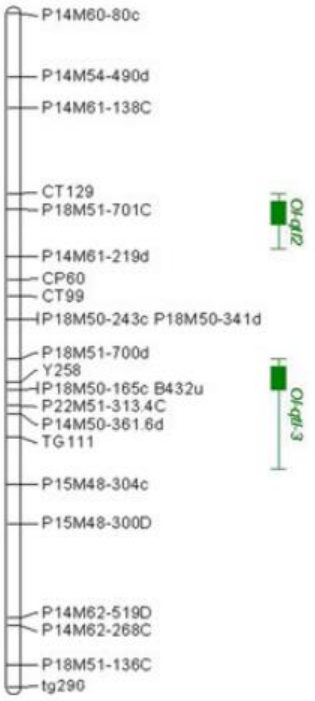


Figure 9. The positions of Ol-qtl2 and Ol-qtl3 on chromosome 12 in cM. The inner bar shows a one LOD support interval, the outer bar shows a two-LOD support interval (Faino et al., 2012).

1.4 Aim of the Project

As described above, different sources of resistance are described in literature. Detailed information of genes offering high resistance levels is available and sources and markers that can help introgress the resistance in a modern breeding line are described. Although these sources and markers are described, it is unknown whether this information is used for this purpose and how this has influenced breeding for modern cultivars. The aim of this project therefore is to get more insight in the level of resistance of modern cultivars and the allele frequency of the known resistance genes.

The key objectives of this project are:

1) Identify levels of resistance in a set of modern cultivars
2) Determine the allele frequency of resistance genes
3) Test markers and develop new markers where necessary
4) Investigate the relationship between *Ol-1* and *Ol-5*

2. Material and methods

2.1 Collect data of resistant varieties

To get insight in the varieties which were previously on the market, we requested the variety registration list at Naktuinbouw (the Netherlands Inspection Service for Horticulture). The list included all resistant cultivars that are admitted to the Dutch variety register (NRR) and contained the date of acceptance and status. The list was used as an indication of previously introduced varieties with a resistance claim, but it was not mandatory to mention the *On* resistance when subscribing to the NRR. Of note, the level of resistance was not given or checked by the Naktuinbouw.

A second list was composed, including all varieties in the current portfolio of the seed companies: De Ruiter (Monsanto), Rijk Zwaan, Syngenta, Enza Zaden, Hazera and Nunhems (Bayer). This list includes information on the level of resistance and the tomato category and only contains information that is publicly available, i.e. only includes cultivars that are mentioned in the catalogue of the seed companies.

To get insight in the year of introduction of the cultivar, registration dates of the intermediate *On*-resistant varieties of the seed companies were checked in the list of Naktuinbouw.

2.2 Plant material

For the disease test 10 modern cultivars were selected, based on category, seed company, year of introduction and availability, and ordered at Tomatoworld. They were grown in a greenhouse compartment together with 7 controls (**table 2**). Of each accession 7 plants were raised, of which 6 plants were grown out in the compartment. Due to a lack of germination, of Merlice and Belido, only 5 plants could be grown. Additionally, 2 plants of Belido, were slow in development and their DNA could not be isolated. These 2 plants were used for the disease test, but were difficult to score.

Table 2. Cultivars and controls used for the disease test and DNA isolation

	Accession	Source of resistance	Category	Seed company	Year of introduction
Control	PV033375	*Ol-1*			
Control	PV093068	*ol-2*			
Control	PV043255	*Ol-3*			
Control	PV093056	*Ol-4*			
Control	PV043258	*Ol-5*			
Control	PV043154	*Ol-qtl2* and *Ol-qtl3*			
Control	Moneymaker	Susceptible			
Cultivar	Merlice	-	Large	De Ruiter Seeds	2013
Cultivar	Rebelski	-	Beef	De Ruiter Seeds	2012
Cultivar	Brioso	-	Coctail	Rijk Zwaan	2009
Cultivar	Kanavaro	-	Beef	Enza Zaden	2013
Cultivar	Annaïsa	-	Coctail	Enza Zaden	2016
Cultivar	Maxeza	-	Large	Enza Zaden	2017
Cultivar	Diamantino	-	Medium	Enza Zaden	2013
Cultivar	Belido	-	Cherry	Syngenta	2017
Cultivar	Climstar	-	Large	Syngenta	2014
Cultivar	Funtelle	-	Cherry	Syngenta	In progress

To study the interaction between *Ol-1* and *Ol-5*, disease index results and DNA were available of 40 samples derived from plants of the accession PV033045. This accession was grown, by accident, in a previous experiment (Arce, 2018) instead of an *Ol-1* control. After inoculation, it was found segregating for resistance. It is expected that the resistance is based on the *Ol-5* gene as the BC3S2 population can be traced back to source S. *habrochaites* PI247087 (**Appendix A**).

The line was found segregating showing plants with a high degree of resistance (DI=0), plants that were slightly infected (DI= 0.25 – 1.25) and heavy infected plants (DI=2 – 3). The results at 11dpi can be seen in **figure 10**. Line PV033375, harboring the Ol-1 gene, was tested in the same experiment. The result of PV033045 suggests a 1:2:1 segregation in a semi-dominant population as 8 plants scored a DI of 0, 19 plants a DI between 0.25 and 1.25 and 8 plants a DI between 2 and 3. DNA was isolated of PV033045 and PV033375, additionally DNA was available of Moneymaker, a near isogenic line (NIL) containing *Ol-1* and a NIL containing *Ol-5* (**Table 3).**

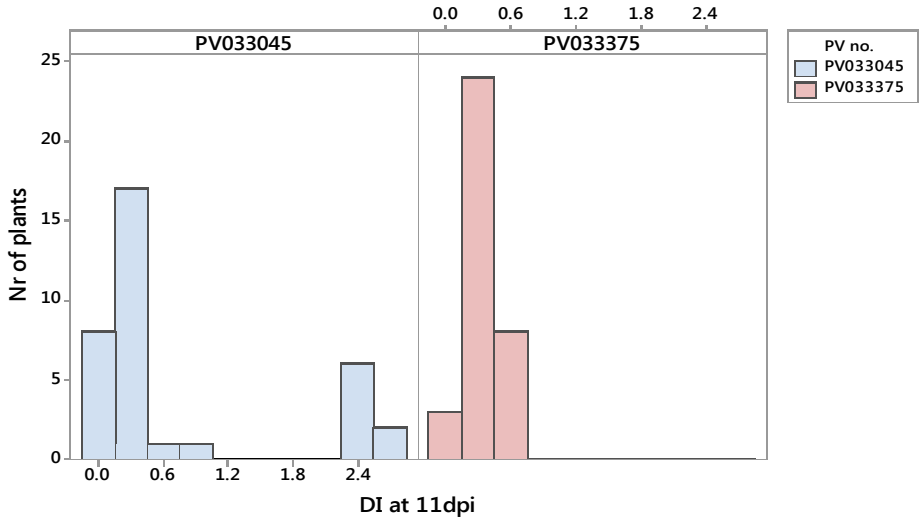


Figure 10. Disease index score of segregating population PV033045. Results are scored at 11 dpi together with *Ol-1* control PV033375.

Table 3. DNA samples used to investigate the interaction between Ol-1 and Ol-5

DNA	Resistance	Population	Origin
PV033045	Expected *Ol-5*, *Ol-3*	BC3S2	*S. habrochaites* PI247087
PV033375	*Ol-1*	BC1S2	*S. esculentum* 95-3666 from S&G seeds
NIL-Ol1	*Ol-1*	NIL	PV093052
NIL-Ol5	*Ol-5*	NIL	PV043258
Moneymaker	none	Inbred	

In a second experiment, 30 plants of PV033045 were grown, under the same conditions and with the same controls as the cultivars and controls showed in **table 2**.

2.3 Disease test

Three-week-old plants were infected with an *On* isolate, which was maintained on Moneymaker tomato plants. Spores were washed from leaves and diluted to a concentration of $2.5 \cdot 10^4$/ ml. The inoculum was uniformly sprayed on the tomato plants by making use of a spray bottle. The level of

infection was measured by scoring the disease index (DI) in a range of 0 to 3 as illustrated in **figure 11**. Score 0 represents no symptoms. Score 0.25-1: the plant is slightly affected; very few colonies are visible and possibly older colonies become greyish or brownish, indicating that the defense system is induced. Score 1-2: moderate sporulation is visible, but not as heavy as the susceptible control. And finally, the score is between 2 and 3 as abundant white sporulation is visible. Scoring is typically performed between 7 and 14 days post inoculation (dpi) (Bai, 2004).

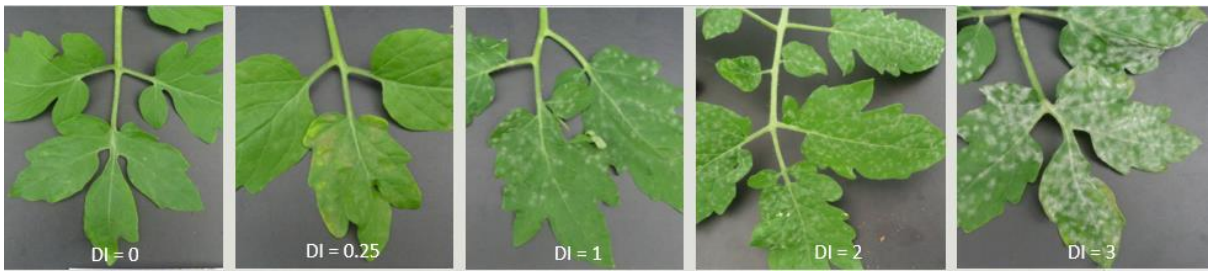


Figure 11. Disease index scores. The disease index scores are based on the infection severity of the tomato leaves.

2.4 Primers

Markers were selected from literature, except for marker 45, which was developed by our own department based on the most recent information about the location of *Ol-1*. Primers (Biolegio, Netherlands) were diluted to create a 100 µmol stock solution, from which a 10 µm work solution is made by further diluting in water.

Table 4. Overview of used markers for identification of *Ol*-genes in tomato cultivars claimed to be *On*-resistant

Marker name	Ol-gene	Forward and Reverse primer sequence	Polymorphism	Ann. Temp	Product size Ol-like (bp)	Product size MM-like (bp)
Ingene marker	*ol-2*	F- ACCCTTAAGAAACTAGGGCAAA R- ACCATCATGAACCCATGTCT	deletion	56°C	178	197
tg25	*Ol-5*	F- TAATTTGGCACTGCCGT R- TTGTYATRTTGTGYTTATCG	SCAR	52°C	350	300
648	*Ol-5*	F- TACTAGTCATGTATTCCCTTTTCCA R- ACATCCTTTTCGAGGTTCATC	SCAR	56°C	145	160
U217233	*Ol-5*	F- AGGCATAGCAATTCTATGGATGGG R- TTGGAACGTGCAGCAGATTGTC	RsaI	55°C	346+294+268+249+166+68+23	522+294+267+249+70+23
P21M47	*Ol-5, Ol-1*	F- TAACAATCTCGACCATAGTTCC R-CCATACCCGAATTTCCTTCC	DdeI / HaeIII	56°C	190+126 196+90+30	316 226+90
P13M49	*Ol-1*	F-TGCTAAGAATCAGAAACCACACCT R-ACAACAAGCTGATCCACCTAAAGA	XcmI	56°C	500	200+300
45	*Ol-1*	F- ATCCATTAATCTCCCATTCCGTCT R- GCGGATAAACTTCACCAGTCGAAA	EcoRI / MboI	60°C	280 + 140 + 27 407 + 40	420 + 27 331 + 76 + 40
32.5Cla	*Ol-4*	F- ACACGAAACAAAGTGCCAAG R- CCACCACCAAACAGGAGTGTG	HinfI	56°C	327 + 311 + 135	774
60Kb	*Ol-qtl*	F- ATGAAACCAACACAAACGCA R- ACGGCCATAACCAGACAAAG	DdeI	56°C	661	433+240

2.5 Marker analysis

The plants described in **table 2** were grown in a greenhouse and inoculated with *On*. The results were scored according a disease index and DNA was isolated from obtained samples. DNA was used to test described markers and to develop new markers by performing PCRs and gel electrophoresis. This enabled us to combine and compare disease test with marker results. **Figure 10** gives an overview of the whole procedure.

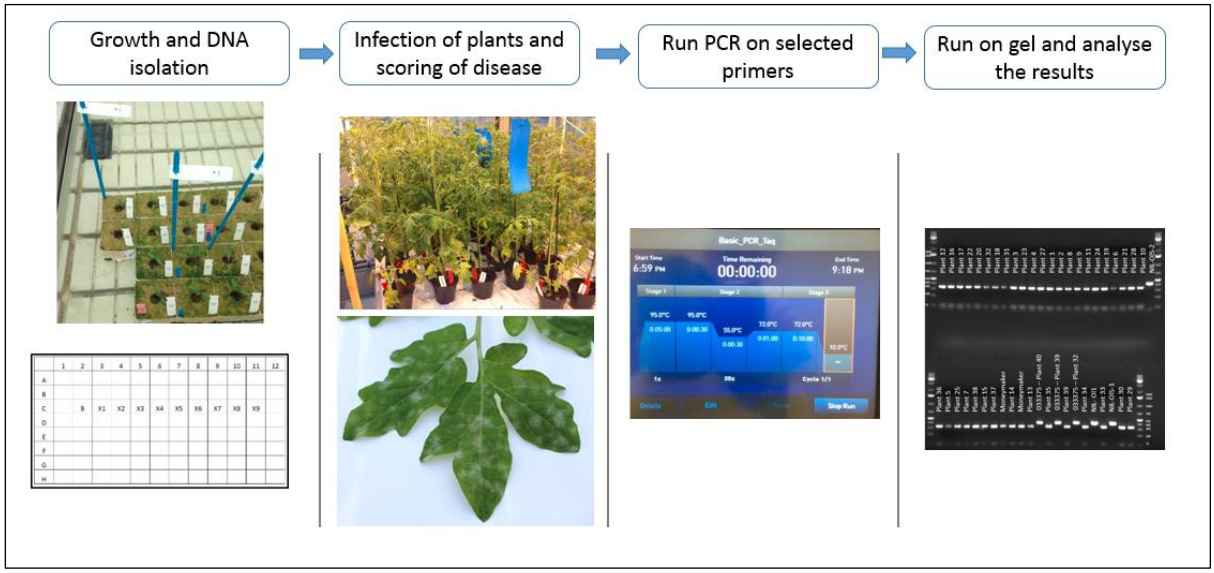


Figure 12. Marker analysis procedure. Consecutively: the plants were grown and the DNA was isolated, the plants where inoculated and the disease was scored according the disease index, the PCR was performed using the selected primers and the PCR product was run on a gel.

2.5.1 DNA isolation

For the DNA isolation, the protocol is followed for genomic DNA isolation using A CTAB buffer (RETCH protocol 1.4 (May 2007). This protocol can be found in **Appendix B**.

2.5.1 PCR protocol

To perform the PCR, a mastermix was prepared by adding per sample: 13.9 µl sterile water, 2 µl 10x buffer Dream taq, 1 µl dNTP and 0.5 µl of the forward and reverse primer. Subsequently, the Taq (0.1 µl per sample) was added to the solution and the mix was vortexed and centrifuged. From the isolated DNA, a work solution was made by diluting it 5 times. Of this work solution, 2 µl per sample was pipetted in a 96 well plate and per sample 18 µl of the mastermix is added. Finally, the plate with samples was centrifuged. **Table 5** shows the reagents for the PCR solution.

Table 5. Overview of solutions used to create a PCR sample. The total volume is 20 µl per sample.

Name	Volume per sample
H_2O (milli-Q)	13.9 µl
Dreamtaq buffer (10x)	2 µl
dNTP (5mM)	1 µl
Forward primer	0.5 µl
Reverse primer	0.5 µl
Dreamtaq	0.1 µl
DNA	2 µl

For the PCR, either a Veriti 96 well Thermal Cycler (Applied Biosystems) or a SimpliAmp Thermal Cycler (Applied Biosystems) was used. The program consists of 6 steps, explained in **table 6**, of which step 2 to 4 are repeated 35 times. Step 3 is the annealing temperature which was standard set at 56°C, but differed based on the primer used.

Table 6. Settings to run a PCR program. The temperature of step 3 can differ depending on the primer used. Total run time is approximately 1 hour and 25 minutes.

Step	Temperature	Time	
1	95°C	5 min.	
2	95°C	30 sec.	
3	56°C	30 sec.	Repeat 35x
4	72°C	1 min.	
5	72°C	10 min.	
6	10°C	∞	

2.5.2 Enzyme digestion

For CAPS markers, the PCR product was digested by a restriction enzyme overnight. A separate mastermix was created containing the enzyme and the enzyme buffer in milli-Q water, see **table 7**. Of the PCR product 4 µl is pipetted in a new plate and per well 6 µl of the mix is added. The restriction enzyme and enzyme buffer used depended on the CAPS marker it concerns.

Table 7. Overview of mixture created for enzyme digestion

Name	Volume per sample
H_2O (milli-Q)	4.9 µl
Enzyme buffer	1 µl
Restriction enzyme	0.1 µl
PCR product	4 µl
Total	**10 µl**

2.5.3 Gel electrophoresis

The PCR product was mixed with 20% loading dye and run on an agarose gel. The gel was prepared based on either TBE + 1% agarose or TAE + 1.5% agarose and 1.5% ethidium bromide. In case the band was very weak or the difference between the two bands was very small, a 1.5% TAE–containing gel was used to improve accuracy.

2.6 Sequencing

To acquire detailed information about the PCR product, some samples were sequenced by the GATC biotech company. The samples for sequencing were prepared by adding 2.5 µl of the primer to 7.5 µl of PCR product. The PCR product was checked on forehand on amplification by running it on a TBE gel with 1% agarose.

3. Results

3.1 Occurrence of resistance

3.1.1 Varieties registered as *On* resistance

In the last 15 years, between 2003 and 2017, a total of 1569 tomato varieties have been registered in the Dutch variety registration list (NRR). Of these varieties, 11,7% was claimed to be resistant to *On*. Because the registration only offers the possibility to claim cultivars as resistant or susceptible, intermediate resistance is not recorded. Thus, it is important to keep in mind that cultivars registered as resistant can also be intermediate resistant. **Figure 13** shows that the number of cultivars with and without *On* resistance claim varies over the years. In the last 5 years on average 17,2% of the cultivars that were introduced had an *On* resistance claim. The total number of cultivars includes also open field cultivars, in which *On* resistance is less important. When only the high-tech glasshouse varieties would be considered, the proportion of resistant cultivars would be considerably higher (Smilde, 2018).

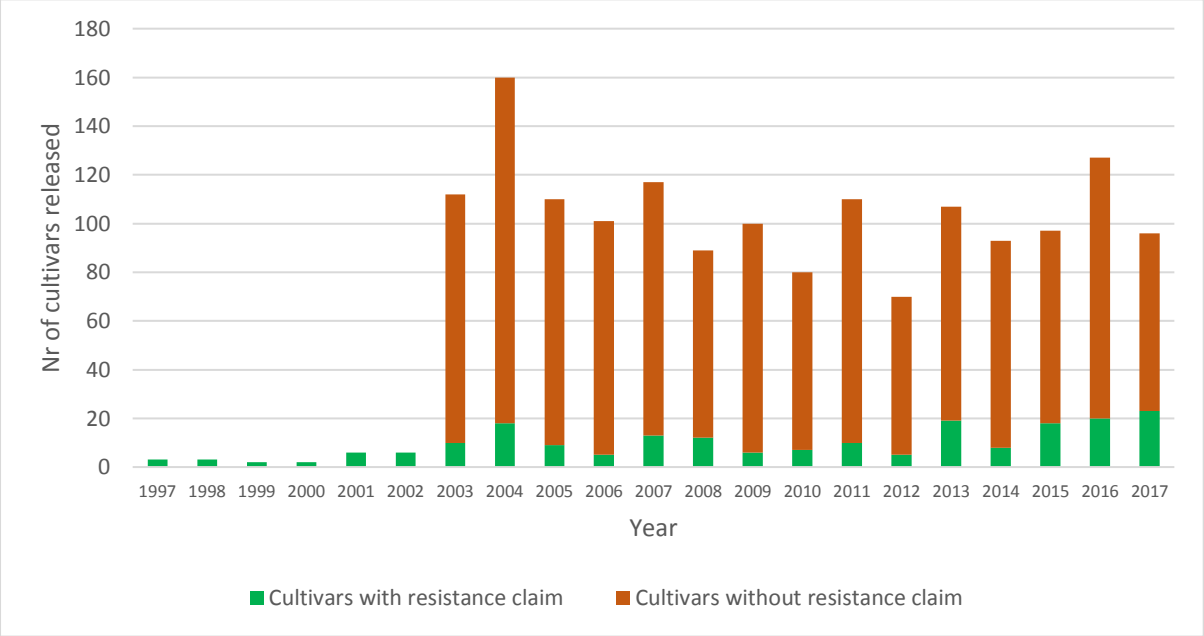


Figure 13. Frequency of registered tomato varieties (NRR) with and without *On* resistance claim. In 1997 the first varieties with *On* resistance were introduced (green). From 1997 up to 2002 Information of cultivars without an *On* resistance claim (red) is missing. The total number of cultivars includes open field varieties which underestimates the proportion of *On* resistant varieties within the glasshouse sector.

The first varieties with an *On* resistance claim were registered more than 20 years ago. Since then 213 varieties have been registered, of which 88 (41,3%) in the last 5 years. Additionally, 15 varieties are currently in the process of registration, of which 12 are offered under coded names and therefore expected to be recently developed. Another 13 varieties have a resistance claim and are listed in the seed companies' portfolio, but are not known in the NRR. Conversely, not all registered varieties are traced back to the portfolio of the seed companies. **Figure 14** shows the total number of varieties with IR claim per year and indicates whether or not the variety is found in the portfolio of the seed companies ("Website seed companies," 2018).

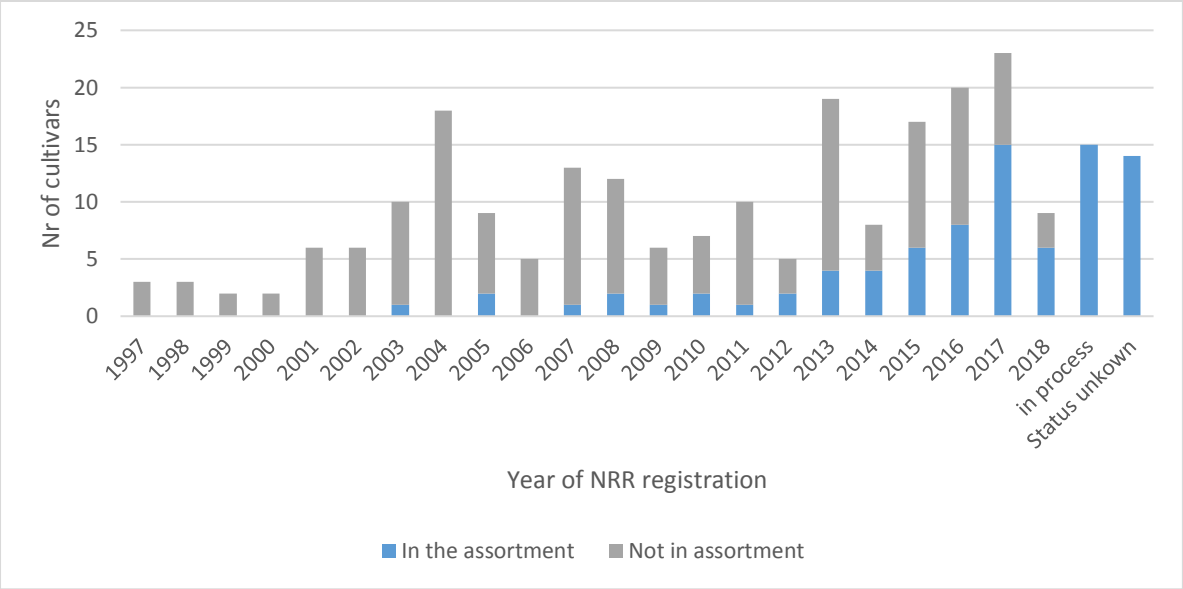
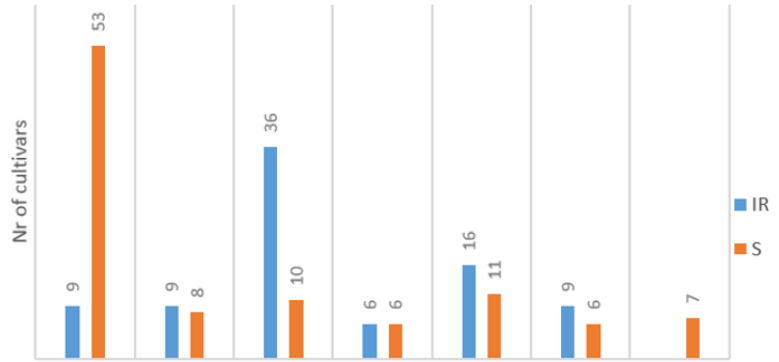


Figure 14. Overview of number of registered *On*-resistant cultivars per year. Grey bars is the proportion of *On* resistant varieties (Personal communication Smilde, 2018). Blue bars indicate cultivars that are listed in the portfolio of seed companies, in April 2018. It concerns the assortment for the Dutch, non-biological, high tech tomato greenhouse production of the following seed companies: De Ruiter Seeds (Monsanto) (www.deruiterseeds.nl), Rijk Zwaan (www.rijkzwaan.nl), Enza Zaden (www.enzazaden.nl), Axia (www.axiaseeds.nl), Nunhems (Bayer) (www.nunhems.nl) and Hazera (www.hazera.nl).

3.1.1 Varieties in the portfolio of seed companies

Of all the varieties in the seed companies portfolio, 84,8% was introduced in the last 5 years and only 7,1% of *On*-resistant varieties is older than 10 years (**Figure 14**). When all the varieties of the 7 major seed companies are taken into account, 46% are claimed to be IR ("Website seed companies," 2018). All the seed companies have both susceptible and intermediate resistant (IR) varieties in their portfolio. The percentage of cultivars with IR, however, differs between the companies and categories, as illustrated in **Figure 15A**. Within the portfolio of Enza Zaden, for example, 77% is IR against *On*, while the number of cultivars with *On* offered by De Ruiter Seeds (Monsanto) and Syngenta is only 27%. The category cherry and mini plum (<30 gram) contain a relative low number of IR varieties (17%), while medium sized tomatoes (70-140 gram) contains, with 78%, the highest. The other categories contain between 50% and 60% of IR varieties except for San Marzano and Coeur de Boeuf varieties, which are susceptible (**Figure 15A**). All seed companies offer IR varieties in the categories Medium (70-140 gram) and large (140-230 gram), but cherry and mini plum (<30 gram), cocktail (30-70 gram), plum (>70 gram) and beef (>230 gram) IR varieties are only offered by some (**Figure 15 A and B**).

A



B

Seed company	IR	S	IR	S	IR	S	IR	S	IR	S	IR	S	IR	S	Total IR	Total S	% IR of total
Enza Zaden	5	4	2		7			1	2		1				17	5	77%
Rijk Zwaan		13	6		13	1	1		2	1	3				25	15	63%
Hazera	1	6			6	1		1	3	2	1				11	10	52%
Nunhems / Bayer		1			3			2	1	1					4	4	50%
Axia	1	2	1	2	1	2	1		2	1		1			6	8	43%
De Ruiter Seeds / Monsanto		11		6	5	5	3	1	2	5	3	3		4	13	35	27%
Syngenta	2	16			1	1	1	1	4	1	1	2		3	9	24	27%
															85	101	46%

Figure 15. Overview of modern cultivars with and without *On* resistance in the portfolio of seed companies in April 2018. The varieties are shown per category (A) and per seed company (B) and divided in intermediate resistant varieties (IR) and susceptible varieties (S).

3.2 Allele frequency in modern cultivars

After identifying the intermediate resistant *On*-cultivars in the market, 10 selected cultivars and 9 controls were tested on disease severity and the allele frequency by marker analysis. The means, standard deviations and confidence intervals can be found in **table 7**.

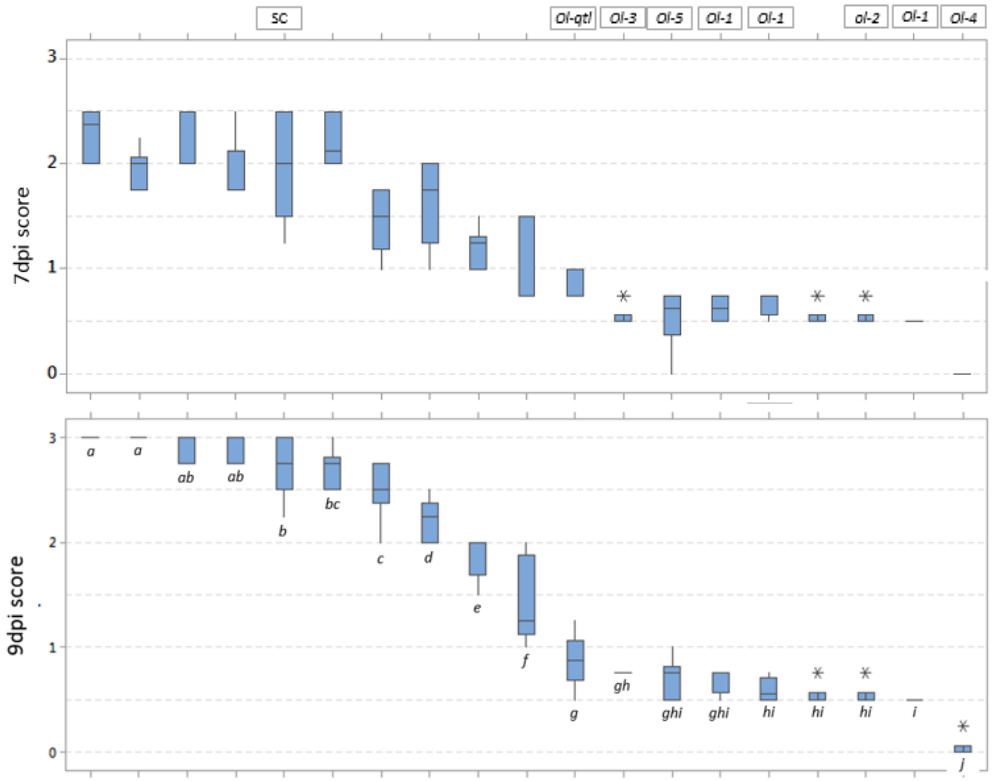
Table 7. Means, standard deviations and confidence intervals of the DI scores at 9dpi

Genotype	N	Mean DI	StDev DI	95% CI	
Annaïsa	6	2.5	0.27	2.35	2.65
Belido	5	2.2	0.21	2.03	2.37
Brioso	6	3.0	0.00	2.85	3.15
Climstar	6	2.7	0.19	2.56	2.86
Diamantino	6	2.8	0.13	2.68	2.98
Funtelle	6	3.0	0.00	2.85	3.15
Kanavaro	6	1.9	0.21	1.72	2.03
Maxeza	6	2.9	0.13	2.77	3.07
Merlice	5	1.5	0.41	1.28	1.62
Moneymaker	10	2.8	0.26	2.63	2.87
PV033373	4	0.5	0.00	0.31	0.69
PV033374	4	0.6	0.12	0.40	0.77
PV033375	4	0.7	0.13	0.50	0.87
PV043154	6	0.9	0.26	0.72	1.03
PV043255	6	0.8	0.00	0.60	0.90
PV043258	6	0.7	0.19	0.56	0.86
PV093056	6	0.0	0.10	-0.11	0.19
PV093068	6	0.5	0.10	0.39	0.69
Rebelski	6	0.5	0.10	0.39	0.69

Pooled StDev = 0.186887

The results per accession were normally distributed and the standard deviations were not significantly different (p-value Levene's test = 0.018). As shown in the ANOVA table, there was a significance difference between the mean DI scores of the accessions (p = 0.000), indicating that the genotype has a predictive value. With the Fisher's LSD test the accessions are categorized in groups *a* to *j* to show the phenotypes that are different from each other. Information about the ANOVA and the Fisher LSD test can be found in **appendix C**.

A



B

Donor	Marker																		
Ol-1	45	a	a	a	a	a	a	a	a	h	a	b	b	b	b	b	h	a	b	b
Ol-5	648	a	a	a	a	a	a	a	a	a	a	a	a	b	a	a	a	a	a	a
ol-2	ingene	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	a	a
Ol-4	32.5Cla	a	a	a	a	a	a	a	a	a	h	a	a	a	a	a	a	a	a	b
Ol-qtl1	60Kb	a	a	a	a	a	a	a	a	a	a	b	a	a	a	a	a	a	a	a

Figure 16. Phenotypic and genotypic analysis of *On* resistance in modern cultivars. The DI results at 7 and 9dpi are shown in boxplots (A). Asterisks indicate outliers and the letters in Italic in the 9dpi graph show the significant differences between groups. The accessions that function as a control are indicated by their resistance gene above the 9dpi boxplot the indication SC stands for susceptible control. Regarding the marker analysis (B) a: homozygous for the *esculentum* allele, b: homozygous for the donor allele, h: heterozygous

At 9dpi the DI of modern cultivar Rebelski is assigned to the same group as the controls of Ol-1, Ol-3, Ol-5 and ol-2, but does not share a group with any other modern cultivar. Belido, Kanavaro and Merlice, all fall in a separate group (*d*, *e* and *f*), in between the resistant controls and the susceptible control Moneymaker. The level of resistance of Annaïsa (*c*) is just below Moneymaker. The level of resistance of Moneymaker (*b*) can be related to that of Climstar (*bc*), Diamantino (*ab*) and Maxeza (*ab*). Finally, Funtelle and Brioso fall in a separate group, showing an even higher average DI than Moneymaker.

The accessions are genotyped by markers 45, 648, ol-2 marker 32.5Cla and 60Kb to identify and/or confirm the resistance alleles. The results of the disease test, combined with marker analysis are shown in **figure 16**.

Rebelski and Kanavaro were found to be heterozygous for marker 45 and therefore harbor the same donor allele on this position. Because the donor allele can be present in more backgrounds, Rebelski and Kanavaro were tested on 3 other markers in the *Ol-1* / *Ol-5* region: U217233, P13M49 and P21M47 (**Figure 6**), together with controls of PV033373 and Moneymaker **(Table 8)**.

Table 8. Marker analysis of Rebelski and Kanavaro in the *Ol-1* / *Ol-5* region. PV033375 functioned as a resistant control, harboring *Ol-1*, Moneymaker functioned as a susceptible control. Marker analysis a: homozygous for the MM allele, b: homozygous for the donor allele, h: heterozygous

Marker	Rebelski	Kanavaro	PV033375	Moneymaker
P13M49	a	a	a	a
P21M47	h	a	b	a
U217233	h	a	b	a

Rebelski was additionally found heterozygous for markers P21M47 and U217233, while Kanavaro was only heterozygous for marker 45. Marker P13M49 was not found to be polymorphic between these alleles as it showed Moneymaker-like for all the accessions including the control PV033375. DI scores and the marker analyses results are included in **Appendix D**.

3.3 Interaction of *Ol-1* with *Ol-5*

As there is still much unknown about the *Ol-1/Ol-5* region on chromosome 6, further research was done on PV033045, which was expected to contain the *Ol-5* gene without presence of the *Ol-1* gene. To confirm the absence of *Ol-1,* plants 1 to 35 were tested for marker 45, U217233 and P21M47 which are located towards the Ol-1 gene, on the right side of the Ol-5 region. These three markers were all *esculentum*-like, indicating that *Ol-1* is not present and the *Ol-5* introgression is likely to the left of U217233 (**Figure 6**). The disease index and the marker scores of the plants can be found in **Appendix E**. No band was visible on two plant samples (15 and 26), probably due to poor DNA concentration.

The presence of *Ol-5* was analyzed by molecular markers tg25 and 648. Co-dominant marker, at the most left border, tg25 (**Figure 6**) was unfortunately found to be very inconsistent on a TBE gel. When running the marker on a 2% agarose gel with ethidium bromide, the separation improved, but the bands were weak. The experiment was repeated 3 times to obtain clear results of every sample. A high correlation was obtained between the results of marker tg25 and the disease test. When the marker was *esculentum*-like (a) the DI was 2.6 ± 0.12, when the marker scored the donor allele (b) the DI was 0 ± 0.09 and when the marker scored heterozygous (h) the DI was 0.3 ± 0.21.

Marker 648, was expected to show a band at 139 bp for the donor allele and a band at 163 bp for the *esculentum* allele. This band at 139 bp was confirmed in resistant controls NIL-Ol1 and NIL-Ol5, however no bands were visible in samples of plants that scored highly resistant in the disease test, indicating that no amplification took place (**Figure 17**). This suggests that there might be an unknown

polymorphism at this locus, located in the ALS3 gene. Because it was shown that this gene plays an important role in *Ol-1* mediated resistance (Gao et al., 2014) we decided to design two new primers in this region.

The two primers (named ALS3-1 and ALS3-2) were annealed to the exons of the ALS3 gene and were designed based on polymorphisms between the sequence of Heinz compared to a contig read of a *habrochaites* draft genome (**Table 9**). Surprisingly, like in the case of marker 648, the primers did not amplify again for the highly resistant plants containing the donor allele (**Appendix F**), making it impossible to clone this region. Based on these findings however, we suggested there could be an unknown introgression at this region.

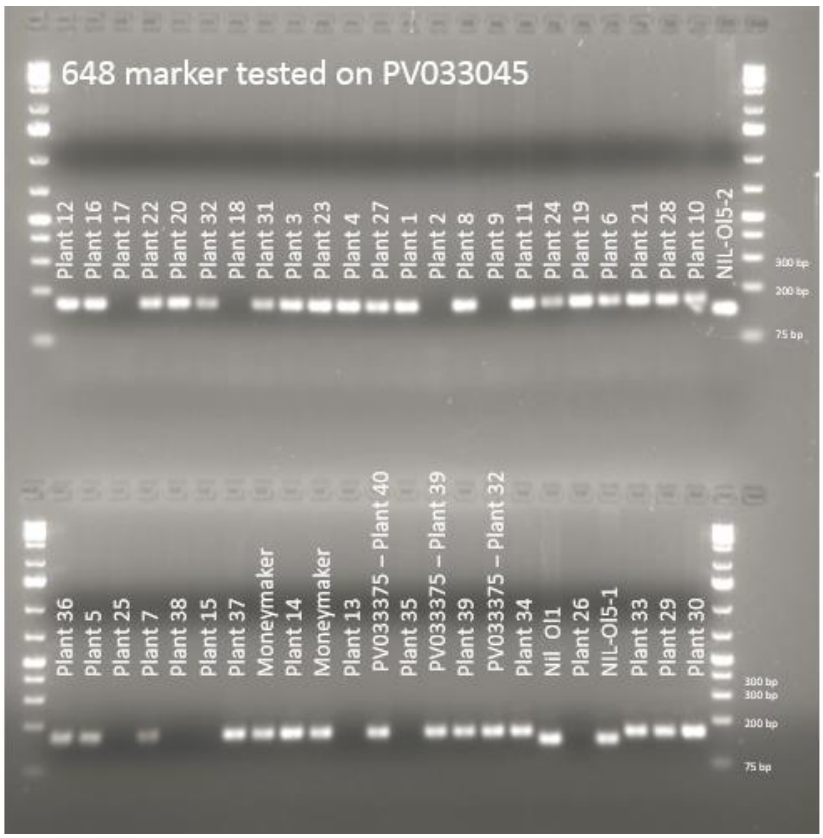


Figure 17. Marker 648 tested on PV033045. Plants 2,9, 13, 17, 18, 25, 35, 38 are homozygous dominant (based on marker tg25) and were not amplified. No band is visible for plant 15 and 26 due to improper DNA isolation.

Table 9. Primers designed within the ALS3 gene

Name primer set	Location	Forward and Reverse primes sequence	Product size (bp)
ALS3-1	Within exon of ALS3 gene	F- TGTCTGAACAATGGCTGCAT R- CACCGGTTTCTTTGATTCAG	+/- 1000
ALS3-2	within exon of ALS3 gene	F- CAGGTATTCCTGTGGCGAGT R- TTCCTTTCAGTAACACCAATGC	+/- 1300

To search the size of the introgression, seven primer pairs were developed, four at the right and three at the left of the ALS3 gene (**Appendix G**). The primer pairs are, based on the Heinz genome, in the exons of known genes. Per primer pair, 10 samples were sent for sequencing: 3 samples with a DI of

0 at 18dpi, 3 samples with a DI of 3 at 18dpi, 2 controls of Moneymaker and 1 of NIL-Ol1 and NIL-Ol5. Primer 3 and primer 7 turned out to be successfully sequenced. A fragment of these sequences can be found in **Table 10** and **Table 11**.

Table 10. Alignment of primer 3. The alignment shows SNPs at position 192 and 195. The 6 plants originate from line PV033045, in which plant 11, 37 and 14 are susceptible and plant 2, 25 and 38 are resistant.

		192 195
Primer 3		190 200 210
Translate Consensus		TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
Heinz genome	*Solyc06g059750 Exon 2(1>1250)*	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
Habrochaites	contig_22270 trimmed(1>1247)	TTTATGCCATCTTTTAGGAAGTTTCTATATTTGATCAAGTGTTAGTAG
Moneymaker	41GJ58_D04.ab1(25>301)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_E02.ab1(24>552)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_D05.ab1(24>300)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_E03.ab1(25>511)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
NIL-Ol1	41GJ58_D06.ab1(35>308)	TTTATGCCATCTTTTAGGAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_E04.ab1(22>588)	TTTATGCCATCTTTTAGGAAGTTTCTATATTTGATCAAGTGTTAGTAG
NIL-Ol5	41GJ58_D07.ab1(23>285)	TTTATGCCATCTTTTAGGAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_E05.ab1(24>464)	TTTATGCCATCTTTTAGGAAGTTTCTATATTTGATCAAGTGTTAGTAG
Plant 11	41GJ58_C10.ab1(21>549)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_D08.ab1(34>464)	TTTCTCCCATCTTTAAGAAAGTTTCAATATTTGATCAAGTGTTAGTAG
Plant 37	41GJ58_C11.ab1(26>371)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_D09.ab1(23>517)	TCTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
Plant 14	41GJ58_C12.ab1(26>483)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_D10.ab1(26>539)	TTTATGCCATCTTTAAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
Plant 2	41GJ58_D01.ab1(30>480)	TTTATGCCATCTTTTAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_D11.ab1(52>439)	TTAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
Plant 25	41GJ58_D02.ab1(29>480)	TTTATGCCATCTTTTAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
	41GJ58_D12.ab1(33>546)	TTTATGCCATCTTTTAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG
Plant 38	41GJ58_E01.ab1(52>450)	TTA- GCCTTCTTTTAGAAAGTTTCTATTTTGATCAAGTGTTAGTAG
	41GJ58_D03.ab1(33>483)	TTTATGCCATCTTTTAGAAAGTTTCTATATTTGATCAAGTGTTAGTAG

Table 11. Alignment of primer 7. The alignment shows SNPs at position 324 and 353. The 6 plants originate from line PV033045, in which plant 11 and 14 are susceptible and plant 2, 38 and 25 are resistant. Of plant 25 only the forward sequence could be captured.

		324 353
Primer 7		320 330 340 350 360
Translate Consensus		CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
Heinz genome	*Solyc06g060410 Heinz(1>1063)*	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
Habrochaites	contig_8080_Trimmed RC(1>1063)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGACGCTACTCT
Moneymaker	41GJ60_A04.ab1(44>518)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
	41GJ60_B02.ab1(24>522)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
	41GJ60_A05.ab1(25>392)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
	41GJ60_B03.ab1(24>514)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
NIL-Ol1	41GJ60_A06.ab1(27>391)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGACGCTACTCT
	41GJ60_B04.ab1(25>516)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGACGCTACTCT
NIL-Ol5	41GJ60_A07.ab1(33>475)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGACGCTACTCT
	41GJ60_B05.ab1(31>519)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGACGCTACTCT
Plant 11	41GJ58_H10.ab1(33>385)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
	41GJ60_A08.ab1(20>485)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
Plant 14	41GJ58_H12.ab1(41>517)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
	41GJ60_A10.ab1(23>513)	CTGGATATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
Plant 2	41GJ60_A01.ab1(34>478)	CTGGACATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
	41GJ60_A11.ab1(23>473)	CTGGACATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
Plant 38	41GJ60_A03.ab1(26>467)	CTGGACATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
	41GJ60_B01.ab1(22>469)	CTGGACATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT
Plant 25 (frw only)	41GJ60_A02.ab1(42>470)	CTGGACATTGAAGAACAGAAGCCCCACAGAACCGGCGCTACTCT

Primer pair 3 shows a polymorphism at location 192, that is identical to the pattern in resistant plants (2, 25 and 38), but not to the susceptible plants (11, 37 and 14). A second polymorphism, however, at position 195, is not found back in the susceptible plants. Primer 7 shows a polymorphism at position 324 that is present in the resistant plants, but not in the resistant controls and at position 353 a polymorphism that is in the resistant controls but not in the resistant plants of PV033045. The location of primer 3 and 7 can be found in **figure 18**.

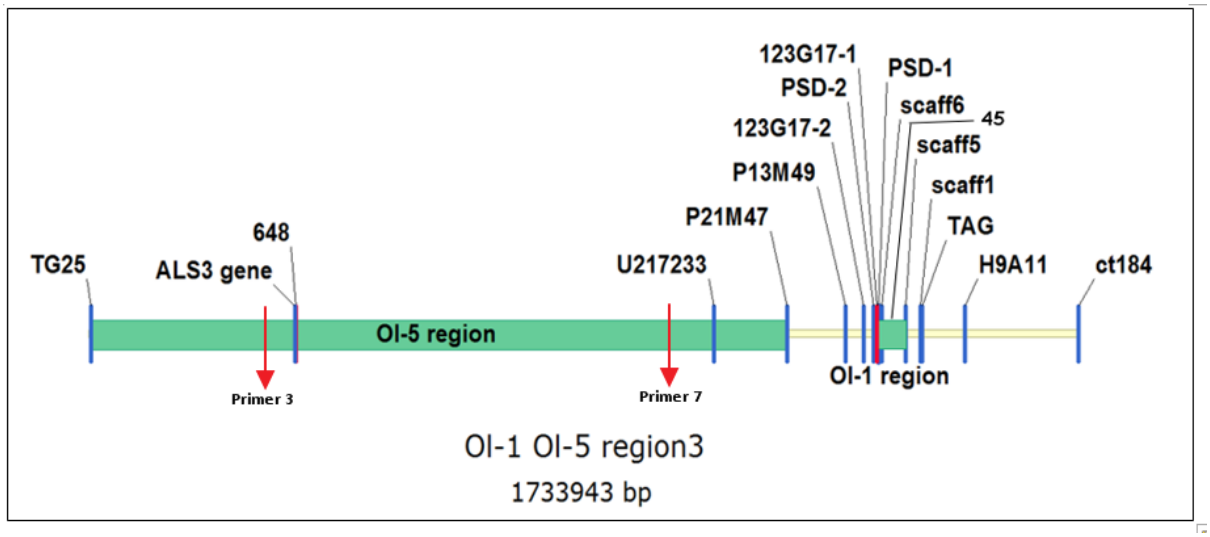


Figure 18. Location of primer pairs 3 and 7 in the *Ol-1* / *Ol-5* region. The region is visualized using Vector NTI and includes the ALS3 gene and several marker locations (Wolters, 2018)

To further investigate the role of the genes in this area, a second grow out was performed on 29 plants. As expected, the line segregated in the disease test (avg. 0.95 ± 1.25 at 9dpi), but not in the 1:2:1 deviation, as observed in the previous experiment. At 9dpi, half of the plants showed no infection symptoms, while one third of the plants were heavily infected and 4 plants showed only mild symptoms (**table 12**).

Table 12. Frequency of disease index scores of PV033045, PV043258 (*Ol-5* control) and Moneymaker at 9dpi

Disease Index score	PV033045	PV043258	Moneymaker
0	15		
0.25 – 1.25	4	6	
1.25 – 2.25			
2.25 – 3.00	10		10

Leaf samples were taken and the DNA was isolated for marker analysis. Surprisingly, in contrast to plants 1 to 35, all DNA amplified when tested on marker 648 (**Appendix H)**. The bands however, were not consistent in length. Because the difference was very subtle, the PCR product of 3 samples with a shorter band (plant 50, 62, 63) and 3 samples with a longer band (plant 49, 51, 61) were sequenced. The sequence of the shorter band was aligned with the Heinz sequence ("Solgenomics network," n.d.)

showing 2 large deletions and 3 SNPs **(Figure 19)**. The sequence of the longer band could not be retrieved and was therefore not aligned.

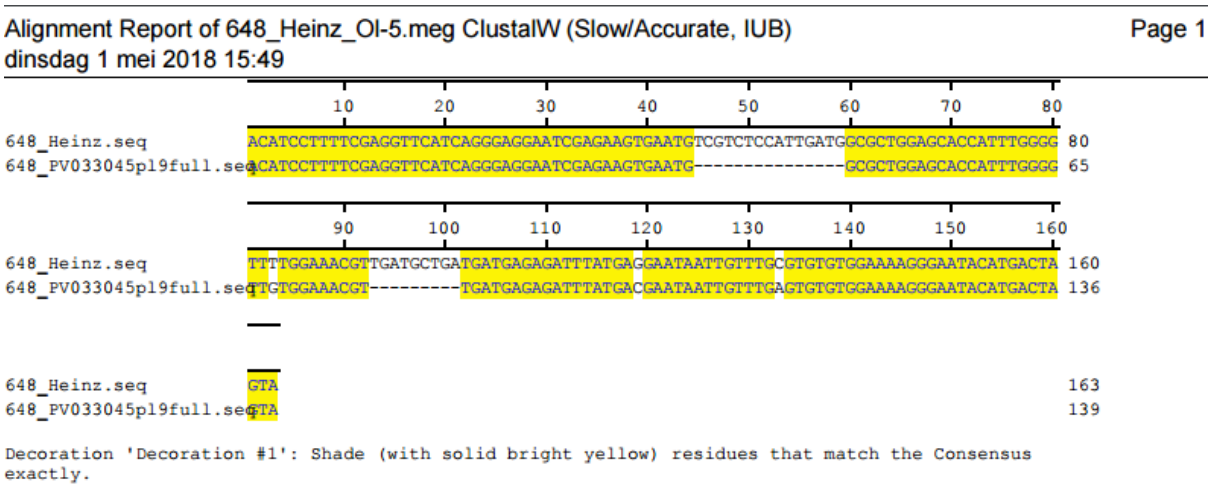


Figure 19. Alignment of the resistant PV033045 plant to the sequence of Heinz for marker 648. The alignment shows two deletions and 3 SNPs.

4. Discussion

4.1 Occurrence of (intermediate) resistant cultivars: a slow trend upwards

The first occurrence of *On* was in 1986 (Paternotte, 1988), after which no resistance cultivars were available for a long period of time. Lindhout reported in 1994 for the first time about promising sources of resistance in different *Solanaceae* species (Lindhout, Pet, & Van der Beek, 1994; Lindhout, Van der Beek, et al., 1994) and Bai *et al*. reported in 2005 that that only a few resistant cultivars were on the market (Bai et al., 2005). Since then, as can be seen in **figure 13 and 14**, there has been a gradual increase in the number of intermediate resistant *On* varieties. Currently, 213 tomato varieties with *On* resistance are registered in the NRR, of which 88 in the last 5 years. This represents 17% of the total number of introduced tomato varieties. This seems to be low, but the total number includes varieties that are not mentioned for the high-tech greenhouse sector and therefore it is expected that the true percentage of cultivars with *On* resistance for this sector is higher. This is confirmed by the assortment offered on the websites of the seven major seed companies who are breeding for the Dutch high-tech glasshouse production. According to this information, 46% of the varieties is intermediate resistant. This 46% includes varieties that are newly developed and not registered at the NRR yet. Therefor we assume that this 46% is a more accurate number than the 17%.

Most of the *On* resistant varieties in the assortment of seed companies are registered in the last 5 years (85%). These varieties are all registered as IR, and none of them as high resistance. This is remarkable as the *ol-2* and *Ol-4* genes are mapped at least 7 years ago. Resistance gene *ol-2,* known for its high level of resistance due to a loss of function in the *SlMLO1* gene, was cloned in 2008 (Bai et al., 2008). Dominant monogenic gene *Ol-4*, blocking early development of the fungus by making use of a fast HR, was fine-mapped in 2011 (Seifi, 2011). The resistances of *ol-2* and *Ol-4* are so severe that a high resistance claim should be possible. That highly resistant varieties are not on the market up to now, can indicate that these 2 genes are not in the commercial material of the seed companies. Introgression of these resistance genes in a modern background might be more time-consuming than expected, for example due to linkage drag. As all of the seed companies offer IR resistant varieties, breeding for *On* resistance is expected to be a major goal but still “work in progress”.

Regarding categories, medium truss has the highest percentage of IR varieties. All breeding companies are active in this category. They possibly give priority to this tomato type as it is medium sized and therefore can easily be crossed with larger and smaller sized tomatoes as soon as it is backcrossed in a modern background. Tomato varieties smaller than 30 grams (cherry and mini-plum) contain the fewest varieties with IR resistance. Only 9 out of 62 varieties are claimed to be IR. The plant and fruit type of these varieties are, in general, different compared to the plant type of the other categories. A cherry or cherry plum type will therefore not quickly be crossed with a medium truss type harboring the resistance. Another reason why varieties smaller than 30 grams have fewer varieties with IR might be that other traits are found to be more important, e.g. taste, visual appearance, shelf life and yield. Therefore, the priority of *On* might be higher in the other categories.

4.2 Allele frequency in modern cultivars, more than *Ol-1* to *Ol-6*

Ten varieties, belonging to 4 different seed companies, were examined in our disease test and the leaf material was analyzed for marker expression. In the disease test, variation was found in the level of resistance (**Table 12**). Only the varieties Rebelski, Kanavaro and Merlice were heterozygous for one or more markers.

4.2.1 Presence of *Ol-1* and *Ol-6*

Rebelski was found heterozygous for marker 45, U217233 and P13M49 indicating that there is a large *habrochaites*-like introgression on the locus of *Ol-1.* As the disease test shows the same level of resistance as the *Ol-1* controls, it is likely that this gene confers the resistance. Kanavaro was found to be heterozygous for marker 45 as well, but *esculentum*-like for markers U217233 and P133M49. The results of the disease test, however, showed a lower level of resistance as the *Ol-1* controls (**Table 12**). An explanation might be that *Ol-1* is expressed, but that another gene in the region is lacking to interact with it, as suggested by Seifi *et al*. in 2011 (Seifi, 2011). However, it is more likely that Kanavaro contains the same *habrochaites*-like polymorphism as the *Ol-1* gene, but it is derived from a different source. As shown in **figure 16**, the controls of *Ol-qtl1, Ol-3, Ol-4* and *Ol-5* contain the *habrochaites*-like polymorphism but lack the *Ol-1* gene. It is therefore most likely that Kanavaro does not contain the *Ol-1* gene and the heterozygous score at marker 45 is a false positive or a recombination event between *Ol-1* and marker 45.

Table 11. Results of the accessions, indicating the average DI and the presence of resistance alleles, based on origin (controls) and marker analysis (modern cultivars). DI is the average of the DI score of 6 plants of each accession and the color indicates the severity of the infection.

Accession	DI 7dpi	st dev	DI 9dpi	st dev	resistance allele present in accession
PV093056	0.000	0.000	0.042	0.102	*Ol-4*
PV033373	0.500	0.000	0.500	0.000	*Ol-1*
PV093068	0.542	0.102	0.542	0.102	*ol-2*
Rebelski	0.542	0.102	0.542	0.102	Likely *Ol-1*
PV033374	0.688	0.125	0.588	0.118	*Ol-1*
PV033375	0.625	0.144	0.688	0.125	*Ol-1*
PV043258	0.542	0.292	0.708	0.188	*Ol-5*
PV043255	0.542	0.102	0.750	0.000	*Ol-3*
PV043154	0.833	0.129	0.875	0.262	*Ol-qtl*
Merlice [1]	1.050	0.411	1.450	0.411	Possibly *Ol-6*
Kanavaro	1.208	0.188	1.875	0.209	Unknown
Belido [2]	1.650	0.418	2.200	0.209	Unknown
Annaïsa	1.458	0.292	2.500	0.274	Unknown
Climstar	2.208	0.246	2.708	0.188	Unknown
Moneymaker	1.975	0.463	2.750	0.264	None
Diamantino	1.917	0.303	2.833	0.129	Unknown
Maxeza	2.333	0.258	2.917	0.129	Unknown
Brioso	1.958	0.188	3.000	0.000	Unknown
Funtelle	2.292	0.246	3.000	0.000	Unknown

[1] The average is calculated based on 5 plants instead of 6, due to emergence

[2] The average is calculated based on 5 plants instead of 6, because 2 of these 5 plants were difficult to analyze as they were late in development on the moment of inoculation

Marker results of Merlice showed that it is heterozygous for marker 32.5Cla which is closely linked to *Ol-4*. *Ol-4* is known to be a single dominant gene based on hypersensitive response followed by programmed cell death. This mechanism is known to confer a high resistance, as no hyphae or secondary appressoria are formed (Bai et al., 2005). This high level of resistance is confirmed in the disease test by control PV093056, with an average disease index score of 0.04 ± 0.10 at 9dpi. Merlice, however, was more susceptible than the resistant control with an average DI score of 1.45 ± 0.41 at 9dpi. Therefore, the resistance of Merlice is unlikely to be mediated by the same mechanism as *Ol-4* control PV093056.

A possible explanation would be that the marker result of Merlice is a false positive, coming from a recombination between the *Ol-4* gene and the 32.5Cla marker. However, results of Seifi et al., 2011 have shown that in a population of 2000 plants, no recombinants were found between markers 32.5Cla and REX-1, flanking the *Mi-1* gene cluster that harbors the *Ol-4* gene. Therefore, it is unlikely that recombination has taken place between *Ol-4* and 32.5Cla.

A second possibility is that the polymorphism at the 32.5Cla location originates from another source than accession *S. peruvianum* LA2172. The gel picture in **figure 19** shows an extra band around 470 bp, indicating that this could be another allele of the *Ol-4* gene. As *Ol-6* is mapped at the same location as *Ol-4*, and shows a lower level of resistance at seedling stage (Bai et al., 2005), *Ol-6* would be a candidate for this source of resistance.

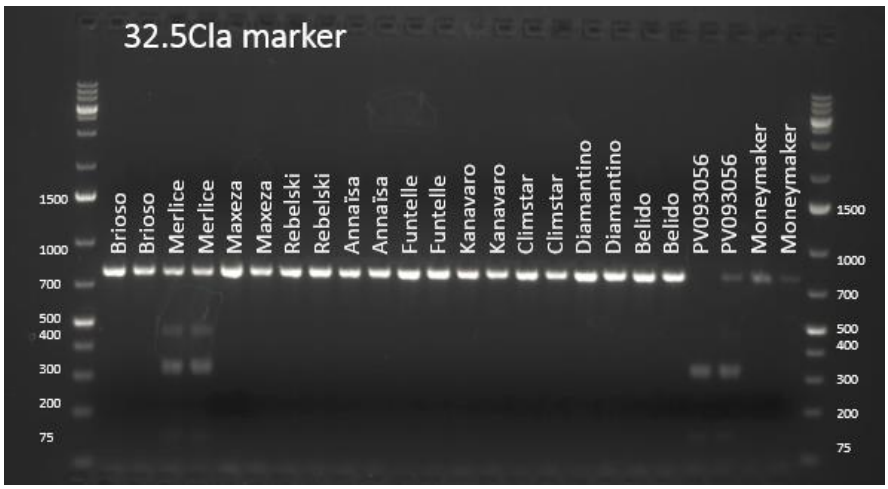


Figure 19. Gel picture of marker 32.5Cla tested on modern cultivars, Moneymaker and *Ol-4* control PV093056. The control PV093056 shows a band at 327 and 311 bp (visible as 1 band) and 135bp. The band at 774bp of the second sample of PV093056 is present because the enzyme HinfI did not fully digest the PCR product. Merlice shows bands at 774bp (*esculentum*-like) and 327 and 311 bp (visible as 1 band) and 135bp (*habrochaites*-like) and a 5th band around 470bp.

4.2.2 Can adult plant resistance be the unknown mechanism?

Now that the resistance allele of Rebelski is identified and we speculated about the resistance allele of Merlice, there are still 8 other varieties of which the resistance is unknown. It is remarkable that 5 out of 10 varieties, all claimed to be intermediate resistance, have a similar or lower resistance level than the susceptible control Moneymaker. Based on the disease test Climstar, Diamantino, Maxeza, Brioso and Funtelle could be considered as susceptible varieties. The varieties Annaïsa, Belido and Kanavaro were all more heavily infected than any of the controls, but not as heavily as Moneymaker. These results indicate that the disease test was not discriminative enough for these varieties.

The disease test is performed on 3 to 6 weeks old plants, while the disease claim of the seed companies might be based on plants in a mature stage. This would suggest that there are genes which are not expressed at a young plant stage, and could be expressed in a mature growth stage. This form of adult plant resistance (APR) was already described by the work of Mieslerova, Lebeda and Kennedy in 2003, in which line OR4061 (Rijk Zwaan) showed fast and abundant sporulation on 6 – 8 weeks old plants, but gave a low infection rate at 4 months old plants. This study concluded that valuable sources of

resistance may be overlooked, when they are only tested at the juvenile stage (Mieslerová, Lebeda, & Kennedy, 2004).

Little is known about the origin and genetic background of this form of resistance, while it is possibly present in many modern cultivars. APR was already reported in tomato in 1997, when studying resistance against *Cladosporium fulvum* in which the *Cf-9* gene cluster showed this particular resistance response (Parniske et al., 1997). In other crops, like wheat and broccoli, more research was done on APR. For example, in the work of Li *et al.,* an overview is given of the QTLs for APR against leaf rust and powdery mildew in wheat. The overview consists of 50 studies conducted during a period of 15 years prior to publication and includes many genes that are either race-specific, based on a hypersensitive reaction, or non-race-specific resistance (Li et al., 2014). In *Brassica oleracea,* downy mildew resistance genes have been both identified at the seedling stage and the adult plant stage. In 2004, Farinhó *et al.* reported specifically about the mapping of a dominantly inherited gene to downy mildew that is exclusively expressed at the adult stage (Farinhó et al., 2004).

Further research is required to establish if APR is the form of resistance that is giving modern tomato cultivars protection to *On*. Studying these varieties can identify new loci and elucidate unknown mechanisms.

4.3 The *Ol-1* and *Ol-5* interaction: choice of the population is crucial

Initially, PV033045 seemed to be the perfect line to investigate the interaction between *Ol-1* and *Ol-5*. The line segregates for a large *habrochaites*-like insertion at the *Ol-5* region, without covering the *Ol-1* gene and information from the pedigree confirmed the presence of the *Ol-5* gene. An unknown allele was found with marker 648 at the ALS3 locus. If we could prove that the high level of resistance was offered solely by *Ol-5*, evidence would be found that *Ol-1* and *Ol-5* can independently exist from each other. Although several new alleles were identified at this location, the marker 32.5Cla, close to *Ol-4,* was found to co-segregate with the population (**table 12**). Based on these findings, we speculate that the resistance is at least partially conferred by *Ol-4*.

Currently, there is still a lot unknown about the *Ol-1* and *Ol-5* region. The gene responsible for the *Ol-1*-mediated resistance is fine mapped but not identified and the *Ol-5* region still spans a big region, containing many candidate genes. Therefore, many questions regarding this defense mechanism remain unanswered, and there is no evidence that both genes can exist with or without each other. Further work is required to unravel this mystery.

The sequence of primer pair 3 and 7 of PV033045 has shown that there is a different allele at this location compared to the NIL-Ol1 and NIL-Ol5 controls. As a follow-up of this research, another backcross could be made between a resistant PV033045 plant and Moneymaker to find progeny containing a crossing over event between the *Ol-4* region and the *Ol-5* region. By making use of marker assisted selection, it would be easy to select such a plant at a young stage. When the progeny is selected without the *Ol-4* gene, but including the *Ol-5* region, the plants can be used to continue studying the true nature of *Ol-5*. However, new markers should be developed, as markers 648 and tg25, located in the *Ol-5* region are both not functioning well. Because, both markers are based on a small insertion or deletion, it will be difficult to turn them from a SCAR into a CAPS marker. Primer pairs 3 and 7, however, show many polymorphisms that can provide a basis for these new markers.

Table 12. Correlation between the polymorphism of the 32.5Cla marker and the DI score of the PV033045 population at 18dpi. Polymorphism are a: homozygous for the MM allele, b: homozygous for the donor allele, h: heterozygous

Allele	Nr of plants	Average DI	Standard dev. DI
a	9	3.00	0.00
b	8	0.03	0.09
h	22	0.11	0.13

In summary, this study provides an overview of modern cultivars with intermediate resistance against *On.* It gives insight in the genetic background of 10 cultivars, of 4 different seed companies, based on the identified resistance genes *Ol-1* to *Ol-6*. From these cultivars, the genetic background of "Rebelski" was traced back to *Ol-1,* based on markers 45, U217233 and P13M49. Cultivar "Merlice" was *habrochaites*-like at the *Ol-4* locus, based on the marker 32.5Cla, but did not show the same level of resistance as *Ol-4.* We therefore assumed that the resistance is allelic to *Ol-4* and can possibly be *Ol-6.* The genetic background of the other cultivars could not be identified. As they showed a high infection rate in the disease test, the resistance might be a form of APR. In a second study the *Ol-5* region was studied based on the segregating population PV033045. Although, no clear conclusions could be drawn from the disease test, a new allele was identified at the *Ol-5* region. Isolating this region from *Ol-4* with new markers in the *Ol-5* region, would make it possible to further identify this region.

5. Bibliography

Appiano, M. (2016). *Susceptibility pays off: Insights into the mlo-based powdery mildew resistance*. (M. Appiano, Ed.).

Aquaah, G. (2012). *Principles of Plant Genetics and Breeding* (2nd ed.). Sussex, West: Wiley - Blackwell.

Arce, L. (2018). MSc thesis, title unknown.

Bai, Y. (2004). *The genetics and mechanisms of resistance to tomato powdery mildew (Oidium neolycopersici) in Lycopersicon species*. Wageningen University and Research.

Bai, Y., Huang, C., Hulst, R. Van Der, Meijer-dekens, F., Bonnema, G., & Lindhout, P. (2003). QTLs for Tomato Powdery Mildew Resistance (Oidium lycopersici) in Lycopersicon parviflorum G1 . 1601 Co-localize with Two Qualitative Powdery Mildew Resistance Genes Hrh Ã9DÃ. *Molecular Plant-Microbe Interactions*, *16*(2), 169–176.

Bai, Y., Hulst, R. Van Der, Bonnema, G., Marcel, T. C., Meijer-dekens, F., Niks, R. E., & Lindhout, P. (2005). Tomato Defense to Oidium neolycopersici : Dominant Ol Genes Confer Isolate-Dependent Resistance Via a Different Mechanism Than Recessive ol-2. *Molecular Plant-Microbe Interactions*, *18*(4), 354–362.

Bai, Y., Pavan, S., Zheng, Z., Zappel, N. F., Reinstädler, A., Lotti, C., … Panstruga, R. (2008). Naturally Occurring Broad-Spectrum Powdery Mildew Resistance in a Central American Tomato Accession Is Caused by Loss of Mlo Function, *21*(1), 30–39.

Bloem, E., Haneklaus, S., & Schnug, E. (2015). Milestones in plant sulfur research on sulfur-induced-resistance (SIR) in Europe. *Frontiers in Plant Science*, *5*(January), 1–12. http://doi.org/10.3389/fpls.2014.00779

Boonekamp, G. (2018). LTO Glaskracht ruim 99% biologische bestrijding. *18 Apr 2018*.

Brown, J. K. M., & Hovmøller, M. S. (2002). Aerial Dispersal of Pathogens on the Global and Continental Scales and Its Impact on Plant Disease. *Science*, *297*(July), 537–542.

Causse, M., Giovannoni, J., Mondher, B., & Zouine, M. (2016). *The Tomato Genome*. (C. Kole, Ed.). Compendium of Plant Genomes.

Centraal Bureau voor de Statistiek. (2017). Retrieved March 22, 2018, from www.cbs.nl

Ciccarese, F., Amenduni, M., Schiavone, D., & Cirulli, M. (1998). Occurrence and inheritance of resistance to powdery mildew (Oidium lycopersici) in Lycopersicon species. *Plant Pathology*, *47*, 417–419.

College voor de toelating van gewasbeschermingsmiddelen en biociden. (2018). Pesticides Exemption Scheme (RUB). Retrieved November 7, 2018, from https://english.ctgb.nl/topics/low-risk-products/pesticides-exemption-scheme

Consonni, C., Humphry, M. E., Hartmann, H. A., Livaja, M., Westphal, L., Vogel, J., … Panstruga, R. (2006). Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetics*, *38*(6), 716–720. http://doi.org/10.1038/ng1806

Cossardeaux, J. (2018). Pesticides: la tomate francaise se met sous l'abri de nouveaux labels. *Les Echos*.

De Bruine, T. (2018). Een kilo zaad is duurder dan een kilo goud. *Omroep West*. Retrieved from

https://www.omroepwest.nl/nieuws/3580096/Een-kilo-zaad-is-duurder-dan-een-kilo-goud

European Seed Association. (2017). ESA website. Retrieved February 21, 2018, from https://www.euroseeds.eu/system/files/publications/files/esa_16.0794.1.pdf

Faino, L. ., Azizinia, S. ., Hassanzadeh, B. H. ., Verzaux, E. ., Ercolano, M. R. ., Visser, R. G. F. ., & Bai, Y. . (2012). Fine mapping of two major QTLs conferring resistance to powdery mildew in tomato. *Euphytica*, *184*, 223–234. http://doi.org/10.1007/s10681-011-0551-6

Farinhó, M., Coelho, P., Carlier, J., Svetleva, D., Monteiro, A., & Leitão, J. (2004). Mapping of a locus for adult plant resistance to downy mildew in broccoli (Brassica oleracea convar. italica). *Theoretical and Applied Genetics*, *109*, 1392–1398. http://doi.org/10.1007/s00122-004-1747-0

Food and Agriculture Organisation (FAO). (2018). Retrieved February 26, 2018, from www.fao.org

Gao, D., Huibers, R. P., Loonen, A. E. H. M., Visser, R. G. F., & Wolters, A. A. (2014). Down-regulation of acetolactate synthase compromises Ol-1 - mediated resistance to powdery mildew in tomato, 1–11.

Giovanni, C. De, Dell, P., Bruno, A., Ciccarese, F., Lotti, C., & Ricciardi, L. (2004). Identification of PCR-based markers (RAPD , AFLP) linked to a novel powdery mildew resistance gene (ol-2) in tomato. *Plant Science*, *166*, 41–48. http://doi.org/10.1016/j.plantsci.2003.07.005

Glawe, D. A. (2008). The Powdery Mildews : A Review of the World ' s Most Familiar (Yet Poorly Known) Plant Pathogens. *Annual Review of Phytopathology*, *46*, 27–51. http://doi.org/10.1146/annurev.phyto.46.081407.104740

Groenten en fruit. (2018). Retrieved March 22, 2018, from www.gfactueel.nl

Heuvelink, E., Costa, J. M., & Lindhout, P. (2005). *Tomatoes*. (Heuvelink E., Ed.). Crop Production Science in Horticulture.

Huang, C. C. ., Van De Putte, P. M. ., Haanstra-Van Der Meer, J.G.; Meijer-Dekens, F. ., & Lindhout, P. (2000). Characterization and mapping of resistance to Oidium lycopersicum in two Lycopersicon hirsutum accessions: evidence for close linkage of two Ol-genes on chromosome 6 of tomato. *Heredity*, *85: 511-52*.

Jaarverslag Kwaliteits Controle Bureau 2017. (2017).

Jacob, D., David, D. R., Sztjenberg, A., & Elad, Y. (2008). Conditions for Development of Powdery Mildew of Tomato Caused by Oidium neolycopersici. *Phytopathology*, *98*(24), 270–281.

Jones, H., Whipps, J. M., & Gurr, S. J. (2001). Pathogen profile The tomato powdery mildew fungus Oidium neolycopersici. *Molecular Plant Pathology*, *2*, 303–309.

Kashimoto, K. ., Sameshima, T. ., Matsuda, Y. ., Nonomura, T. ., Oichi, W. ., Kakutani, K. ., … Toyoda, H. . (2003). Infectivity of a Japanese isolate of Oidium neolycopersici KTP-01 to a European tomato cultivar resistant to O. lycopersici. *Journal of General Plant Pathology*, *69*(6), 406–408.

Li, Z., Lan, C., He, Z., Singh, R. P., Rosewarne, G. M., Chen, X., & Xia, X. (2014). Overview and Application of QTL for Adult Plant Resistance to Leaf Rust and Powdery Mildew in Wheat. *Crop Science*, *54*(october), 1907–1925. http://doi.org/10.2135/cropsci2014.02.0162

Lindhout, P., Pet, G., & Van der Beek, J. (1994). Screening wild Lycopersicon species for resistance to powdery mildew (Oidium lycopersicum). *Euphytica*, *72*, 43–49.

Lindhout, P., Van der Beek, H., & Pet, G. (1994). Wild Lycopersicon species as sources for resistance to powdery mildew (Oidium lycopersicum): Mapping of resistance gene Ol-1 on chromosome 6

of Lycopersicon hirsutum. *Acta Hortic*, *376*, 387–394.

Llorens, E., Agustí-brisach, C., González-hernández, A. I., Troncho, P., Vicedo, B., Yuste, T., … Lapeña, L. (2017). Bioassimilable sulphur provides effective control of Oidium neolycopersici in tomato , enhancing the plant immune system. *Pest Management Science*, *73*(5), 1017–1023. http://doi.org/10.1002/ps.4419

Mieslerová, B., Lebeda, A., & Kennedy, R. (2004). Variation in Oidium neolycopersici development on host and non-host plant species and their tissue defence responses. *Annals of Applied Biology*, 237–248.

Nieuw Frans predicaat voor residuvrije AGF. (2018). *Www.agf.nl*.

Parniske, M., Hammond-kosack, K. E., Golstein, C., Thomas, C. M., Jones, D. A., Harrison, K., … Jones, J. D. G. (1997). Novel Disease Resistance Specificities Result from Sequence Exchange between Tandemly Repeated Genes at the Cf-4 / 9 Locus of Tomato. *Cell*, *91*(6), 821–832.

Paternotte, S. J. (1988). Occurrence and chemical control of powdery mildew (Oidium sp.) in tomatoes. *Med. Fac. Landbouwwet Rijksuniv. Gent*, *53*, 657–661.

Pavan, S., Zheng, Z., Borisova, M., Berg, P. Van Den, Lotti, C., Giovanni, C. De, … Bai, Y. (2008). Map-vs . homology-based cloning for the recessive gene ol-2 conferring resistance to tomato powdery mildew, 91–98. http://doi.org/10.1007/s10681-007-9570-8

Peet, M. M., & Welles, G. (2005). Greenhouse Tomato Production. In H. E. (Ed.), *Tomatoes* (pp. 257–304). Crop Production Science in Horticulture.

Qian, T., Dieleman, J. A., Elings, A., De Gelder, A., Van Kooten, O., & Marcelis, L. F. M. (2011). Comparison of climate and production in closed, semi-closed and open greenhouses. *Acta Horticulturae*, (893), 807–814.

Saving energy and sustainable energy in greenhouse horticulture. (2018), 6. Retrieved from https://www.kasalsenergiebron.nl/en/

Seifi, A.;Nonomura, T.; Matsuda, Y.; Toyoda, H.; Bai, Y. . (2012). An avirulent tomato powdery mildew isolate induces localized acquired resistance to a virulent isolate in a spatiotemporal manneritle. *Molecular Plant-Microbe Interactions*, *25*(3), 372–378.

Seifi, A. (2011). *Characterization of tomato genes for resistance to Oidium neolycopersici*. Wageningen University and Research.

Seifi, A., Gao, D., Zheng, Z., Pavan, S., Faino, L., Visser, R. G. F., … Bai, Y. (2013). Genetics and molecular mechanisms of resistance to powdery mildews in tomato (Solanum lycopersicum) and its wild relatives, 641–665. http://doi.org/10.1007/s10658-013-0314-4

Seifi, A., Kaloshian, I., Vossen, J., Che, D., Bhattarai, K. K., Fan, J., … Bai, Y. (2011). Linked , if Not the Same , Mi-1 Homologues Confer Resistance to Tomato Powdery Mildew and Root-Knot Nematodes, *24*(4), 441–450.

Smilde, D. (2018). Personal communication on the allowance of tomatoes with On resistance.

Solgenomics network. (n.d.). Retrieved May 1, 2018, from www.solgenomics.net

The Tomato Genome Consortium. (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, *485*, 635–641. http://doi.org/10.1038/nature11119

Website seed companies. (2018). Retrieved April 4, 2018, from www.deruiterseeds.com/nl; www.rijkzwaan.nl; www.syngenta.nl; www.enzazaden.nl; www.hazera.nl; www.axiaseeds.nl;

www.nunhems.nl

Whipps, J., & Budge, S. (2000). Effect of humidity on development of tomato powdery mildew (Oidium lycopersici) in the glasshouse. *European Journal of Plant Pathology*, *106*(4), 395–397.

Whipps, J. M., Budge, S. P., & Fenlon, J. S. (1998). Characteristics and host range of tomato powdery mildew. *Plant Pathology*, *47*, 36–48.

Willer, H., & Lernoud, J. (Eds. . (2016). *The World of Organic Agriculture. Statistics and Emerging Trends 2016*. Frick and Bonn.

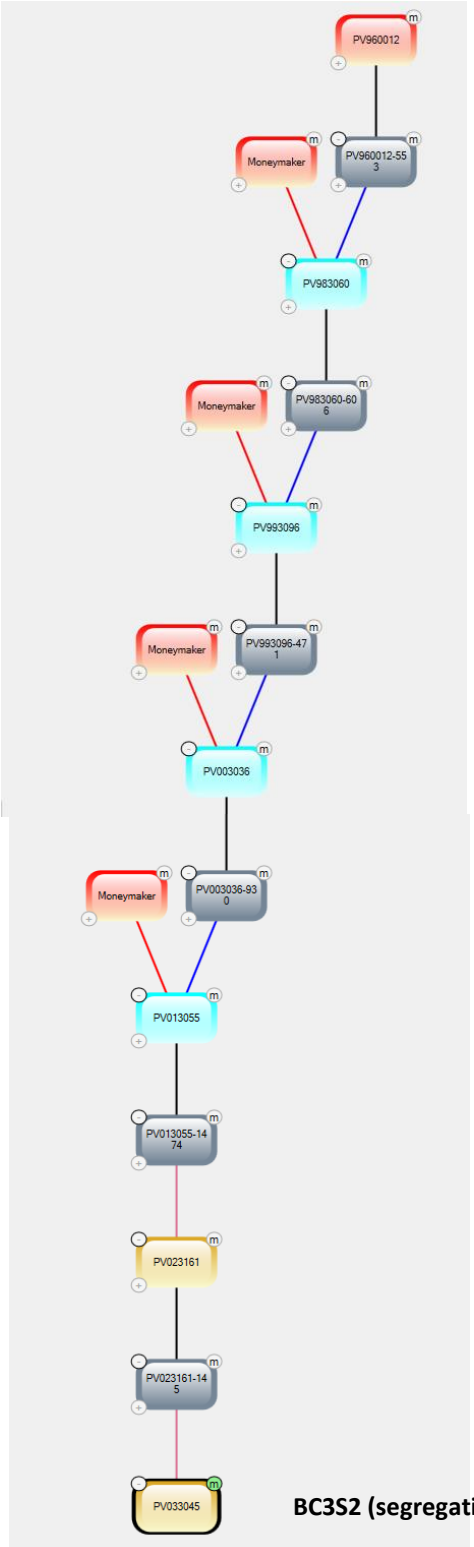
Williams, J. S., & Cooper, R. M. (2004). The oldest fungicide and newest phytoalexin – a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathology*, *53*, 263–279. http://doi.org/10.1111/j.1365-3059.2004.01010.x

Wolters, A. (2018). Personal Communication. Wageningen.

Zheng, Z., Nonomura, T., Appiano, M., Pavan, S., Matsuda, Y., Toyoda, H., … Bai, Y. (2013). Loss of Function in Mlo Orthologs Reduces Susceptibility of Pepper and Tomato to Powdery Mildew Disease Caused by Leveillula taurica, *8*(7). http://doi.org/10.1371/journal.pone.0070723

Appendix A: Pedigree of PV033045

The tree comprises the BC3S2 family of PV033045 including crosses and the parental lines.



Donor Ol-5 and Ol-3?
Laterrot
L.esc. HIROL 3-21-13-4-4
= S. habr. PI 247087

BC3S2 (segregating)

Appendix B: Protocol for genomic DNA isolation using a CTAB buffer

RETCH protocol 1.4 (May 2007) for genomic DNA isolation.

Step	Protocol
1	Fresh, young leafdisks of approximately 1x1cm were collected in 96 micronic tubes. Two steel balls were placed in every micronic tube. Samples were harvested on ice.
2	When the DNA is extracted from leaf samples at the same day as harvesting, no procedure with liquid nitrogen is needed. Otherwise the material has to be treated with liquid nitrogen and grinded in the shaker for 60 seconds.
3	Add 2 x 250µL CTAB extraction buffer with RNase (per 1ml CTAB 1µl RNase (2mg/ml) is added) to every individual leaf sample. Close the tubes tightly with caps.
4	Use the shaker to mix the de samples for 2 x 60 seconds.
5	Place the micronic tubes and holder in a press and tight the nuts carefully to prevent the lids from popping off, and incubate the samples for 60 minutes in a water bath at 65°C.
6	Cool the samples in ice water for 30 minutes, still keeping the samples in a press.
7	The following steps of the protocol must be performed in a fume hood. Add 250µl chloroform isoamyl alcohol (24:1), mix the suspension by inverting the tubes for approximately 40 times.
8	Separate the phases by centrifuging the samples at 4000RPM for 15 minutes. Thereafter pipet 400µl of the water phase into new, clean tubes. Do not touch the pellet and underlying phase.
9	Add 200µl of isopropanol to the suspension, and close the tubes with the caps. Mix the suspensions by inverting the tubes briefly.
10	Centrifuge the mix for 15 minutes at 4000 RPM in order to obtain pellets on the bottom of the tubes. Briefly throw away the suspension within the tubes, remaining only the pellets.
11	Was the pellets by adding 300µl of 70% ethanol and centrifuge the samples for 15 minutes at 4000 RPM.
12	Dry the pellets for 2 – 3 hours (or during the night). No ethanol should be present anymore within the tubes.
13	Dissolve the DNA in 100µl sterile MQ water by briefly Add 100µ sterile MQ water to the pellets, and dissolve the DNA pellets by briefly vortexing or pipetting the suspension.

Composition of CTAB buffer:

- 100ml 1Molair TRIS pH 7.5
- 140ml 5Molair NaCl
- 740ml MiliQ H_2O
- 20ml 0.5Molair EDTA pH 8.0
- 2% CTAB

Appendix C: ANOVA and Fisher Pairwise Comparisons

ANOVA on the accessions (Genotype) of the disease test at 9dpi

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Genotype	18	122.390	6.79946	194.68	0.000
Error	91	3.178	0.03493		
Total	109	125.569			

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

Genotype	N	Mean	Grouping
Funtelle	6	3.000	A
Brioso	6	3.000	A
Maxeza	6	2.9167	A B
Diamantino	6	2.8333	A B
Moneymaker	10	2.7500	B
Climstar	6	2.7083	B C
Annaïsa	6	2.500	C
Belido	5	2.2000	D
Kanavaro	6	1.8750	E
Merlice	5	1.450	F
PV043154	6	0.875	G
PV043255	6	0.7500	G H
PV043258	6	0.7083	G H I
PV033375	4	0.6875	G H I
PV033374	4	0.5875	H I
Rebelski	6	0.5417	H I
PV093068	6	0.5417	H I
PV033373	4	0.5000	I
PV093056	6	0.0417	J

Means that do not share a letter are significantly different.

Appendix D: Results of disease test and marker analysis

In the disease test all inbred lines, including Moneymaker are found to be less tall than the cultivars. The DI was scored for the individual plants at 7 and 9dpi. Marker analysis a: homozygous for the MM allele, b: homozygous for the donor allele, h: heterozygous

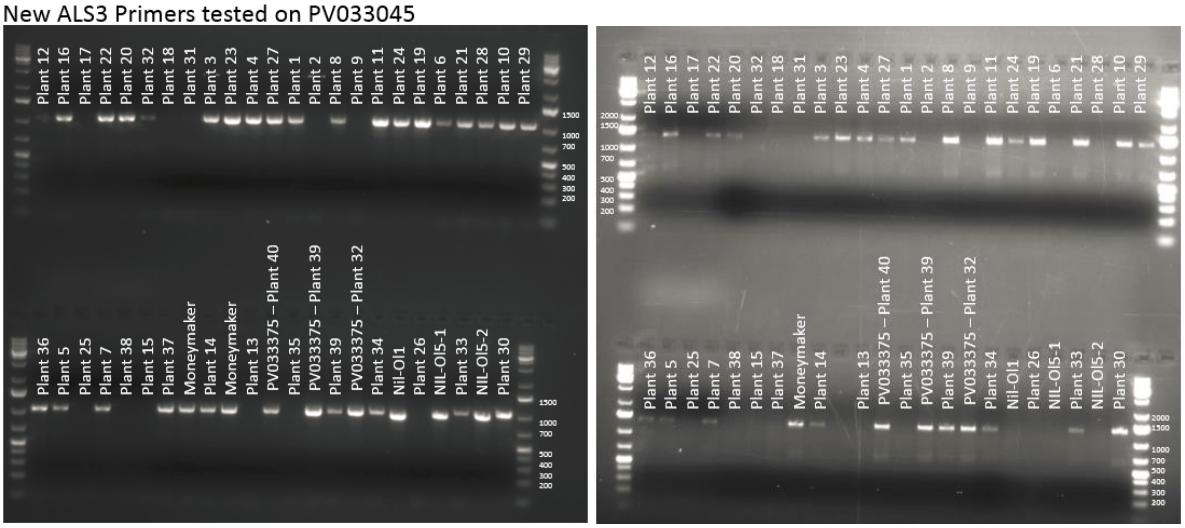
	Genotype	Plant	7dpi Score	9dpi Score	TG25	648	U217233	P21M47	P13M49	45	ol-2	32.5Cla	60KB	Comments
OI-5 INRA BC3S2	PV033045	41	0	0.25	NR	h				a				
	PV033045	42	0	0	NR	b				NR				
	PV033045	43	0	0	NR	h				a				
	PV033045	44	0	0	NR	h				a				
	PV033045	45	0	0.25	NR	h				a				
	PV033045	46	0	0	NR	b				a				
	PV033045	47	2	2.5	NR	h				a				
	PV033045	48	0	0	NR	b				a				
	PV033045	49	0	0	NR	b				a				
	PV033045	50	2.5	2.75	NR	h				a				
	PV033045	51	0	0	NR	b				a				
	PV033045	52	0.5	0.5	NR	h				a				
	PV033045	53	0	0.25	NR	h				a				
	PV033045	54	1.5	2.75	NR	h				a				
	PV033045	55	0	0	NR	h				a				
	PV033045	56	0	0	NR	h				a				
	PV033045	57	0	0	NR	b				a				
	PV033045	58	2.5	2.75	NR	h				a				
	PV033045	59	2	2.75	NR	h				a				
	PV033045	60	2.5	2.75	NR	h				a				
	PV033045	61	0	0	NR	b				a				
	PV033045	62	0	0	NR	h				a				
	PV033045	63	2	2.5	NR	h				a				
	PV033045	64	1.75	2.5	NR	h				a				
	PV033045	65	0	0	NR	b				a				headless, strange small
	PV033045	66	1.75	2.5	NR	h				a				
	PV033045	67	0	0	NR	b				a				
	PV033045	68	0	0	NR	b				a				
	PV033045	69	1.75	2.5	NR	h				a				
OI-5 INRA BC2S3	PV043258	1	0.5	0.5	NR	b				b	a			grey / brown
	PV043258	2	0.5	0.75	NR	b				b				
	PV043258	3	0	0.5	NR					b				
	PV043258	4	0.75	1	NR					b				
	PV043258	5	0.75	0.75	NR					b				grey
	PV043258	6	0.75	0.75	NR					b				
	Moneymaker	1	2	2.5	NR	a	a	a	a	NR	a	a	a	
	Moneymaker	2	1.75	2.5	NR	a	a	a	a	a	a	a	a	
	Moneymaker	3	2.25	2.75										
	Moneymaker	4	2	3										
	Moneymaker	5	2.5	3	NR					a				
	Moneymaker	6	1.5	2.75										
	Moneymaker	7	2.5	3	NR					a				
	Moneymaker	8	1.5	2.75										
	Moneymaker	9	1.25	2.25	NR					a				
	Moneymaker	10	2.5	3	NR					a				

	Genotype	Plant	7dpi Score	9dpi Score	TG25	648	U217233	P21M47	P13M49	45	ol-2	32.5Cla	60KB	Comments
ol-2 BC3S2	PV093068	1	0.5	0.5	NR					a	b			grey
	PV093068	2	0.5	0.5	NR					a	b			
	PV093068	3	0.5	0.5	NR					a	b			
	PV093068	4	0.5	0.5	NR					a	b			
	PV093068	5	0.75	0.75	NR					a	b			
	PV093068	6	0.5	0.5	NR					a	b			
Ol-qtls BC2S3	PV043154	1	1	1	NR				a	b			NR	grey
	PV043154	2	0.75	1	NR				a	b	a		b	leaf joints turn brown, leaves drop off easily
	PV043154	3	0.75	0.75	NR					b				
	PV043154	4	0.75	0.75	NR					b				
	PV043154	5	1	1.25	NR					b				
	PV043154	6	0.75	0.5	NR					b				1st leaf broken off
Ol-4 BC3S6	PV093056	1	0	0	NR					b	a	b		
	PV093056	2	0	0	NR					b		b		
	PV093056	3	0	0	NR					b				
	PV093056	4	0	0	NR					b				
	PV093056	5	0	0	NR					NR				
	PV093056	6	0	0.25	NR					NR				
Ol-3 BC3S3	PV043255	1	0.5	0.75	NR					b	a			grey / brown
	PV043255	2	0.5	0.75	NR					b				
	PV043255	3	0.5	0.75	NR					b				
	PV043255	4	0.5	0.75	NR					b				
	PV043255	5	0.75	0.75	NR					b				
	PV043255	6	0.5	0.75	NR					b				
Ol-1 S&G BC1S3	PV033373	1	0.5	0.5	NR					b				
	PV033373	2	0.5	0.5	NR					b				
	PV033373	3	0.5	0.5	NR					NR				
	PV033373	4	0.5	0.5	NR					NR				
	PV033373	5			NR					b				plant discarded before inoculation
	PV033373	6			NR					b				plant discarded before inoculation
Ol-1 S&G BC1S3	PV033374	1	0.75	0.6	NR					b				
	PV033374	2	0.5	0.5	NR					b				
	PV033374	3	0.75	0.5	NR					b				
	PV033374	4	0.75	0.75	NR					b				grey
	PV033374	5			NR					b				plant discarded before inoculation
	PV033374	6			NR					b				plant discarded before inoculation
Ol-1 S&G BC1S3	PV033375	1	0.75	0.5	NR		b	b	a	b	a			grey
	PV033375	2	0.5	0.75	NR					NR				
	PV033375	3	0.5	0.75	NR					b				
	PV033375	4	0.75	0.75	NR		b	b	a	b				
	PV033375	5			NR					NR				plant discarded before inoculation
	PV033375	6			NR					b				plant discarded before inoculation
	Brioso	1	2	3		a			a	a	a	a	a	
	Brioso	2	2	3		a			a	a	a	a	a	
	Brioso	3	1.75	3							a			
	Brioso	4	1.75	3							a			
	Brioso	5	2	3							a			
	Brioso	6	2.25	3							a			white
	Merlice	1	0.75	1.25			a	a	a		a			1st and 2nd leaf insect damage
	Merlice	2	0.75	1.25			a	a	a		a			
	Merlice	3	1.5	2		a	a	a	a	a	a	h	a	

Genotype	Plant	7dpi Score	9dpi Score	TG25	648	U217233	P21M47	P13M49	45	ol-2	32.5Cla	60KB	Comments
Merlice	4	0.75	1		a	a	a	a	a	a	h	a	
Merlice	5	1.5	1.75			a	a	a		a			
Merlice	6												not germinated
Maxeza	1	2	2.75		a			a	a	a		a	white
Maxeza	2	2.5	3		a			a	a	a		a	
Maxeza	3	2.5	3							a			
Maxeza	4	2.5	3							a			
Maxeza	5	2.5	3							a			
Maxeza	6	2	2.75							a			
Rebelski	1	0.5	0.5		a	h	h	a	h	a	a	a	
Rebelski	2	0.5	0.5		a	h	h	a	h	a	a	a	
Rebelski	3	0.5	0.5			h	h	a		a			
Rebelski	4	0.5	0.75			h	h	a		a			
Rebelski	5	0.75	0.5			h	h	a		a			brown
Rebelski	6	0.5	0.5			h	h	a		a			
Annaïsa	1	1.25	2.5		a			a	a	a	a	a	
Annaïsa	2	1.5	2.75							a			
Annaïsa	3	1	2							a			
Annaïsa	4	1.75	2.75		a			a	a	a	a	a	grey / white
Annaïsa	5	1.75	2.5							a			
Annaïsa	6	1.5	2.5							a			
Funtelle	1	2	3							a			wilting / drooping of older leaves
Funtelle	2	2	3							a			
Funtelle	3	2.25	3							a			
Funtelle	4	2.5	3		a			a	a	a	a	a	
Funtelle	5	2.5	3		a			a	a	a	a	a	white
Funtelle	6	2.5	3							a			
Kanavaro	1	1.5	2		a	a	a	a	a	a	a	a	
Kanavaro	2	1	1.75			a	a	a	h	a			grey
Kanavaro	3	1.25	2			a	a	a	h	a			
Kanavaro	4	1.25	2		a	a	a	a	h	a	a	a	
Kanavaro	5	1.25	2			a	a	a	h	a			
Kanavaro	6	1	1.5			a	a	a	NR	a			brown rings
Climstar	1	2	2.5							a			
Climstar	2	2.5	3							a			
Climstar	3	2.25	2.75							a			
Climstar	4	2	2.5		a			a	a	a	a	a	
Climstar	5	2	2.75		a			a	a	a	a	a	
Climstar	6	2.5	2.75							a			
Diamantino	1	1.75	2.75							a			
Diamantino	2	2	3							a			
Diamantino	3	1.75	2.75							a			
Diamantino	4	1.75	2.75							a			grey, ol-qtl like
Diamantino	5	1.75	2.75		a			a	a	a	a	a	
Diamantino	6	2.5	3		a			a	a	a	a	a	white
Belido	1	1.5	2.25										very small on moment of inoculation
Belido	2	2	2.25		a			a	a	a	a	a	
Belido	3	1	2										very small on moment of inoculation, difficult to score
Belido	4	1.75	2		a			a	a	a	a	a	difficult to score, small
Belido	5	2	2.5							a			
Belido	6												not germinated

Appendix E: ALS3 primer result tested on PV033045

New ALS3 Primers tested on PV033045



The primers were tested on PV033045 and the controls: Moneymaker, Pv033375, NIL-Ol1 and NIL-Ol5.

Appendix F: Results of disease test and marker analysis of PV033045

Plants were grown and evaluated on DI as part of the experiment of Arce MSc thesis, 2018. The DI was scored for the individual plants at 8, 11 and 18dpi. Marker analysis a: homozygous for the MM allele, b: homozygous for the donor allele, h: heterozygous

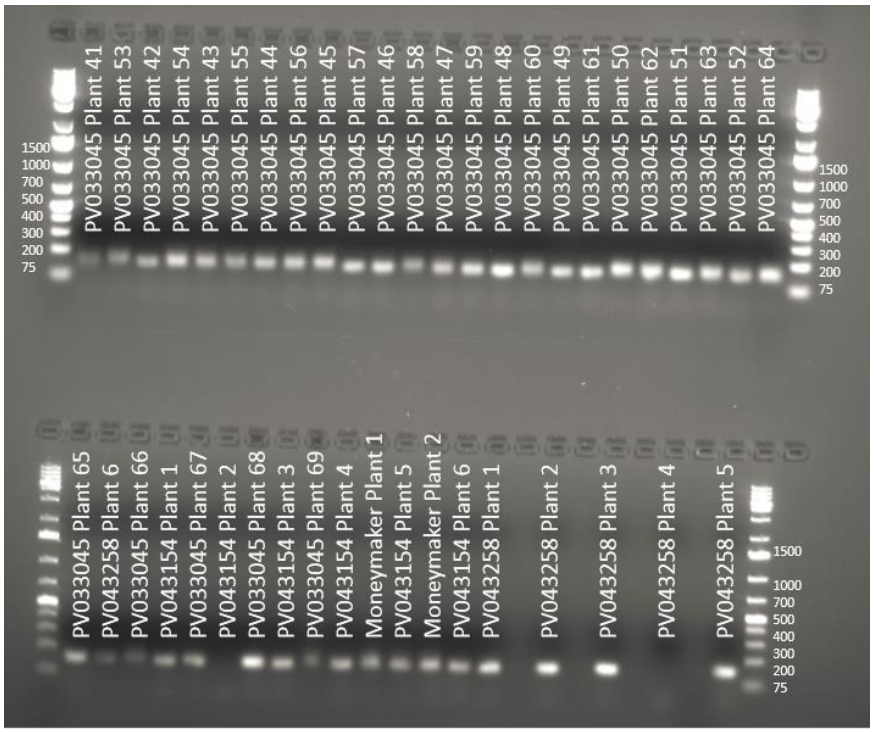
Genotype	Plant	8dpi	11dpi	18dpi	tg25	ALS3-1	ALS3-2	648	U217233	P21M47	P21M47	45	Comments
PV033045	1	0	0.25	0	h	a / h	a / h	a / h	a	NR	a	a	
PV033045	2	0	0.25	0	b	NR	NR	NR	a	a	NR	a	
PV033045	3	0	0.25	0	h	a / h	a / h	a / h	a	a	a	a	
PV033045	4	0	0.25	0.25	h	a / h	a / h	a / h	a	a	a	a	strange collonies
PV033045	5	0.25	0.25	0	h	a / h	a / h	a / h	a	a	a	a	1 white colony
PV033045	6	0.5	0.25	0.25	h	a / h	a / h	a / h	a	a	a	a	strange collonies
PV033045	7	0	0.25	0.25	h	a / h	a / h	a / h	a	a	a	a	strange collonies
PV033045	8	0	0.25	0.25	h	a / h	a / h	a / h	a	a	a	a	strange collony leaf 5
PV033045	9	0	0	0	b	NR	NR	NR	a	a	a	a	
PV033045	10	0	0	0	h	a / h	a / h	a / h	a	a	a	a	
PV033045	11	2.5	2.5	3	a	a / h	a / h	a / h	a	a	a	a	early symptoms@ 7dpi
PV033045	12	0	0.25	0.25	h	a / h	a / h	a / h	a	a	a	a	
PV033045	13	0	0	0	b	NR	NR	NR	a	a	a	a	
PV033045	14	2	2.75	3	a	a / h	a / h	a / h	a	a	a	a	
PV033045	15	0	0.25		NR	NR	NR	NR		a	a		
PV033045	16	2	2.5	3	a	a / h	a / h	a / h	a	a	a	a	
PV033045	17	0	0	0	b	NR	NR	NR	a	a	a	a	
PV033045	18	0	0	0	b	NR	NR	NR	a	a	a	a	
PV033045	19	2	2.75	3	a	a / h	a / h	a / h	a	a	a	a	
PV033045	20	2.5	2.5	3	a	a / h	a / h	a / h	a	a	a	a	
PV033045	21	2	2.5	3	a	a / h	a / h	a / h	a	a	a	a	early symptoms@ 7dpi
PV033045	22	0	0.25	0	h	a / h	a / h	a / h	a	a	a	a	
PV033045	23	1.5	2.5	3	a	a / h	a / h	a / h	a	a	a	a	very white colony
PV033045	24	0.25	1	0.25	h	a / h	a / h	a / h	a	a	a	a	
PV033045	25	0	0	0	b	NR	NR	NR	a	a	a	a	
PV033045	26	0	0.25	0	NR	NR	NR	NR	NR	NR	NR	NT	few strange collonies
PV033045	27	0.25	0.25	0	h	a / h	a / h	a / h	a	a	a	a	few strange collonies
PV033045	28	0	0.25	0	h	a / h	NR	a / h	a	a	a	a	very dry
PV033045	29	0	0.25	0	h	a / h	a / h	a / h	a	a	a	a	
PV033045	30	2	2.5	3	a	a / h	a / h	a / h	a	a	a	a	
PV033045	31	0.25	0	0	h	NR	NR	a / h	a	a	a	a	strange collonies
PV033045	32	0.5	0.5	0.25	h	a / h	NR	a / h	a	a	a	a	strange collonies
PV033045	33	0	0.25	0	h	a / h	a / h	a / h	a	a	a	a	
PV033045	34	0	0.25	0.25	h	a / h	a / h	a / h	a	a	a	a	
PV033045	35	0	0	0.25	b	NR	NR	NR	a	a	a	a	
PV033045	36			0	h	a / h	a / h	a / h	a	a	NR	a	
PV033045	37			3	a	a / h	NR	a / h	a	a	a	a	
PV033045	38			0	b	NR	NR	NR	a	a	a	a	
PV033045	39	0.25		0.25	h	a / h	a / h	a / h	a	a	a	a	
PV033375	32	0.25	0.25	0.5	a	a / h	a / h	a	b	b	b	b	Same as NIL-Ol1
PV033375	39	0	0	0.75	a	a / h	a / h	a	b	b	b	b	Same as NIL-Ol1
PV033375	40	0	0	0.5	a	a / h	a / h	a	b	b	b	b	Same as NIL-Ol1
Moneymaker					a	a / h	a / h	a / h	a	h	a	a	
Moneymaker					a	a / h	empty	a	a	h	a	a	
NIL-Ol1					NR	a / h	NR	b	b	b	b	b	Coming from ABL1 and from G1.1560 / LA2172
NIL-Ol5	1				NR	a / h	NR	b	a	b	a	b	Coming from ABL1
NIL-Ol5	2				NR	a / h	NR	b	a	b	a	b	Coming from ABL1

Appendix G: Primers designed within the Ol-5 region

The primers are, based on the Heinz genome, located within exons of known genes.

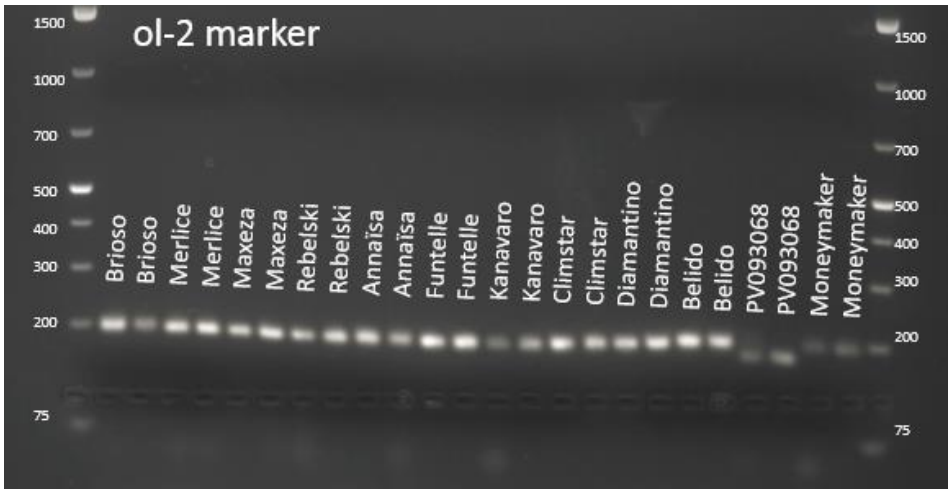
Name primer set	Location	Forward and Reverse primes sequence	Product size (bp)
MS_Ol5.1	Solyc06g054690.2	F- AAACAAGTCTAATAAAAGGTGGAGAG	633
	Between tg25 and ALS3	R- GGATTCACAAAAGGGATCTCA	
MS_Ol5.2	Solyc06g054690.2	F- TGGCAATCAGGCATATGGTA	800
	Between tg25 and ALS3	R- TAGTAGGTCGCCTTTCACAGTC	
MS_Ol5.3	Solyc06g059750.2	F- TGGATGATGCAGCAAAAGAA	649
	Between tg25 and ALS3	R- GGGTTAGGAAACTGCTGTCC	
MS_Ol5.4	Solyc06g059750.2	F- GTGGCGGACTAGGAAGTTTG	409
	Between tg25 and ALS3	R- CACATCCCGGGCTCATATAC	
MS_Ol5.5	Solyc06g060050	F- GAAAACGGGGTGTGAACAGT	800
	Between ALS3 and U217233	R- TGCTTCTTCAAGGTGTCCTG	
MS_Ol5.6	Solyc06g060270	F- TCGATTTCGTCTTCAGATAGCTC	350
	Between ALS3 and U217233	R- CCCAATATCCTTGTGGTGGA	
MS_Ol5.7	Solyc06g060410	F- CCAACGCTCTCTCTGTGTCTC	550
	Between ALS3 and U217233	R- CTTGTTCTTTCCGGCGAAGT	

Appendix H: Gel picture of marker 648 on PV033045 and controls

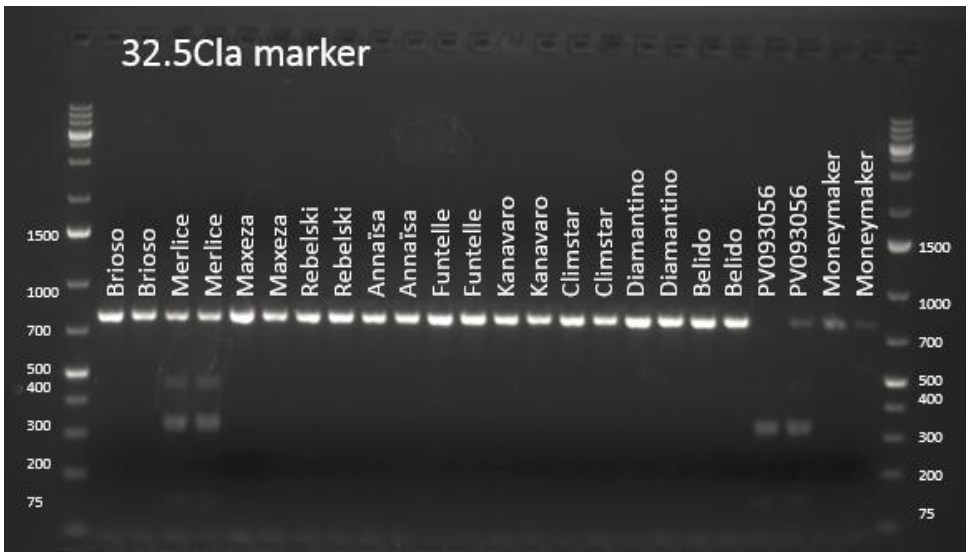


PV043258 is the control for *Ol-5* and Moneymaker is the susceptible control. The bands of the PV033045 plants are not consistent in length.

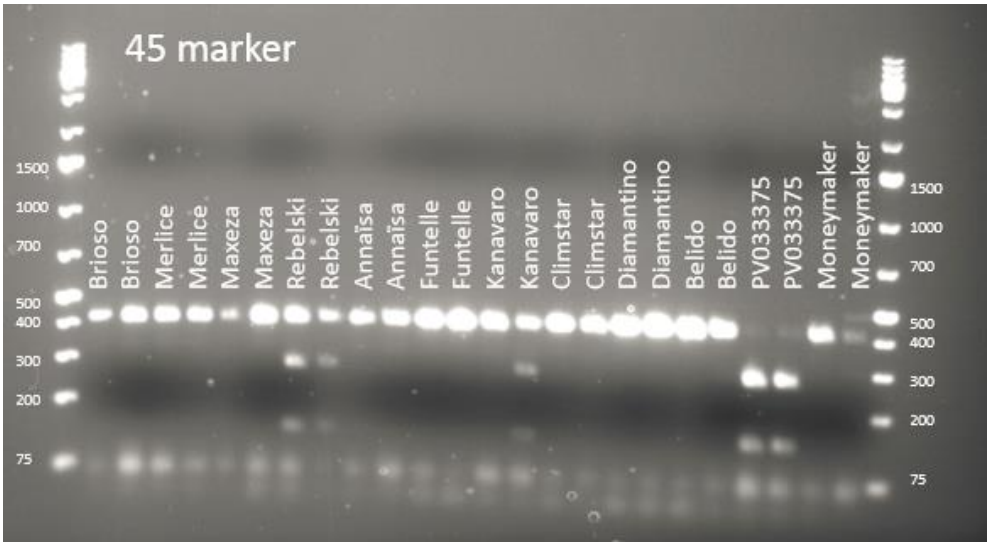
Appendix I: Gel pictures of markers *ol-2*, 32.5Cla, 45 and 648



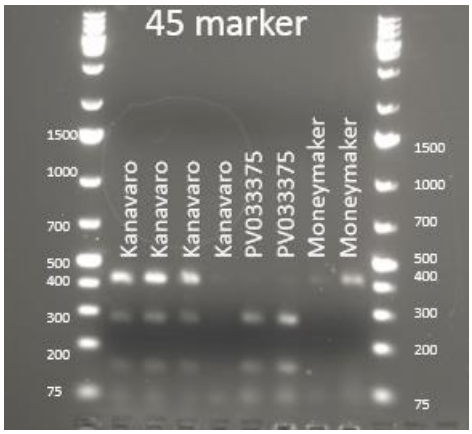
ol-2 marker on modern cultivars. PV093068 and Moneymaker are the controls



32.5Cla marker on modern cultivars. PV093056 and Moneymaker are the controls. Plant 2 of PV093056 shows a little band at 742 bp due as the pcr product was not fully digested by the enzyme hinf1.



45 marker on modern cultivars. PV033375 and Moneymaker are the controls



Repeat of marker 45 on Kanavaro. PV033375 and Moneymaker are the controls

Marker 648 on modern cultivars. PV043258 and Moneymaker are the controls

Acknowledgements

First of all I would like to thank all my supervisors. Prof. Dr. Yuling Bai and dr. Anne-Marie Wolters. Thank you for giving me the opportunity to join the Resistance in Solanaceae group, and for your great supervision and the guidance during my thesis. I learned a lot during this thesis research. Miguel Santillán thank you for introducing me in the world of markers, sequencing and disease tests. I could always pass by with questions and you took the time to answer them. Thank you for the many explanations and the great time we had in the greenhouse and the laboratory.

Also, I would like to thank René van Paasen of Tomatoworld and Diederik Smilde of Naktuinbouw for their contributions. Diederik for giving me insight in the data of the variety registration list and René for sending us seeds of the modern cultivars. This study would not have been possible without you.

I also want to share my gratitude towards all my colleagues at Monsanto Vegetable Seeds who gave me the opportunity to spent time at the University to do the experiments.

Lastly, I would like to thank my family and girlfriend for supporting me through my years of study.