Exploration of wild relatives of tomato for enhanced stress tolerance

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Exploration of wild relatives of tomato for enhanced stress tolerance

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Thesis

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ABSTRACT

Among the different abiotic and biotic stresses, *Botrytis cinerea*, *Phytophthora infestans* and high salt concentrations are world-wide the most destructive. Several wild relatives of tomato were identified as source for tolerance to these stresses. Three introgression line (IL) populations derived from *S. habrochaites* LA1777, *S. pennellii* LA716 and *S. lycopersicoides* LA2951 were employed to identify quantitative trait loci (QTL). For *B. cinerea* resistance twenty four QTLs were identified in *S. habrochaites* LA1777 and *S. lycopersicoides* LA2951. These QTLs resulted in reduced lesion size (LS) and disease incidence (DI) in leaves, stem or fruits. Five QTLs were found in *S. habrochaites* LA1777 for reduced LS in the interaction between tomato and *P. infestans*. For salt tolerance in the seedling stage ten QTLs were identified in *S. pennellii* LA716 and five in *S. lycopersicoides* LA2951. Some QTLs were semi-dominant with a non-additive or even epistatic effect. Many QTLs co-localized indicating that cross talk between coordinating pathways for abiotic and biotic stress might exist. The results provide the basis to combine QTLs with tolerance to abiotic and biotic stresses and for further narrowing down the size of the introgressions. The introgressions from these wild relatives which are involved in tolerance to multiple stresses are of interest for tomato breeders.

CHAPTER 1

## General introduction

Tomato: a dicotyledonous model crop

Tomato is an important economic crop, cultivated widely in the world. It is consumed as fresh fruit but also as various processed products. It has also been in the forefront of modern plant breeding and this has led to its development as a model crop for genetics, fleshy fruit development, secondary metabolism, disease resistance, domestication, and evolution (Labate et al. 2007). Most of the traits in tomato are governed by quantitative variation which has been accumulated over a long period of time by breeders, but little is known about the molecular basis of them. As one of the early species studied by mapping quantitative trait loci (QTL), more than 20 molecular linkage mapping studies involving over 50 traits have been reported up to date (Tanksley and Fulton 2007). Although only in a few instances the loci underlying natural quantitative variation have been cloned, the feasibility of quantitative trait map-based cloning in tomato has been established. Three QTLs controlling fruit size (*fw2.2*), shape (*OVATE*) and total soluble solids (*Brix9-2-5*) respectively, have been cloned (Frary et al. 2000; Fridman et al., 2000; Liu et al. 2002). In practice, advanced backcross QTL strategies, combining QTL analysis with variety development, have been broadly used on exploring exotic germplasm for tomato improvement (Tanksley and Nelson 1996). Furthermore, large databases for tomato have been well documented; for instance, a wealth of collected EST representing more than 30,000 unigenes from 27 different tissues and treatments has been created (Fei et al. 2006); Metabolite networks have been started to be established (Zou et al. 2006; Semel et al. 2007; Schauer et al. 2008; Iijima and Aoki 2009) and the determination of the genome sequence was initiated a few years ago (Mueller et al. 2005). All this provides a basis for improved tomato breeding.

Abiotic and biotic stress

Humans have selected tomato plants to fit their needs, environments and markets for hundreds of years. Tomato is exposed to a variety of increased abiotic and biotic stresses due to human activities, agricultural practices and natural processes. The abiotic stresses mainly affect plant metabolism and restrict plant growth and development. Abiotic stress includes salt/alkali, drought, flooding, chilling, high temperature, air pollution etc. Biotic stress such as pathogens and pests directly damage or destroy part or the whole plant. About 160 serious diseases and more than 110 pathogen species, including plant pathogenic fungi, bacteria, and virus, have been described for tomato (Tanksley and Fulton 2007). In this thesis we mainly focus on salt stress and two globally destructive pathogens, *Botrytis cinerea* and *Phytophthora infestans*, and the recent development for tomato to adapt to these stresses through breeding.

Botrytis cinerea

B. cinerea Pers.:Fr (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel) is an airborne plant pathogen with a necrotrophic lifestyle (Fig. 1), attacking over 200 crop hosts worldwide (Williamson et al. 2007; Cantu et al. 2009). It can cause heavy losses for both pre and post-harvest crop products. The most typical symptoms in tomato are initiated by rapidly enlarging water-soaked necrotic lesions followed by the appearance of grey masses, called grey mold. Tomato fruit may display symptoms of primary, non-expanding lesions referred to as ghost spots (Verhoeff et al. 1979). Sclerotia play an important role for the survival mechanism of *B. cinerea*, and it usually starts to grow in early spring in temperate regions to produce coniophores and multi-nucleate conidia, which serve as a primary source of inoculum within a crop (Williamson et al. 2007). Pectic enzymes produced by the fungus are involved in the development of soft rot. In non-heated or partially heated greenhouses, the pathogen infects primarily leaves, but lesions on the stems are also apparent. In heated greenhouses, the occurrence of leaf and fruit infections is limited but infections on stems are common (Shtienberg et al. 1998).
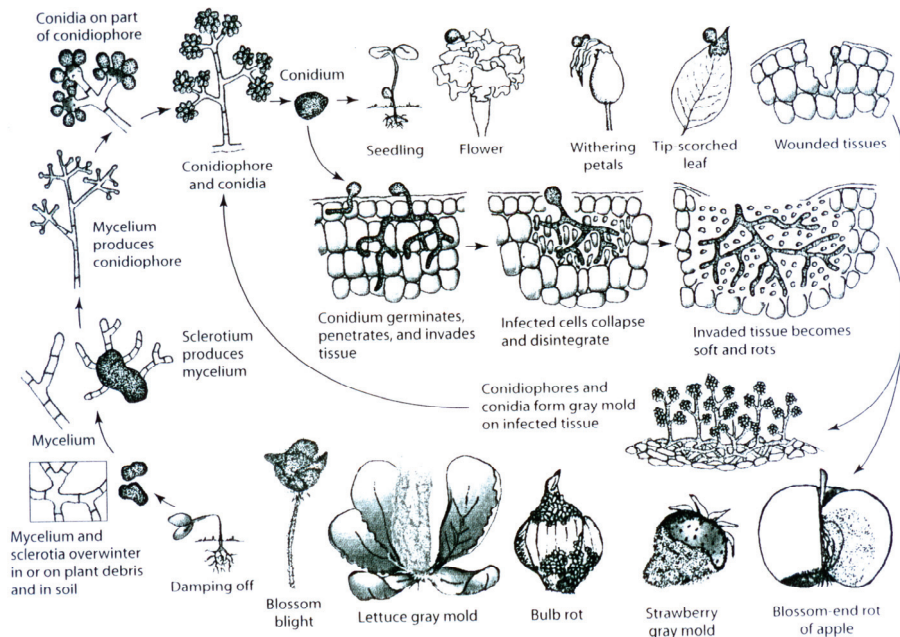
Fig. 1 Infection and symptoms caused by *Botrytis cinerea* during its life cycle. Reprinted from Plant Pathology, Agrios GN (2005), with permission from Elsevier

Resistance to *B. cinerea*

Plant defense mechanisms against necrotrophic pathogens, such as *B. cinerea*, are complex and differ from those that are effective against biotrophes. In general *B. cinerea* lands on an intact plant surface, and then appressoria secrete a spectrum of phytotoxic metabolites of low molecular weight as well as phytotoxic proteins and breach the cuticle (Colmenares et al. 2002). The ability to induce programmed cell death plays a pivotal role in the success of *B. cinerea*. The tetraspanin *BcPls1*, a membrane protein, is required for appressorium-mediated penetration of *B. cinerea* into host plant leaves (Gourgues et al. 2004). In contrast, the host plant attempts to prevent pathogen invasion and produces outgrowth by activating multiple defense pathways including the production of antifungal metabolites and pathogenesis related proteins (Van Baarlen et al. 2004). Three distinct phases during the infection of tomato leaf and several enzymes with high level of mRNA expression have been observed (Benito et al. 1998). Resistance to *B. cinerea* in an abscisic acid-deficient *S. sitiens* mutant also involves the timely production of hydrogen peroxide and cell wall modification of the epidermis (Asselbergh et al. 2007a). However, the successful protection of host plants against this

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fungus is severely hampered by the lack of resistance genes in the hosts and the considerable phenotypic diversity of the fungus (Oirdi and Bouarab 2007).

Commercial tomato varieties are more susceptible to *B. cinerea* than most wild species. Disease control in greenhouse production frequently relies on fungicides or on biocontrol. Exotic germplasm has proven to be a valuable source for disease resistance. After screening of wild relatives of tomato, several accessions including *S. chilense* LA1932, LA2747, *S. peruvianum* LA2745, *S. habrochaites* LA2314 and LYC4, *S. pimpinellifolium* 1246, *S. lycopersicoides* LA2951, *S. neorickii* G1.1601 have shown quantitative resistance to *B. cinerea* in stems or leaves (Egashira et al. 2000; Nicot et al. 2002; Guimarães et al. 2004; Finkers et al. 2007; ten Have et al. 2007; Finkers et al. 2008). In order to precisely detect the resistance involved in wild species, a quantitative tomato stem segment assay was developed (ten Have et al. 2007). Recently, progress has been made and three QTLs reducing stem lesion growth and disease incidence have been identified in a F_2 population, which was derived from a crossing between the susceptible parent *S. lycopersicum* cv. Moneymaker with the partial resistant accession *S. habrochaites* LYC4 (Finkers et al. 2007a). After the development of an introgression line population, they detected seven additional loci involved in the resistance (Finkers et al. 2007b). Lately other three QTLs were explored from *S. neorickii* G1.1601 (Finkers et al. 2008). More recently four QTLs responsible for leaf resistance on chromosome 1 through 4, and two QTLs susceptibility on chromosome 5 and 11 were identified from *S. lycopersicoides* LA2951(Davis et al. 2009).

However, the accumulating evidence from tomato, such as the complicated character of infection of this fungus, being the major post-harvest pathogen of tomato fruits, no registered post-harvest fungicide for *Botrytis* control in tomatoes and the observed low relationship between leaf and stem resistance (Nicot et al. 2002; Fallik et al. 2003; Guimarães et al. 2004), strongly force further investigation of resistance on different organs including leaf, stem and fruit, respectively.

Phytophthora infestans
Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is an oomycete and one of the most devastating diseases of tomato, as well as potato. It resulted in the notorious Irish potato famine in 1845-1846. Agrios (2005) has predicted that this disease is still one of the most likely to cause severe losses in the future. Late blight affects all above-ground parts of the tomato plant. Under favorable conditions with cool and wet weather, plants can become severely blighted in a few days or completely destroyed within a few weeks. Symptoms can be described as rapidly expanding leaf lesions, invasive brown to black stem lesions, and

irregular brown, rough, firm and zonate fruit lesions which frequently encompass much or all of the fruits (Jones et al. 1991). This pathogen has effective life cycles both in asexual and sexual stages. During the asexual stage, only one mating type A1 or A2 is involved. Asexual spores (zoospores) and sporangia can not survive in soil or on dead plant debris. In contrast, sexual stage happens only if both A1 and A2 mating types are present. Sexual spores (zoospores) can survive both in soil and on dead plant debris (Fig. 2).
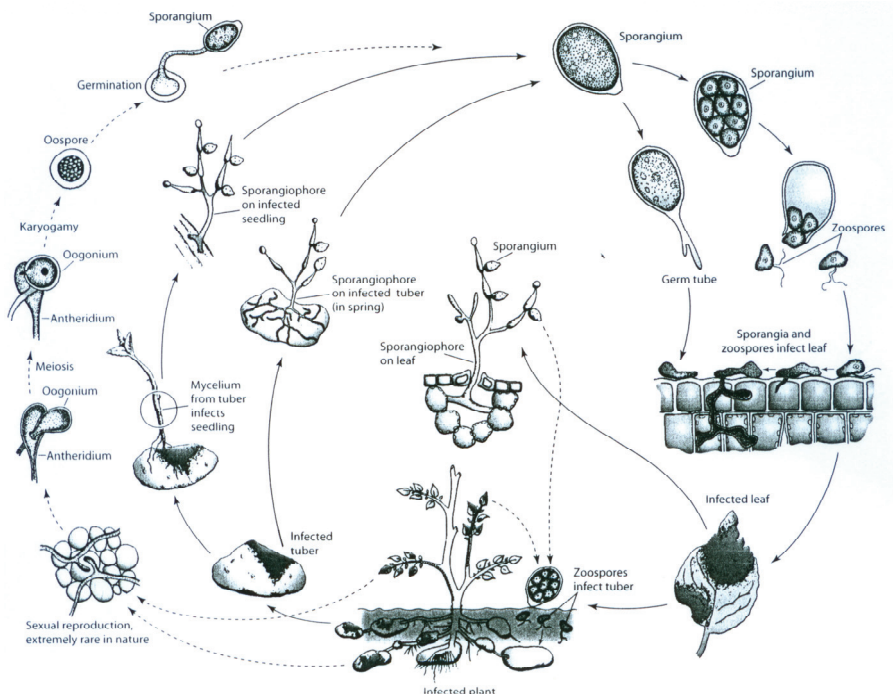
Fig. 2 The life cycle of *P. infestans* in tomato and potato. Reprinted from Plant Pathology, Agrios GN (2005), with permission from Elsevier.

Resistance to *Phytophthora infestans*

Resistance to late blight in tomato has been proven to be both qualitative and quantitative. Wild species are a source for high levels of resistance to late blight especially in two Solanaceous crops; *S. pimpinellifolium* and *S. habrochaites*. Up to now, the resistance factors derived from *S. pimpinellifolium* have a qualitative character. Five named genes have been identified from several accessions of *S. pimpinellifolium*: *Ph*-1 (Conover and Walter, 1953), *Ph*-2 (Peirce 1971), *Ph*-3 (Black et al. 1996), *Ph*-4 (Labate et al. 2007) and *Ph*-5 (Foolad et al.

2006). *Ph*-1 is a single dominant allele conditioning a high level of resistance to *P. infestans* tomato race 0 (T0) but now the rapidly developed strains have overcome its resistance. The partially dominant allele *Ph*-2 shows a good early resistance but often fails late in the season (Turkensteen 1973). *Ph*-3 gives a high level of resistance but some isolates have been identified overcoming it (Labate et al. 2007). *Ph*-4 and *Ph*-5 are recently reported genes and show resistance to a wide range of *P. infestans*. These five genes have been mapped to chromosome 7, 10, 9, 2 and 1 respectively (Peirce 1971; Moreau et al. 1998; Chunwongse et al. 2002; Foolad et al. 2006; Labate et al. 2007). Plant age strongly influences the expression of *Ph*-2 and *Ph*-3 in particular (Turkensteen 1973; Moreau et al. 1998; Chunwongse et al. 2002). Generally older plants of the same genotype tend to show greater resistance than younger plants. *Ph*-3 shows a high resistance level in five week old plants compared with *Ph*-2, the resistance of which is not fully expressed in five-week-old plants (Turkensteen 1973; Moreau et al. 1998; Chunwongse et al. 2002). Furthermore, quantitative resistance was also found within wild species, especially in *S. habrochaites* (Chunwongse et al. 2002; Brouwer et al. 2004). A total of eight QTLs, which give most consistent results in replicated experiments or across assay methods, were identified in two BC_1 populations derived from *S. habrochaites* accession LA2099 (Brouwer et al. 2004). The evidence from potato and other crops indicate that polygenic or quantitative resistance that is not associated with HR may provide a strategy for durable resistance to multiple races of a particular pathogen because race-specific genes are easily overcome by new races (Lindhout 2002; Colton et al. 2006; Sinha et al. 2006). Hence, the exploration of QTLs especially from other wild species for the resistance to *P. infestans* would provide a potential gene pool for durable resistance.

Salt tolerance in tomato
Salinity is an increasingly important environmental constraint to crop production mostly in the arid and semi-arid regions of the world (Boyer 1982; Tanjie 1990). It is estimated that about 20% of cultivated lands and 33% of irrigated agricultural lands are afflicted by high salinity in the world (Epstein et al. 1980; Tanjie 1990). The salinized agricultural areas are increasing at a rate of 10% annually (Cuartero et al. 2006). Furthermore, secondary salinisation caused by large use of chemical fertilization or inadequate irrigation management has resulted in severe yield reduction in vegetable crops (Cuartero and Fernández-Muñoz 1999). Hence, salt tolerance has become globally important for crop improvement. However, most crop plants including *S. lycopersicum* (the cultivated tomato) are sensitive or moderately sensitive to salinity stress. Accumulated evidence suggests that plant response to abiotic stress is generally complex; it is often controlled by more than one gene (Blum 1988; Foolad 2004)

and highly influenced by environmental variation (Ceccarelli and Grando 1996; Richards 1996). In addition, stress tolerance appears to be a developmentally-regulated, stage-specific phenomenon (Greenway and Munns 1980; Shannon 1985; Johnson et al. 1992; Foolad 1999).

Wild relatives of tomato adapted to dry or salt/alkali conditions have evolved tolerance against salt stress and provide the potential for the improvement of tomato. Several related wild species, including *S. pimpinellifolium, S. peruvianum*, *S. cheesmanii*, *S. habrochaites* and *S. pennellii,* have been identified and could contribute salt tolerance during the different development stages of tomato (Rush and Epstein 1976; Phills 1979; Tal and Shannon 1985; Taleisnik-Gertel and Tal 1986; Jones 1986; Foolad and Lin 1997). Taking into account that salinity in surface soils can be higher than in the subsoil, a serious problem can occur at the germination stage. Salt tolerance during seed germination has been detected with various populations, including F_2, BC_1, BC_1S_1 and recombinant inbred line (RIL) derived from *S. pennellii* LA716 and S. *pimpinellifolium* LA 722 (see review in Foolad 2004). Loci located on chromosomes 1, 3, 7, 8 and 12 were identified in the LA716 population and QTL on chromosomes 1, 2, 5, 7, 9 and 12 were detected in the LA722 population. The results proved that salt tolerance during seed germination was controlled by a few QTLs with major effects and several QTLs with small effects, and some QTLs were conserved across species whereas others were species-specific (see review in Foolad 2004).

For tomato production under saline conditions, salt tolerance during the vegetative stage is more important than salt tolerance during seed germination because most tomato crops are established by seedling transplantation rather than direct seeding in the field. However in different countries like for instance china seeds are sown directly to soil and the available water is often containing too many salts hence tolerance at the seedling stage is important   under these conditions. By different strategies using a cross between *S. lycopersicum* and *S. pimpinellifolium* LA722, four QTLs were detected on chromosomes 1, 3, 5 and 9 in a BC_1S_1 population, and five QTLs on chromosomes 1, 3, 5, 6 and 11 after selective genotyping of a BC_1 population and seven QTLs were identified on chromosomes 3, 4, 5, 7, 8, 9 and 12 in RILs (F_9) (Foolad et al. 1997; Foolad and Chen 1999; Foolad et al. 2001). The overall results from these three studies indicate that the stable QTLs on chromosomes 3, 5 and 9 can be used for introgression into cultivated tomato (see review in Foolad 2004). Several other QTLs were identified for fruit-related traits under salt tolerance (Monforte et al. 1996, 1999).

Very limited research has been conducted to identify QTLs for salt tolerance during reproduction in tomato. Breto et al. (1994) identified a few QTLs which appeared to be associated with fruit yield, fruit number and/or fruit size under salt tolerance. However,

because of the extreme difference in fruit size between the parents of this F_2 population, QTL identification was most likely confounded by the effects of genes controlling fruit size, and thus, the identified QTLs should be considered with caution and should be verified in advanced generations before use in MAS. More recently, a few, salinity specific QTLs for fruit yield were identified through two *Solanum* populations of F_7 lines, which were not associated with detrimental effects (Villalta et al. 2007). They might be used to increase tomato salt tolerance but the effect of the genetic background is crucial to breed for wide adaptation using wild germplasm (Villalta et al. 2007).

Comparison of QTLs indicated that in most cases the location of QTLs for salt tolerance during seed germination were different from that during the vegetative stage (Foolad 1999), suggesting the involvement of different genes controlling salt tolerance during seed germination and vegetative stage in this population. Salt tolerance, at any given stage of plant development in tomato is genetically not correlated with tolerance at other developmental stages (Asins et al. 1993; Foolad 1999). This has also been found in other plant species (see review in Foolad 2004). Although transgenic approaches could also provide an alternative way to improve tomato tolerance to abiotic and biotic stress and some degree of promising progress in plants have been made, there are few reports in tomato (Cuartero and Fernández-Muñoz 1999). Furthermore, implementation of transgenic abiotic and biotic-tolerance crops in practice is unlikely to occur in the near future due to legal restriction and lack of consumer acceptance. In conclusion, the great progresses have been made in tomato for salt tolerance but most of the researches are limited within very few wild species such as *S. pimpinellifolium*.

Introgression line (IL) populations for exploring desirable traits

The breeding history of the cultivated tomato has narrowed its genetic base. Reduced genetic variation of commercial cultivars, mainly caused by the repeated intercrossing of adapted elite materials, is the reason that they are more prone to be susceptible to abiotic and biotic stresses. Non-adapted or wild relatives of modern varieties have been used as an essential resource to explore the hidden valuable genes (Rick 1973; Rick and Chetelat 1995; Hajjar and Hodgkin 2007). However, most of these valuable traits are often controlled by QTLs (Tanksley and Fulton, 2007). Molecular markers allow the analysis of the genetic control of quantitative traits with complex characters by mapping QTL, which requires a segregating population that is derived from the cross between two parents with different traits of interest. The most common populations for QTL analysis are F_2 or BC_1. Advanced backcross (AB) QTL analysis was also proposed (Tanksley and Nelson 1996) as a novel plant breeding

scheme designed to integrate the processes of QTL discovery and variety development. Recombinant inbred lines (RIL) as a permanent population can be conducted for one or more interesting traits in multiple environments. However, the efficiency of detecting a particular QTL in a segregating population is still low partly because other QTLs are segregating and major QTLs are masking the minor ones. For this reason, Eshed and Zamir (1994a) proposed the use of introgression line (IL) libraries. It provides a powerful tool for QTL exploration (Eshed et al. 1992; Zamir 2001; Lippman et al. 2007).

IL populations

An IL population consists of a series of lines harboring a unique segment from a wild progenitor introgressed into a uniform, cultivated genetic background. Ideally all lines together present the complete genome of the wild progenitor. Up to now, several IL populations have been publicly reported and they are respectively derived from *S. pennellii* LA716 (Eshed and Zamir 1994a), *S. habrochaites* LA1777 (Monforte and Tanksley 2000), *S. habrochaites* LA407 (Francis et al. 2001), *S. habrochaites* LYC4 (Finkers et al. 2007), *S. chmielewskii* LA1840 (Labate et al. 2007; Prudent et al. 2009) and *S. lycopersicoides* LA2951(Canady et al. 2005). In addition, Doganlar et al. (2002) developed a set of 196 IBLs (*S. lycopersicum* × *S. pimpinellifolium* LA1589) which could also present good starting material for the development of a complete IL genome population (Labate et al. 2007). Moreover, IL populations also have been developed in Arabidopsis and other crops including melon, lettuce, wheat, rice, barley as reviewed by Labate et al. (2007). These clues hinted that IL populations are playing an important role for exploring exotic germplasm and showed the prospects for understanding the genetic base of complex plant traits.

Mapping in IL populations

The efficiency of IL libraries in detecting and mapping QTLs is due to the near-isogenic nature of the lines. The advantages of ILs are: (1) multiple replicates are possible, which enables a more reliable estimation of the effect of the QTL; (2) epistatic effects from the donor parent are eliminated and the detection of QTLs with small effects is possible; (3) the permanent nature of these lines also allows several laboratories to collect data for different traits on the same lines, thereby facilitating the integration of data from independent studies and the creation of a comprehensive phenotypic database for general access (Zamir 2001); (4) ILs can also be used to obtain more precise estimates of the GxE interaction (Eshed and Zamir 1995; Monforte et al. 2001; Gur and Zamir 2004; Lecomte et al. 2004; Chaïb et al. 2006). In an IL population, the number of replicated measurements has a larger impact on

mapping power and at least five replications should be analyzed to obtain enough statistical power (Keurentjes et al. 2007). One disadvantage of this type of population is the long time required for their development; however, the availability of numerous marker-screening technologies has currently made the construction of such libraries a more efficient process that can be completed after ten generations of crossing and marker analysis (Young 1999). Currently the *S. pennellii* IL library has been extensively used to identify QTLs for several traits including yield and yield-related traits, fruit quality, biotic and abiotic stress.

Biotic stress

Wild tomato species have to compete with all kinds of other organisms and therefore have to evolve defensive mechanisms (Rick 1973; Rick and Chetelat 1995; Hajjar and Hodgkin 2007). *S. habrochaites* has been proven to be a source of genes or QTLs conferring resistance to different kinds of pathogens and insects. Some of them have been explored and incorporated into modern varieties. The IL populations are constructed from *S. habrochaites* LA1777, LA407 and LYC4, *S. pimpinellifolium* LA1589 and *S. pennellii* LA716 and therefore valuable loci for different kinds of diseases and insect resistances can be studied in more detail. The genome-wide scan of the *S. pennellii* LA716 IL population identified six independent *Fusarium* resistance loci (Sela et al. 2001). These loci confer varying degrees of resistance to different races of this pathogen. This IL population also has been used for the exploration of resistance to *P. infestans* (Smart et al. 2007) and *Xanthomonas campestris pv. Vesicatoria* (Astua-Monge et al. 2000). A quantitative locus on IL6-2 was identified to account for 25% of the phenotypic variance in the former population and one qualitative locus *Xv4* was found in the latter one. Furthermore, resistance to Taiwanese race 1 strains of *Ralstonia solanacearum* has been reported for IL1-1, IL6-2, IL8-1 and IL10-3 (Hong Hai et al. 2008). QTLs conferring resistance to *B. cinerea* have been successfully identified using another *S. habrochaites* LYC4 IL population (Finkers et al. 2007). A primary study was conducted to evaluate the resistance to *Alternaria solani* with *S. habrochaites* LA1777 IL population, but only few lines presented partial resistance (Graham et al. 2005). However, *S. habrochaites* accession LA1777 has been reported to be resistant to TYLCV, but no begomovirus resistance in the LA1777 IL population was found. It suggests that some limitations of capturing all genes exist in this IL population, mainly due to a single wild plant being used for outcrossing (Momotaz et al. 2007). In addition, *S. lycopersicoides* LA2951 has been proven to be resistant to *B. cinerea* and viruses (Guimarães et al. 2004; Zhao et al. 2005) and four QTLs for resistance *B. cinerea* have been identified (Davis et al. 2009). Wild species *S.*

pennellii LA716, *S. habrochaites* LA1777, *S. habrochaites* LA407, *S. habrochaites* LY4C and *S. chmielewskii* LA1840 may also have potential as source for resistance.

Abiotic stress

In order to cope with adverse environmental conditions, wild tomato species have implemented various mechanisms during their evolution to adapt to dry, cold, wet, hot, etc. conditions. For example, *S. pennellii* accessions are used to living in arid and semi-arid environments in South America and hence can tolerate drought tolerance quite well (Rick 1973). *S. habrochaites* and *S. lycopersicoides* accessions are from high altitude and thus possess cold tolerance (Rick 1973, 1988). Other wild species also possess some kinds of positive factors to cope with abiotic stress (Rick 1973; Flowers 2004; Zhao et al. 2005; Hajjar and Hodgkin 2007). In the aforementioned studies, salt tolerance has been extensively explored from one wild species namely *S. pimpinellifolium.* This wild species also showed drought tolerance and four QTLs have been identified to be associated with seed germination under drought stress (Foolad et al. 2003). A dominant QTL controlling shoot turgor maintenance under root chilling was confirmed on chromosome 9 from *S. habrochaites* LA1778 (John et al. 2005). These previous studies hinted that it is possible to unravel potential abiotic stress tolerance from six established IL populations. Excellent work was conducted using a *S. pennellii* IL population to explore potential loci conferring drought tolerance (Gur and Zamir 2004). Three independent introgressions from Chromosome 7 (IL7-5-5), Chromosome 8 (IL8-3), and Chromosome 9 (IL9-2-5) that affect the components of Brix × yield (BY) have been used to verify their effects on drought tolerance. IL7-5-5 showed a dominant effect on yield about 12% to 22% under dry conditions as both homozygous IL and heterozygous ILH. IL8-3 was greatly inferior to cv. M82 for yield (about 34% less) as homozygous IL but the ILH increased yield with about 25%. However, IL9-2-5 showed an additive effect (Gur and Zamir 2004) and this line also provided tolerance to chilling stress due to elevated ascorbic acid content in fruits (Stevens et al. 2008). Recently, a dominant QTL (QWUE5.1) making $\delta^{13}C$ useful as a proxy for plant water-use efficiency and explaining 25.6% of the total phenotypic variance was mapped to an interval about 2.2 cM on chromosome 5 (Xu et al. 2008). Introgressions originating from *S. pennellii* were introduced into lines of processing tomato, and the resulting hybrid, AB2, is presently a leading variety in California (Lippman et al. 2007). Therefore IL populations have shown the advantage to challenge all kinds of abiotic stress through gene or QTL mapping strategies.

Fruit quality

Wild tomato species exhibit great biochemical diversity and are a rich source of genes affecting both fruit development and chemical composition, and hence harbor many traits for improving fruit quality. Recently QTLs involved in these factors have been extensively explored using *S. pennellii* and *S. habrochaites* IL populations. Up to now about 1300 QTLs measured in fruits were identified mainly from *S. pennellii* LA716 population. The detailed information is listed in Table 1. However, tomato fruit quality is a complex character due to its number of components and environmental dependency. The established metabolomics combined with genetic, physiological and biochemical profiling may provide a valuable tool for practical breeding (http://tomet.bti.cornell.edu/).

Table 1 Genes/QTLs identified for fruit quality using IL populations

No. Gene/QTL	IL population	Trait	Trait measured	Reference
1	*S. pennellii* LA716	1	Fruit aroma	Tadmor et al. (2002)
16	*S. pennellii* LA716	1	Intensity of red internal color of ripe fruit	Liu et al. (2003)
68	*S. pennellii* LA716	8	Metabolites and brix	Causse et al. (2004)
2	*S. pennellii* LA716	1	Soluble solid	Baxter et al. (2005a)
20	*S. pennellii* LA716	3	Nutritional and antioxidant	Rousseaux et al. (2005)
88	*S. pennellii* LA716	23	Volatile compounds	Tieman et al. (2006)
14	*S. pennellii* LA716	1	Ascorbic acid biosynthesis and metabolism	Zou et al. (2006)
889	*S. pennellii* LA716	74	Primary metabolites	Schauer et al. (2008)
1	*S. pennellii* LA716	1	Fruit flavor	Matsui et al. (2007)
12	*S. pennellii* LA716	1	Ascorbic acid	Stevens et al. (2007)
127	*S. pennellii* LA716	74	candidate gene for fruit chemical composition	Bermudez et al. (2008)
30	*S. habrochaites* LA1777	33	Fruit volatile composition	Mathieu et al. (2009)
17	*S. habrochaites* LA1777	1	fruit ripening-associated ethylene emissions	Dal Cin et al. (2009)
27	*S. chmielewskii* LA1840	5	Fruit dry matter and sugar	Prudent et al. (2009)

Yield and yield-associated traits

It is relatively straightforward to identify wild accessions that contain genes for resistance to pathogens but it is difficult to identify accessions that are likely to contain genes for the improvement of quantitative traits such as yield since wild tomato species have small sized

fruits (Eshed and Zamir 1995). In this respect, non-adapted germplasm is almost always inferior to elite varieties. Although QTLs for increased yield have been identified from these relatives of several crop plants through commonly used populations (Swamy and Sarla 2008), each line from an IL population carries only a small fraction of the wild species genome, and most of the fertility problems can be eliminated hence IL provide the advantage for yield-associated trait measurement. Using *S. pennellii* LA716 IL population, at least sixteen QTL for plant weight, twenty two for percentage green fruit weight, eleven for total yield and fourteen for total soluble solids yield were identified (Eshed and Zamir 1995). Out of these QTLs, eight QTLs for increasing horticultural yield of processing tomatoes were confirmed (Eshed and Zamir 1996a). Lately 13 QTLs for fruit weight were identified (Causse et al. 2004). Hanson et al. (2007) proved that ILH heterozygous for *S. habrochaites* segments at the bottom of chromosome 1 can increase yield with about 20%. Meanwhile other yield-related QTLs also have been explored. For example, 22 QTL primarily affecting leaf dissection and 8 QTL primarily affecting leaf size were identified in *S. pennellii* IL population (Holtan and Hake 2003). Using a *S. chmielewskii* LA1840 IL population, 41 QTLs for fruit weight, 50 QTLs for fruit physiological traits and 12 QTLs for plant developmental traits were identified (Prudent et al. 2009). However, the exploration of *S. lycopersicoides* would be difficult because many lines present obvious fertility problems. For this population, sub-NIL or another strategy is needed to break the linkage drag.

Analysis of QTL effects in IL lines

Due to the single introgression in each IL line, it is possible to analyze QTL effects under the assumption that one locus controls the trait. The ILs are genetically almost identical to the recurrent genotype, and therefore all the variation that differentiates the IL from the recurrent parent can be associated with the introgressed segment. QTLs can be caused by single genes (Tanksley and Fulton 2007). Compared to other population types, IL lines provide a convenient tool to understand QTLs without whole-genome epistatic interactions. Using *S. pennellii* IL lines, Eshed and Zamir (1996b) proved that the effect on yield of the double heterozygous ILs was smaller than the sum of the effects of the corresponding single heterozygotes. Three independent introgressions from Chromosome 7 (IL7-5-5), Chromosome 8 (IL8-3), and Chromosome 9 (IL9-2-5) that affect components of BY showed dominant, overdominant and additive effects on yield (Gur and Zamir 2004). Analysis of the metabolomic QTLs showed that 174, 61 and 80 out of 322 QTLs present dominant, additive and recessive characters respectively (Schauer et al. 2008). These results from yield and metabolites provide the potential power for using IL lines to understand the mechanism of

interaction of different genes. This will be invaluable information for tomato improvement in the near future.

Fine mapping by IL lines

An IL line carries a short and single introgression of wild species. Each of the introgression lines is nearly isogenic to the cultivated tomato and all lines together provide complete coverage of the wild species genome. Moreover the segment presents quite distant relation with the cultivar or other wild species in this region. Hence it could be conveniently used for fine mapping of desirable genes over the whole genome (Eshed and Zamir 1994b). On the other hand, high density maps (for example F2.2000) have been constructed and much information related to IL populations has been deposited in databases (http://solgenomics.net/). All of this genomic information provides a robust platform for gene fine mapping. Using *S. pennellii* IL, fine mapping of several yield-related QTLs and a QTL for carbon isotope composition have been conducted through the segregation of IL×M82 (Eshed and Zamir 1995; Xu et al. 2008). In the same manner, several qualitative genes including *Ph-2* and *Ph-3* for resistance to *Phytophthora infestans*, and *Ve* for *Verticillium dahliae* race 1, and *obscuravenosa* for reducing the transmission of light through leaf veins were further mapped (Moreau et al. 1998; Diwan et al. 1999; Chunwongse et al. 2002; Jones et al. 2007). Based on *S. habrochaites* IL population, a QTL affecting several agronomically important traits at the end of chromosome 1 and QTL *Brix9-2-5* for tomato sugar content have been fine-mapped (Fridman et al. 2000; Monforte and Tanksley 2000). Novel parthenocarpy QTLs have been fine-mapped through *S. habrochaites* LYC4 IL population (Gorguet et al. 2008). More recently, other fine-mapped single genes or QTLs have been cloned using this strategy in tomato (Cong et al. 2008; Orsi and Tanksley 2009).

Map-based cloning by IL lines

IL lines have proven to be invaluable starting materials for the positional cloning of single genes and genes underlying QTLs. On the one hand an identified IL line can be used for backcrossing with the recurrent parent and the recombinant population can be used for map-based cloning. This approach has been used to clone QTL *Brix9-2-5* and *fw2.2*. The introgression line with the mapped gene/QTL can also be crossed with another IL line, which contains another introgression in that region. Genes like *fasciated* and *tanger* were cloned with this strategy. Up to now, more than 30 genes or QTLs have been cloned in tomato and one third of these genes/QTLs have been cloned with the use of introgression lines (Table 2).

Table 2 Some genes/QTLs cloned using IL lines

Gene/QTL	Product/features	Phenotype	Chr.	Reference
fw2.2	Similar to human oncogene c-H-ras	Fruit weight	6	Frary et al. (2000)
Beta/og^c	Lycopene β-cyclase	β-carotene synthesis	6	Ronen et al. (2000)
Brix9-2-5	Apoplastic invertase	Sugar content	9	Fridman et al. (2000)
sun	IQ67 domain-containing protein	Elongated fruit shape	7	Isaacson et al. (2002)
tanger	Carotenoid isomerase	Carotenoid desaturation	10	Isaacson et al. (2002)
wf	b-ring carotene hydroxylase	White flower	3	Galpaz et al. (2006)
Cwp1	a protein of DUF833 domain family	Cuticular water permeability	4	Hovav et al. (2007)
Style2.1	putative transcription factor	Style length	2	Chen et al. (2007)
fasciated	YABBY-like transcription factor	Fruit size	11	Cong et al. (2008)
Sw4.1	ABC transporter	Seed size	4	Orsi and Tanksley (2009)

QTL pyramiding by IL lines

Many QTLs involved in different traits have been mapped in the last decade. Hence it is possible to pyramid these QTLs into elite cultivars for tomato breeding by marker assisted selection (MAS). However, linkage drag and other genetic effects like pleiotropy and additivity still need to be considered when combining them in one genetic background. In recent years, QTL pyramiding has been a successful approach for new variety release. For example, three *S. pennellii* segments were pooled, using marker assisted selection, into a single cv. M82 line designated IL789. After crossing with IL789 the hybrids had increased yield under both wet and dry environments (Gur and Zamir 2004); one of the hybrids AB2, is presently a leading variety in California for at least the last five years (http://www.ptab.org/ranking11.htm). More recently, gene expression, candidate gene identification and metabolomics have been conducted on tomato fruit productivity and quality improvement (Baxter et al. 2005a; Baxter et al. 2005b; Bermudez et al. 2008; Iijima and Aoki 2009). These results led to a comprehensive metabolic profiling and phenotyping of interspecific introgression lines (Schauer et al. 2006), and the results can give an integrated understanding for tomato improvement.

Outline of this thesis

The research described in this thesis, focuses on the identification of tomato QTLs conferring resistance to two major pathogens and salt tolerance in three different IL populations. The possibilities of using the IL lines to identify genomic regions in wild species which could have a positive effect on tolerance against two devastating pathogens (*P. infestans* and *B. cinerea*) and tolerance against salt stress is presented. Not only were already identified QTLs confirmed but also new ones were identified. In particular the research into qualitative

resistance to *B. cinerea* has proven to be a challenging and laborious task but will yield many novel leads for further research.

Chapter 2 presents the identification of QTLs conferring resistance to *B. cinerea* in a set of 22 wild species. Using both *S. habrochaites* LA1777 and *S. lycopersicoides* LA2951 populations a large number of QTLs were identified for two traits; lesion size and disease incidence. Three different tissues, leaves, stems and fruits, were subjected to disease tests and most QTLs were observed for fruits. The found QTLs were compared with previously reported QTLs.

Chapter 3 gives the identification of QTLs conferring resistance to late blight in the *S. habrochaites* LA1777 population. Although complete resistance could not be observed the qualitative nature of the identified QTLs shows promise for the future if more of these QTLs will be deployed by pyramiding.

Chapter 4 describes the identification of QTLs conferring salt tolerance during seedling stage using both *S. pennellii* LA716 and *S. lycopersicoides* LA2951 populations. A total of 15 QTLs were identified of which three were found in both populations. Also here possibilities lie to create more salt tolerant tomatoes in the future by introgressing and pyramiding the different QTLs.

Chapter 5 gives an overview on the experimental chapters focusing on the use of the results for tomato breeding for enhanced levels of tolerance to abiotic and biotic stresses. It also gives an overview of the different QTLs found for the different traits under investigation both in this study and in other published studies. It furthermore discusses the fact that once a QTL is identified in an IL zooming in on the QTL by further delineating the region using sublines often results in complete loss of the QTL effect. Although this phenomenon is not entirely new (Dr. M. Jeuken et al., Wageningen UR Plant Breeding, The Netherlands; unpublished results) it occurred in our studies for all investigated traits and could pose problems in trying to introgress interesting regions while keeping linkage drag at a minimum.

CHAPTER 2

Identification of QTLs for resistance to *Botrytis cinerea* using *Solanum habrochaites* LA1777 and *Solanum lycopersicoides* LA2951 introgression line populations

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Abstract

Botrytis cinerea is the causal agent of grey mold, infection with *B. cinerea* results in severe damage in hundreds of different plant species. Tomato (*Solanum lycopersicum*) is susceptible but wild relatives of tomato can show partial resistance. Screening of a collection of twenty two wild accessions showed that the accession *S. lycopersicoides* LA2951 and two *S. habrochaites* accessions, namely PI134417 and LA1392, exhibited a large reduction of leaf lesion size (LS) and disease incidence (DI). Two introgression line (IL) populations were used to examine the level of susceptibility to *B. cinerea* in leaves, stems, green fruit and ripe fruit. Three QTLs (*Rbchq1a*, *Rbchq1b* and *Rbchq8*) were identified from *S. habrochaites* LA1777, which are located on chromosome 1 and 8, and respectively reduce lesion size (LS) on leaves and stems, and disease incidence (DI) on stems. Three robust QTLs (*Rbclq4*, *Rbclq11* and *Rbclq3a*) were identified from *S. lycopersicoides* LA2951, are located on chromosome 4, 11 and 3 and resulted in smaller LS and DI on leaves. Isolate specific resistance loci were found for the *S. lycopersicoides* LA2951 leaf resistance and no resistance was detected on stems. The results clearly showed that loci from both *S. habrochaites* LA1777 and *S. lycopersicoides* LA2951 contribute to ripe fruit resistance. In total, fifteen QTLs located on chromosomes 1, 2, 3, 4, 5, 6, 7, 9, 10 and 12 were identified from the *S. habrochaites* LA1777 IL population. Twelve QTLs (*Rbchq1b*, *Rbchq2*, *Rbchq4a*, *Rbchq4b*, *Rbchq5*, *Rbchq6a*, *Rbchq7a*, *Rbchq7b*, *Rbchq9*, *Rbchq10a*, *Rbchq10b* and *Rbchq12*) are responsible for reducing LS with 22.6-67.3%. The presence of three QTLs (*Rbchq3*, *Rbchq6b*

and *Rbchq7c*) resulted in a reduction of 17.8-67.3 % of the DI on ripe fruits. Combining loci might provide a robust resistance over different environments. Three QTLs located on chromosome 3, 6 and 12 were identified from *S. lycopersicoides* LA2951 IL population. All of them are responsible for reducing LS on ripe fruits with 35.9-76.3%. Furthermore it was shown that loci derived from the wild species *S. habrochaites* LA1777 increase the susceptibility while the loci from *S. lycopersicoides* LA2951 decrease it in green fruit. The identified QTLs in this paper are an excellent starting point for introducing higher levels of resistance to *B. cinerea* in tomato.

Key Words: tomato, *Botrytis cinerea*, quantitative resistance, *Solanum habrochaites*, *Solanum lycopersicoides*, introgression lines

Introduction

The fungus *B. cinerea* Pers.: Fr (teleomorph: Botryotinia fuckeliana (de Bary) Whetzel) is a necrotrophic pathogen that attacks different plant tissues and has an extraordinarily wide host range of over 200 plant species. Especially grapevine, tomato and soft fruit crops (Williamson et al. 2007) can be severely infected resulting in significant economic losses in both pre- and post-harvest products (Choquer et al. 2007). A wide range of infection mechanisms allows *B. cinerea* to penetrate all kind of tissues; the fungus can spread fast under cool damp weather conditions with a temperature between 10-20°C (Prins et al. 2000). *B. cinerea* is notoriously difficult to control by biological agents and chemical fungicides. Infection is usually easier because of the presence of wounds such as pruned leaves and side shoots. Also direct penetration of epidermal cells is possible (Williamson et al. 2007), furthermore growth can take place on senescent and dead tissues especially on flowers, which subsequently causes fruit rot before ripening.

Commercial tomato cultivars, especially when cultivated in heated greenhouses, are susceptible to *B. cinerea* although some cultivars have some level of resistance (Farley et al. 1976). Wild tomato species have proven to be a valuable reservoir of resistance genes, and resistance genes to at least 42 diseases have been identified (Rick and Chetelat 1995). More than half of the identified resistance factors have been introgressed into modern tomato lines. Adaptation of species to higher latitudes with lower temperatures and higher humidity can result in species of wild tomato with higher levels of resistance or tolerance to biotrophic and/or necrotrophic pathogens, because the environment in which they grow is very favorable for fungus development. Testing accessions which grow under such circumstances has shown

that accessions of *S. chilense*, *S. peruvianum*, *S. habrochaites* and *S. pimpinellifolium* have partial resistance to *B. cinerea* (Davis et al. 2009;Decognet et al. 2009;Egashira et al. 2000;Finkers et al. 2007a;Guimarães et al. 2004). This allows breeding for tomato cultivars with higher levels of resistance to *B. cinerea*. However, the resistance mechanisms and the variability of this fungus are complex and there is only a low correlation between leaf and stem resistance (Davis et al. 2009;Decognet et al. 2009). Clear evidence for isolate specific resistance was found in *Arabidopsis thaliana* (Katherine et al. 2004;Rowe and Kliebenstein 2008). The resistance from wild species *S. lycopersicoides* can be seasonal dependant (Davis et al. 2009).

Molecular marker technologies make it more feasible to identify quantitative trait loci (QTL) and to introduce them efficiently into breeding lines. QTL mapping is a particularly powerful tool for genetic dissection of quantitatively inherited traits with low heritability (Doerge 2002). Since the first comprehensive molecular map of tomato (Tanksley et al. 1992), tomato has, as a model for dicotyledonous plants with berry fruit, led the way in QTL mapping which was not only beneficial for tomato improvement but also for understanding quantitative variation in other species. Up to now, a series of QTLs derived from several wild tomato species for different disease resistances have been identified. Substantial progress has been made in tomato in identifying QTLs, causing reduction of susceptibility to *B. cinerea*, in *S. habrochaites* LYC4, *S. neorickii* G1.1601(Finkers et al. 2008;Finkers et al. 2007a;Finkers et al. 2007b) and *S. lycopersicoides* LA2951 (Davis et al. 2009). This was partly possible because of the development of introgression line libraries of the wild *Solanum* species in a background of *S. lycopersicum*. Such permanent mapping populations are a valuable tool to study the nature of QTL variation and for that reason a number of introgression line populations (ILs) have been developed from wild tomato species (Canady et al. 2005;Eshed and Zamir 1995;Finkers et al. 2007b;Francis et al. 2001;Monforte and Tanksley 2000). Compared to plants in an F_2 population, all plants from the same IL are genetically homozygous and numerous genetically identical seeds can be obtained after self pollination. This allows more thorough studies including many replications. The principles of the IL approach were nicely demonstrated in tomato (Lippman et al. 2007). Studies using tomato ILs have identified a number of QTL for improved horticultural traits (Baxter et al. 2005a;Rousseaux et al. 2005).

Whether absolute resistance can be achieved remains questionable. *B. cinerea* is difficult to control due to its genetic diversity and complexity and many classes of fungicides have failed (Williamson et al. 2007). The necrotrophic lifestyle, in combination with the many modes of attack, the wide range of hosts and the different tissues that can be infected

make *Botrytis* difficult to control. The recently identified QTLs from *S. habrochaites* LYC4 and *S. neorickii* G1.1601 (Finkers et al. 2008;Finkers et al. 2007b) were identified in a stem assay. A leaf assay was used to identify the QTLs from *S. lycopersicoides* LA2951 (Davis et al. 2009). The QTLs from *S. habrochaites* LYC4 have proven to be stable, but whether they also play a role in fruits and with different isolates has not been studied yet. In this paper, several wild tomato species have been screened for resistance to *B. cinerea*, with an isolate collected in a greenhouse in China. Among these accessions, *S. habrochaites* and *S. lycopersicoides* show higher levels of partial resistance. Hence, two introgression line populations derived respectively from *S. habrochaites* (Monforte and Tanksley 2000) and *S. lycopersicoides* (Canady et al. 2005) were screened for resistance to *B. cinerea* in leaves, stems and pre- and post-harvest fruits.

Material and methods

Plant material
Twenty two accessions of wild tomato species and three susceptible tomato cultivars (Table 1) were used in this study. Seeds of all accessions were kindly provided by TGRC (Tomato Genetic Resource Center, C.M. Rick, UC Davis, USA) (LA numbers) and the Department of Agriculture, Plant Genetic Resources Unit at Geneva, N.Y.

Two introgression line populations were used in this study. One is the *S. habrochaites* introgression line population developed by Monforte and Tanksley (2000), the introgressions originate from *S. habrochaites* LA1777 (a self-fertile, homozygous green fruited, indeterminate accession) and the background originates from *S. lycopersicum* E6203 (a red fruited, determinate, processing-type tomato). In total 93 of the 98 introgression lines together with two parental controls were screened in 2005, 2006 and 2007. The *S. lycopersicoides* introgression line population has been developed by Canady et al. (2005). Here the introgressions originate from *S. lycopersicoides* LA2951 (a self-fertile indeterminate growing tomato-like nightshade species closely related to tomato with green fruits) and they are in a background of *S. lycopersicum* VF36 (a red fruited, determinate, beef-type tomato). A total of 38, 56 and 74 lines (originally 90 lines within the *S. lycopersicoides* library) were screened respectively in 2005, 2006 and 2007. Originally 34% of the introgressions within the *S. lycopersicoides* library could not be maintained in homozygous condition, but for some of these lines, like LA4278, we were able to obtain homozygous progeny through repeated manual self pollination albeit with a low seed production.

Table 1 Evaluation of different tomato accessions for leaf resistance to *B. cinerea*

Species	Accession	Total no. of plants	N^a	Successful infection	Leaf LS (cm^2)	Leaf DI(%)
S. pimpinellifolium	LA1629	13	38	24	$1.38\pm0.23^*$	63.2 ± 7.1^{ns}
	LA1579	9	23	16	$1.81\pm0.18^*$	69.6 ± 8.5^{ns}
	LA1357	11	31	13	2.20 ± 0.21^{ns}	$41.9\pm7.7^*$
	LA1246	17	49	29	$1.85\pm0.13^*$	59.2 ± 6.2^{ns}
S. chilense	LA2747	11	15	8	$1.41\pm0.22^*$	53.3 ± 11.4^{ns}
S. cheesmaniae	LA0429	22	65	28	$1.24\pm0.12^*$	53.0 ± 5.4^{ns}
S. habrochaites	LA2314	26	78	20	$1.36\pm0.14^*$	$25.6\pm5.0^*$
	LA1341	21	62	9	$2.00\pm0.24^*$	$14.5\pm5.6^*$
	LA1392	26	78	3	$1.16\pm0.36^*$	$38.0\pm5.0^{**}$
	LA1353	1	3	2	1.61 ± 0.51^{ns}	66.7 ± 2.54^{ns}
	LA1347	6	17	9	$1.77\pm0.23^*$	64.7 ± 10.4^{ns}
	LA1343	6	17	11	$1.91\pm0.21^*$	64.7 ± 10.4^{ns}
	PI126445	23	64	9	$1.27\pm0.21^*$	$15.6\pm5.3^*$
	PI247087	18	54	11	$1.47\pm0.21^*$	$24.0\pm6.0^*$
	LA1777	16	48	20	$1.58\pm0.15^*$	$44.0\pm6.4^*$
	PI134417	22	65	6	$0.89\pm0.23^{**}$	$9.20\pm5.4^{**}$
S. chmielewskii	LA1028	7	21	14	$1.99\pm0.19^*$	66.7 ± 9.6^{ns}
S. peruvianum	LA2745	20	60	22	$1.61\pm0.14^*$	$36.7\pm5.7^*$
	LA3900	18	53	30	$1.80\pm0.13^*$	56.6 ± 6.0^{ns}
S. pennellii	LA1926	13	39	8	$1.53\pm0.22^*$	$20.5\pm7.1^*$
S. lycopersicoides	LA2951	11	32	7	$0.71\pm0.25^{**}$	$21.9\pm7.7^*$
	LA2408	29	72	38	$1.47\pm0.10^*$	54.2 ± 4.7^{ns}
S. lycopersicum	E6203	8	21	20	2.41 ± 0.16^{ns}	93.3 ± 9.0^{ns}
	VF36	7	18	18	2.55 ± 0.17^{ns}	100.0 ± 9.6^{ns}
	cv.M82	9	25	24	2.92 ± 0.15	96.0 ± 8.5

[a] number of inoculation sites; [*] and [**] indicate values in species that are significantly different from *S. lycopersicum* M82 at the 0.05 and 0.01 level respectively.

The seeds were germinated in an incubator at a temperature of 25°C and then sown in 9 cm pots containing peat–vermiculite with some organic fertilizers and placed in the

greenhouse in a completely randomized block design. The greenhouse temperature varied between 15-18°C at night and 20-25°C at day time.

Inoculum preparation

The used strain of *B. cinerea* was isolated from tomato leaves in a greenhouse in 2005 in Beijing, China. A single colony was selected, multiplied and maintained on potato dextrose agar medium at 25°C. Inoculation was periodically done on leaves of a susceptible tomato to ensure pathogenicity. Conidia were harvested from the sporulating *Botrytis* isolate growing on potato dextrose agar medium by washing with 5 ml of sterile water containing 0.05% Tween-80. Inoculation of tomato leaves was carried out with the detached leaflet method as described by Benito et al. (1998).

Detached-leaflet assay

The fully expanded fifth or sixth true leaf was detached with a razor blade and immediately inserted in moist florist foam and incubated in a transparent plastic box. Tap water was added to keep the humidity high. Leaves were placed horizontally to keep the droplets stable on the leaf surface. The leaves were placed in a box covered with a spray-wetted lid. Three top leaflets of each leaf were inoculated and ten droplets (1μl per droplet) were carefully pipetted on the adaxial leaf surface. The boxes were randomly stored at 18-19° C after inoculation. After 24h dark, the regime was changed into 12 hours light and 12 hours dark. The largest length and width (perpendicular to the length) of each lesion were measured 5 days post inoculation (dpi), and the ellipse area was calculated following the formula LS (lesion size) = (length×width×π)/4 (Vleeshouwers et al. 1999). The percentage of successful infection was calculated (disease incidence, DI).

Stem assay

The stem assay was carried out according to Benito et al. (1998). Of 6-8 weeks old greenhouse-grown plants the mid-part of stems were cut into pieces of 5 cm and inserted in moist florist foam. Incubation took place in transparent plastic boxes, covered with a wet lid. Five till ten stem pieces of each genotype were used. Each box contained fifteen genotypes together with a susceptible control. On the top of every stem piece 5 μl inoculum (10^6 conidia ml^{-1}) was placed. The boxes were randomly placed in an incubator at 15°C with no light and a 100% relative humidity. The infection length was measured at 5 dpi with a caliper to calculate the lesion size (LS).

Fruit assay

Fruits were collected and twenty genotypes, including one *S. lycopersicum* control, were tested in each box. For the inoculation, six mature stages (mature green, breaker, turning, red and deep red) were categorized (Yamaguchi 1983). Fruits around mature green and red stages were used as green and ripe fruit inoculation. The fruit surface was slightly punctured by a needle and inoculated with 5 µl inoculum (10^6 conidia ml^{-1}) directly on the puncture. Length was calculated as the detached leaf assay. DI was calculated as the percentage of outgrowing lesions.

Screening of wild species

Depending on the availability of seeds 6 to 29 plants were used from each wild accession. The detached leaflet assay was done in 2005.

Botrytis bioassay on two introgression line populations

The experiments conducted on two introgression line populations for the *Botrytis* bioassay are listed in Table 2.

Table 2 Experiments on two introgression line populations with *B. cinerea* in different tissues

Exp.	Year	No. of plants per genotype	Leaf	No. of leaflets	Total pieces of stem	No. of green fruit	No. of ripe fruit
1	2005	5-18	5th true leaf	3	-	-	-
2	2006	3	5th and 6th leaf	3	6 (2 pieces per plant)	≥5	≥5
3	2007	8	5th and 6th leaf	8	10 (2 pieces per plant)	≥5	≥5
4	2007	8	5th and 6th leaf	8	10 (2 pieces per plant)	≥5	≥5
5	2007	8	5th and 6th leaf	8	10 (2 pieces per plant)	≥5	≥5
6	2007	8	5th and 6th leaf	8	10 (2 pieces per plant)	≥5	≥5
7	2007	8	5th and 6th leaf	8	10 (2 pieces per plant)	≥5	≥5
8	2007	8	5th and 6th leaf	8	10 (2 pieces per plant)	≥5	≥5
9	2007	8	-	-	-	≥5	≥5

-: that particular experiment was not conducted.

Statistical analysis

All statistical analyses were performed using SPSS 15.0. Differences in *B. cinerea* resistance among *S. habrochaites* and *S. lycopersicoides* introgression line populations were analyzed

using the procedure of general linear model (GLM). Mean values of different traits were calculated using the following models: traits= constant + genotype + experiment + genotype × experiment. It was compared to the susceptible control *S. lycopersicum* E6203 or VF36 using a Dunnett test and probabilities smaller than 0.05 were considered as significant. QTL were assigned when the chromosomal segment had a significant difference at the 0.05 level. Phenotypic data of LS and LG were transformed by square root to meet the normal distribution. Trait data for experiments within detached-leaflets, stem and fruit assays were tested for homogeneity of variance using a Levene test. Data within detached-leaflets for fully rotten leaves or fruits were omitted from the analysis.

Nomenclature

We name the identified QTLs as follows: <u>R</u>esistance to <u>B</u>otrytis <u>c</u>inerea from *S. <u>h</u>abrochaites* QTL (*Rbchq*) or <u>B</u>otrytis <u>c</u>inerea from *S. <u>l</u>ycopersicoides* QTL (*Rbclq*) followed by the number of the chromosome. If QTLs are located on the same chromosome, we distinguish them from each other by adding letter a, b, c.

Results

Screening of wild species for leaf resistance to *B. cinerea*

To evaluate the resistance levels of different *Solanum* species, leaves of a total of twenty two accessions and three *S. lycopersicum* cultivars were challenged with *B. cinerea*. LS was measured 5 dpi (Table 1). Only clearly expanded lesions were considered for the disease incidence (DI), the values varied over all tested accessions, ranging from 14.5±5.6% (*S. habrochaites* LA1941) to 69.6±8.5% (*S. pimpinellifolium* LA1579). In general tomato cultivars showed the highest DI. Lesion size was measured in all accessions and varied greatly, the mean value from 0.71±0.25 cm^2 (*S. lycopersicoides* LA2951) to 2.20±0.21 cm^2 (*S. pimpinellifolium* LA1357). All tested accessions showed a significant difference compared to the susceptible control cv. M82 (2.92±0.15 cm^2). Three accessions, *S. lycopersicoides* LA2951, and *S. habrochaites* LA1392 and PI134417, combined a large reduction of LS and of DI (Fig.1). Fortunately an introgression line population was available for *S. lycopersicoides* LA2951 and for another *S. habrochaites* accession LA1777 (a little less reduction of DI and LS compared to *S. habrochaites* LA1392, Table 1).
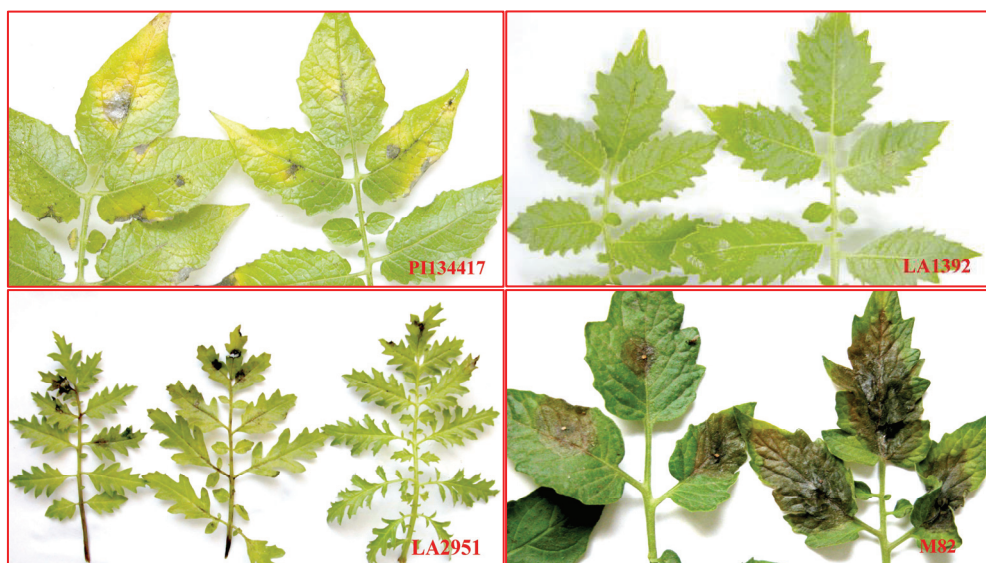
Fig. 1 Screening of wild relatives for *Botrytis* resistance in leaf. *S. habrochaites* PI134417, *S. habrochaites* LA1392 and *S.lycopersicoides* LA 2951 showed clearly reduced LS and DI compared to *S. lycopersicum* M82.

Susceptibility to *B. cinerea* in *S. habrochaites* LA1777 IL population

Leaf assay

In the greenhouse a total of ninety three introgression lines together with the two parents were grown until the first six fully developed true leaves. The fifth and sixth leaves were detached and inoculated. LS and DI at 5dpi (days post inoculation) were measured in seven independent experiments (Table 1). The mean values for lines from the IL population varied from 3.37 ± 0.05 cm^2 to 6.71 ± 0.08 cm^2 between experiments for LS, and from $57.3\pm0.5\%$ to $98.2\pm0.5\%$ for DI. Significant correlations were found between experiments 2, 4, 5, 6 and 7 for both parameters (Tables 3 and 4). Experiments 1 and 3 were excluded from the final analysis of the results but this only influenced the significance of the QTLs.

Table 3 Pearson correlation of different experiments with leaf lesion size (LS) in *S. habrochaites* LA1777 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7
Exp1	1	-0.021	0.097	0.058	0.148	0.166	0.162
Exp2		1	0.113	0.299[**]	0.255[*]	0.329[**]	0.266[**]
Exp3			1	0.149	0.057	-0.048	0.070
Exp4				1	0.241[*]	0.399[**]	0.278[**]
Exp5					1	0.275[**]	0.229[*]
Exp6						1	0.252[*]
Exp7							1

[*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

Table 4 Pearson correlation of different experiments with leaf disease incidence (DI) in *S. habrochaites* LA1777 IL population

| Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7
Exp1	1	-0.081	0.084	0.231[*]	0.063	0.135	-0.057
Exp2		1	0.129	0.224[*]	0.355[**]	0.230[*]	0.296[**]
Exp3			1	0.150	0.390[**]	0.309[**]	0.385[**]
Exp4				1	0.354[**]	0.311[**]	0.340[**]
Exp5					1	0.494[**]	0.943[**]
Exp6						1	0.518[**]
Exp7							1

[*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

The mean LS varied from 3.52±0.21cm^2 to 6.59±0.24 cm^2 in the individual ILs. *S. habrochaites* LA1777 had the smallest LS compared to the ninety three ILs and the susceptible parent *S. lycopersicum* E6203. Four ILs, LA3913, LA3914, LA3916 and LA3937, showed a significantly lower leaf LS than the susceptible parent *S. lycopersicum* E6203 (Fig. 2). Because introgressions, in different lines, in the *S. habrochaites* LA1777 IL population can partly overlap, it can occur that two or more introgression lines show a higher level of resistance due to the same QTL. The introgressions of LA3913, LA3914, LA3916 and LA3915 partly overlap on chromosome 1 as shown in Figure 3 (Monforte and Tanksley 2000). The first three lines had significantly lower LS. LA3915 had a lower, but not significant, LS (*P*=0.962), and LA3995 and LA3998 with apparently the same introgression area were similar to the susceptible control. This phenomenon of lines with similar introgressions of which some do not have the higher levels of resistance is not unique in our studies. All other lines with other introgressions at the end of Chromosome 1 were susceptible. Hence there is evidence that there is a QTL, *Rbchq1*, located near the mid part of chromosome 1 as indicated by an arrow in Figure 3 although not all results are consistent. The QTL of LA3937 with an introgression on Chromosome 4 is not confirmed in the two other ILs with overlapping introgressions since the effect was lower but not significant (Fig. 4). More detailed genotyping of the introgression line population is needed to pinpoint the exact location and nature of the QTL(s) for LS.

The estimated mean DI of the five experiments varied from 66.6±2.7% to 99.3±2.4% in the IL population. The average leaf DI of *S. habrochaites* LA1777 was 35.8±2.8% and of *S. lycopersicum* E6203 it was 93.4±1.7%. Although ninety two lines had on average a lower leaf DI than the susceptible control, in only four lines this effect was significant and the reduction ranged from 12.3% to 26.8% (LA3914, LA3920, LA3941 and LA3966). The QTL of line LA3920 caused a reduction of 17.8%; three other lines, LA3918, LA3919 and LA3999 with introgressions in this region had an almost significant reduction (*P*=0.10, *P*=0.52 and *P*=0.26)

(Fig. 5), the arrow in Figure 5 indicates the most likely region of this QTL. Another example is the QTL of LA3914 with introgressions on the bottom of chromosome 1 in the same region as the LS QTL on this chromosome. Some other lines with introgressions on chromosome 1 had almost a significant reduction (e.g. LA3916) and others not (Fig. 6). The same apparently contrasting results were found for DI in introgression lines LA3941 (Fig. 7) and LA3966 (Fig. 8). Without confirmation in other introgression lines we consider the QTLs in ILs LA3914, LA3941 and LA3966 still as putative.
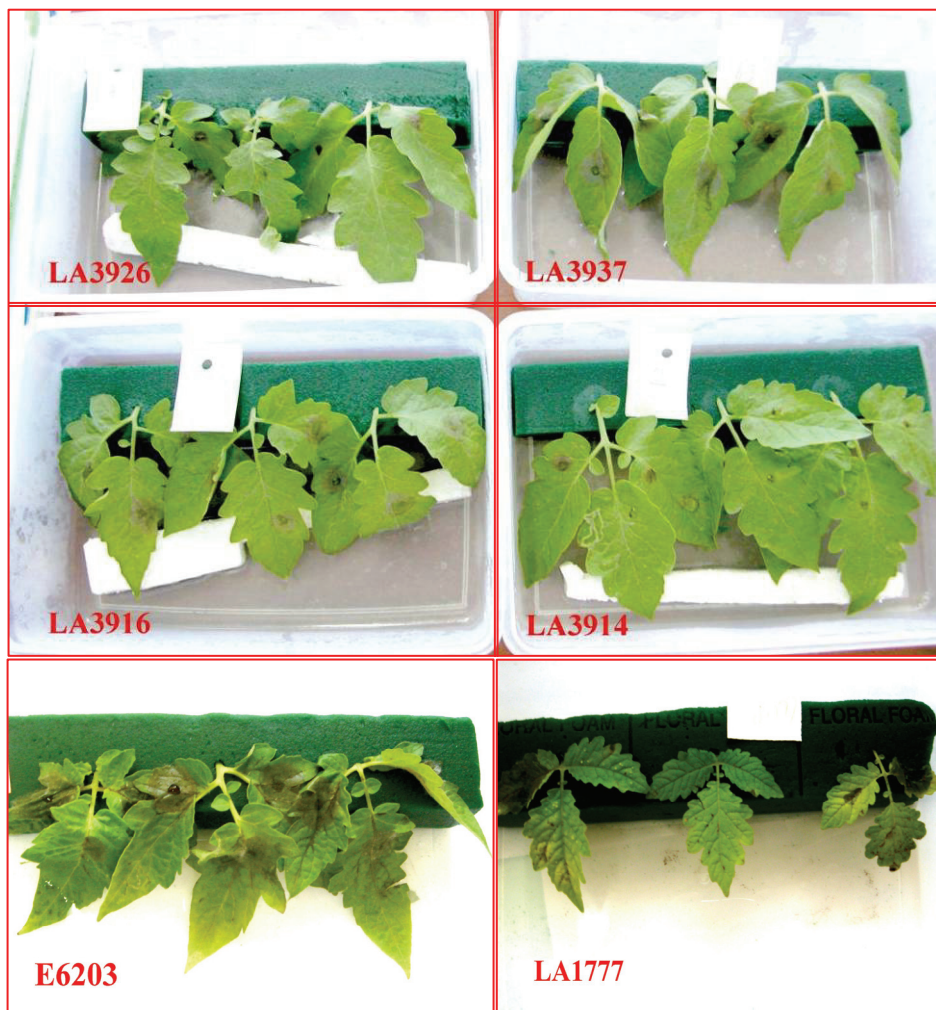
Fig. 2 Screening of *S. habrochaites* LA1777 IL population in leaf. IL lines LA3926, LA3916 and LA3914 show the reduced LS and LA3937 is similar as the susceptible control E6203.
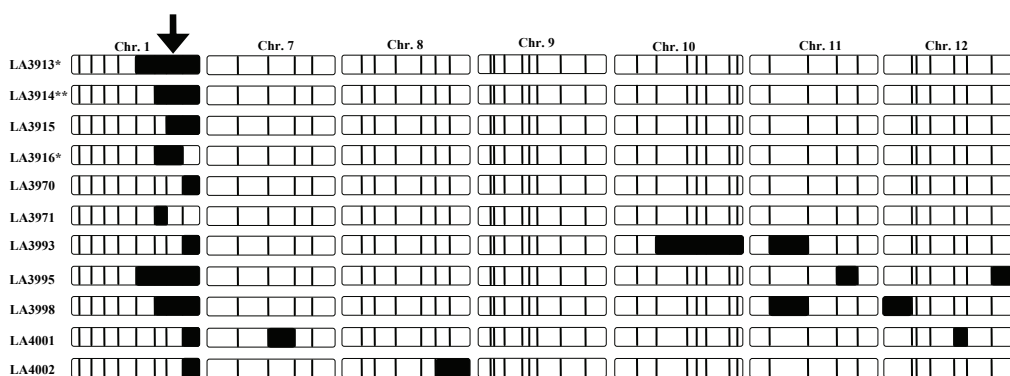
Lines	Leaf LS in each independent experiment (cm^2)					N[a]	Mean LS (cm^2)	Mean DI (%)
	2	4	5	6	7			
LA3913	4.41	4.14	2.79	3.49	3.29	137	3.62 ±0.23[*]	86.6±2.7
LA3914	3.66	5.54	2.76	3.00	2.94	159	3.58 ±0.28[**]	66.6±2.7[**]
LA3915	7.55	5.30	2.81	2.76	2.98	147	4.28 ±0.21(P=0.962)	88.3±2.7
LA3916	5.95	6.13	2.61	1.90	2.99	159	3.92±0.23[*]	78.2±2.6(P=0.146)
LA3970	5.88	7.18	3.92	4.22	5.25	155	5.29 ±0.23	85.6±2.7
LA3971	5.86	6.54	4.00	3.20	4.38	159	4.80 ±0.21	88.3±2.7
LA3993	8.40	7.79	3.76	2.88	5.36	161	5.64 ±0.21	86.3±2.7
LA3995	7.57	9.42	3.60	3.48	4.92	131	5.80±0.29	90.9±3.1
LA3998	7.53	8.63	4.14	3.07	5.69	162	5.81 ±0.21	89.5±2.7
LA4001	7.49	8.06	3.38	2.66	4.90	136	5.30 ±0.28	78.3±3.7(P=0.330)
LA4002	5.14	8.41	4.26	3.89	3.81	118	5.10 ±0.24	87.3±2.6
E6203	7.11	5.87	3.76	3.49	3.60	761	4.76 ±0.13	93.4±1.7
LA1777	4.26	1.14	3.07	0.74	4.89	591	2.82 ±0.30[**]	35.8±2.8[**]

[a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 3 Eleven *S. habrochaites* LA1777 ILs containing introgressions on chromosome 1 and their extra introgressions on other chromosomes. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent leaf LS of each line in five independent experiments, and the mean LS and DI of the five experiments.

Lines	Leaf LS of each experiment (cm²)					Nᵃ	Mean LS (cm²)	Mean DI (%)
	2	**4**	**5**	**6**	**7**			
LA3934	6.88	4.51	3.58	3.39	4.15	151	4.50 ±0.22	87.5±2.7
LA3935	6.84	5.63	3.36	2.68	3.13	148	4.33 ±0.22	92.4±2.7
LA3937	4.11	5.58	2.99	2.34	2.58	152	3.52 ±0.21**	90.3±2.7
LA3977	6.39	7.78	2.69	3.45	4.06	138	4.87 ±0.22	87.2±2.7
LA3978	6.42	7.05	3.29	3.11	3.95	150	4.76 ±0.22	85.4±2.7
LA4007	4.11	5.43	3.78	3.66	5.36	149	4.47 ±0.24	78.9±2.6
E6203	7.11	5.87	3.76	3.49	3.60	761	4.76 ±0.13	93.4±1.7
LA1777	4.26	1.14	3.07	0.74	4.89	591	2.82 ±0.30**	35.8±2.8**

ᵃ:number of inoculation sites; * and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 4 Six *S. habrochaites* LA1777 ILs containing introgressions on chromosome 4 and their extra introgressions in other chromosomes for each line. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent leaf LS of each line in five independent experiments, and the mean LS and DI of the five experiments.
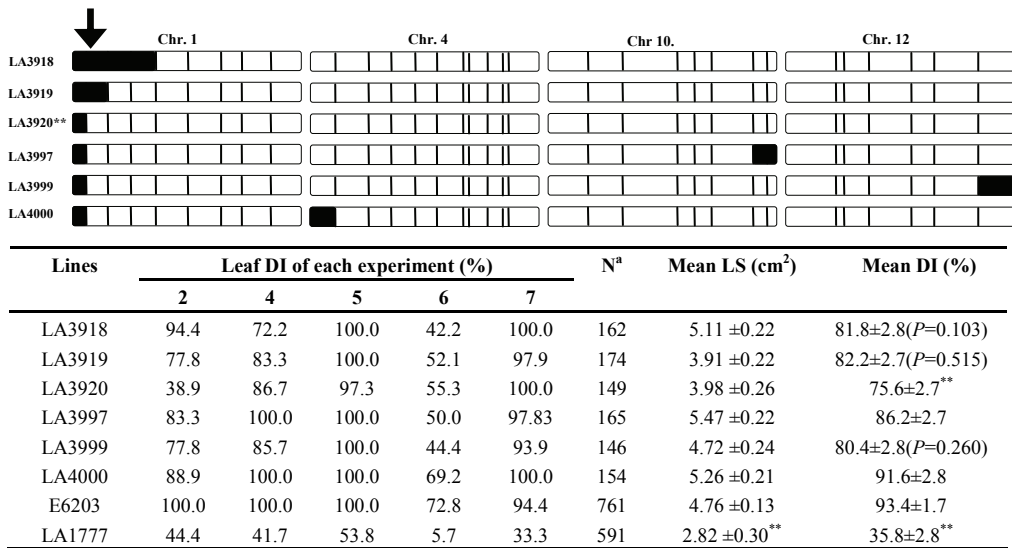

Lines	Leaf DI of each experiment (%)					Nᵃ	Mean LS (cm²)	Mean DI (%)
	2	**4**	**5**	**6**	**7**			
LA3918	94.4	72.2	100.0	42.2	100.0	162	5.11 ±0.22	81.8±2.8(*P*=0.103)
LA3919	77.8	83.3	100.0	52.1	97.9	174	3.91 ±0.22	82.2±2.7(*P*=0.515)
LA3920	38.9	86.7	97.3	55.3	100.0	149	3.98 ±0.26	75.6±2.7**
LA3997	83.3	100.0	100.0	50.0	97.83	165	5.47 ±0.22	86.2±2.7
LA3999	77.8	85.7	100.0	44.4	93.9	146	4.72 ±0.24	80.4±2.8(*P*=0.260)
LA4000	88.9	100.0	100.0	69.2	100.0	154	5.26 ±0.21	91.6±2.8
E6203	100.0	100.0	100.0	72.8	94.4	761	4.76 ±0.13	93.4±1.7
LA1777	44.4	41.7	53.8	5.7	33.3	591	2.82 ±0.30**	35.8±2.8**

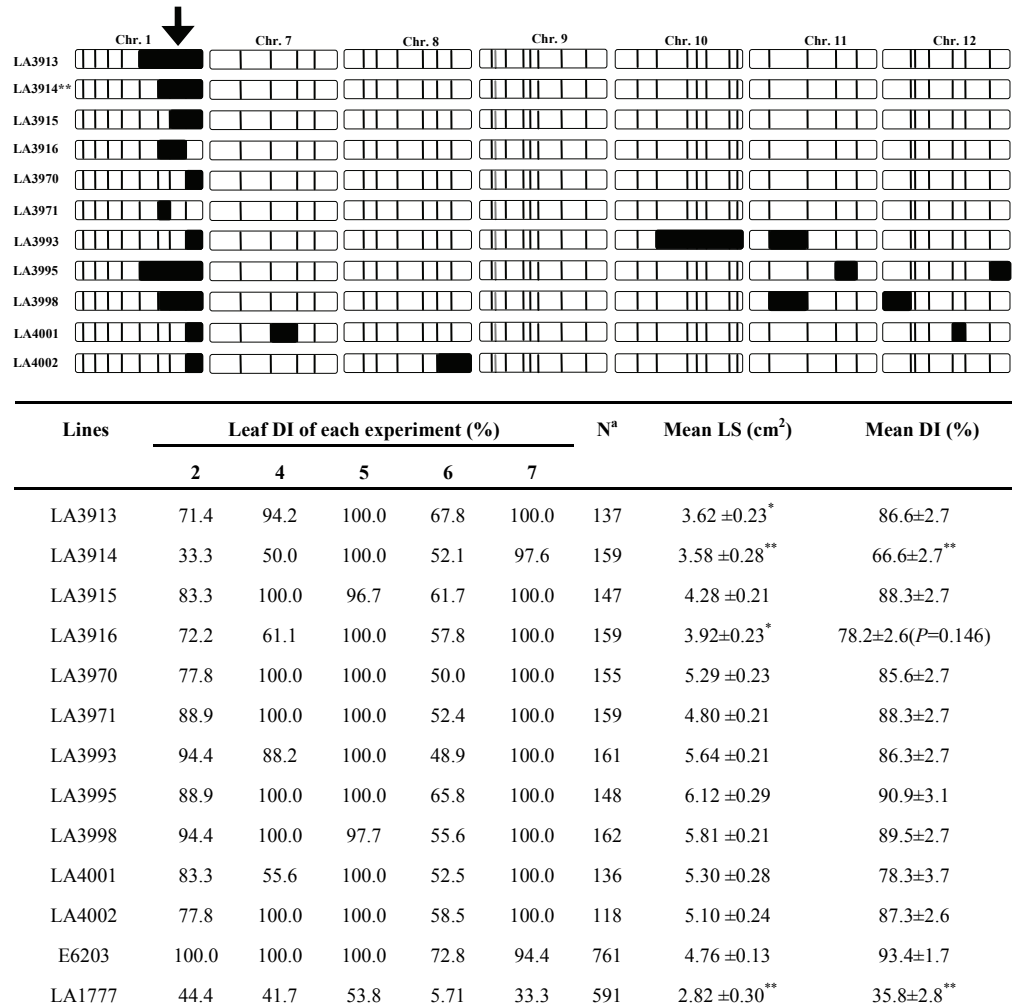ᵃ:number of inoculation sites; * and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 5 Six *S. habrochaites* LA1777 ILs containing introgressions on chromosome 1 and their extra introgressions in other chromosomes for each line. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent leaf DI of each line in five independent experiments, and the mean LS and DI of the five experiments.

Lines	Leaf DI of each experiment (%)					N^a	Mean LS (cm^2)	Mean DI (%)
	2	**4**	**5**	**6**	**7**			
LA3913	71.4	94.2	100.0	67.8	100.0	137	3.62 ±0.23[*]	86.6±2.7
LA3914	33.3	50.0	100.0	52.1	97.6	159	3.58 ±0.28[**]	66.6±2.7[**]
LA3915	83.3	100.0	96.7	61.7	100.0	147	4.28 ±0.21	88.3±2.7
LA3916	72.2	61.1	100.0	57.8	100.0	159	3.92±0.23[*]	78.2±2.6(P=0.146)
LA3970	77.8	100.0	100.0	50.0	100.0	155	5.29 ±0.23	85.6±2.7
LA3971	88.9	100.0	100.0	52.4	100.0	159	4.80 ±0.21	88.3±2.7
LA3993	94.4	88.2	100.0	48.9	100.0	161	5.64 ±0.21	86.3±2.7
LA3995	88.9	100.0	100.0	65.8	100.0	148	6.12 ±0.29	90.9±3.1
LA3998	94.4	100.0	97.7	55.6	100.0	162	5.81 ±0.21	89.5±2.7
LA4001	83.3	55.6	100.0	52.5	100.0	136	5.30 ±0.28	78.3±3.7
LA4002	77.8	100.0	100.0	58.5	100.0	118	5.10 ±0.24	87.3±2.6
E6203	100.0	100.0	100.0	72.8	94.4	761	4.76 ±0.13	93.4±1.7
LA1777	44.4	41.7	53.8	5.71	33.3	591	2.82 ±0.30[**]	35.8±2.8[**]

[a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 6 Eleven *S. habrochaites* LA1777 ILs containing introgressions on chromosome 1 and their extra introgressions in other chromosomes for each line. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent leaf DI of each line in five independent experiments, and the mean LS and DI of the five experiments.
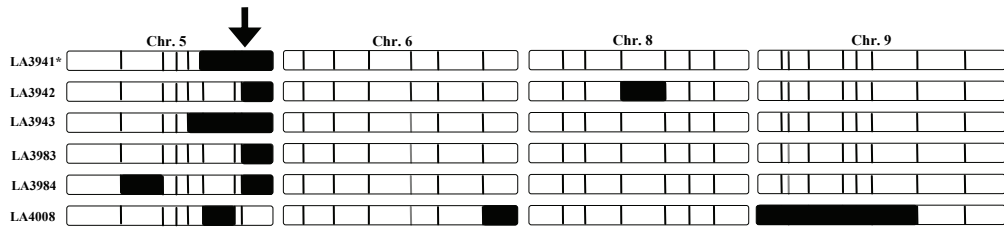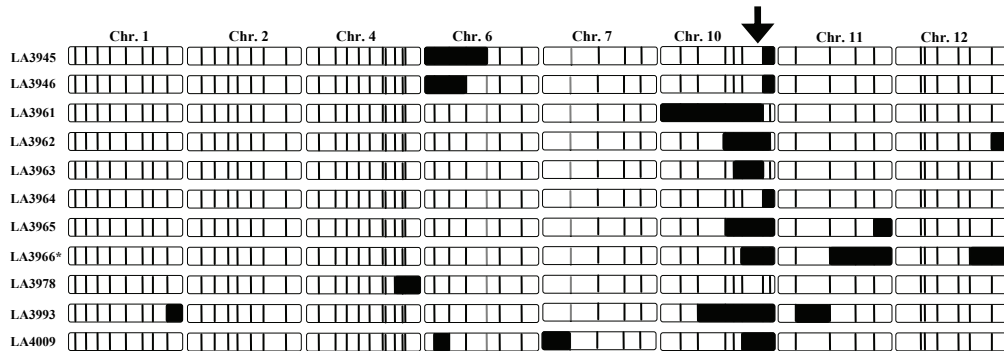
Lines	Leaf DI of each experiment (%)					N[a]	Mean LS (cm²)	Mean DI (%)
	2	4	5	6	7			
LA3941	100.0	47.1	100.0	47.7	97.2	142	4.40 ±0.25	78.4±2.6[*]
LA3942	100.0	73.3	100.0	71.1	100.0	158	4.81 ±0.22	88.9±2.6
LA3943	94.1	64.7	97.7	59.5	100.0	160	5.05 ±0.23	83.2±2.7
LA3983	77.8	86.7	100.0	48.9	100.0	154	5.42 ±0.23	82.7±2.7(P=0.165)
LA3984	100.0	100.0	100.0	57.8	100.0	150	5.79 ±0.22	91.6±3.0
LA4008	94.4	83.3	100.0	48.7	96.2	119	4.40 ±0.24	84.5±2.8(P=0.089)
E6203	100.0	100.0	100.0	72.8	94.4	761	4.76 ±0.13	93.4±1.7
LA1777	44.4	41.7	53.8	5.7	33.3	591	2.82 ±0.30[**]	35.8±2.8[**]

[a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 7 Six *S. habrochaites* LA1777 ILs containing introgressions on chromosome 5 and their extra introgressions on other chromosomes for each line. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent leaf DI of each line in five independent experiments, and the mean LS and DI of the five experiments.

Lines	Leaf DI of each experiment (%)					N[a]	Mean LS (cm²)	Mean DI (%)
	2	4	5	6	7			
LA3945	100.0	94.4	100.0	66.9	93.8	154	5.84 ±0.21	90.8±2.7
LA3946	100.0	58.8	100.0	53.7	100.0	155	5.32 ±0.23	82.5±2.8(P=0.913)
LA3961	94.4	83.3	100.0	72.7	100.0	159	4.90 ±0.21	90.1±3.1
LA3962	83.3	100.0	100.0	63.8	100.0	163	4.98 ±0.21	89.4±2.8
LA3963	94.4	100.0	100.0	47.6	100.0	143	5.77 ±0.22	88.4±2.6
LA3964	72.2	86.8	100.0	55.1	97.1	152	5.45 ±0.23	82.2±2.7(P=0.132)
LA3965	94.4	88.9	100.0	62.8	97.7	163	6.03 ±0.21	88.8±2.7
LA3966	83.3	85.7	100.0	39.5	97.1	147	5.87 ±0.24	81.1±2.7[*]
LA3978	88.9	88.2	100.0	59.5	90.3	150	4.76 ±0.22	85.4±2.7
LA3993	94.4	88.2	100.0	48.9	100.0	161	5.64 ±0.21	86.3±2.7
LA4009	83.3	100.0	100.0	52.9	100.0	95	6.45 ±0.28	87.3±2.7
E6203	100.0	100.0	100.0	72.8	94.4	761	4.76 ±0.13	93.4±1.7
LA1777	44.4	41.7	53.8	5.7	33.3	591	2.82 ±0.30[**]	35.8±2.8[**]

[a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 8 Eleven *S. habrochaites* LA1777 ILs containing the introgressions in chromosome 10 and their extra introgressions in other chromosomes for each line. The map was based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent leaf DI of each line in five independent experiments, and the mean LS and DI of the five experiments.

Stem assay

Stem resistance at 5dpi (days post inoculation) was measured in eight independent experiments as lesion size (LS) and disease incidence (DI). Between experiments, the mean LS varied from 0.77±0.03 cm to 1.90±0.03 cm. Significant correlations between experiments are shown in Table 5. Experiments 3, 5 and 6 were significantly correlated and used for further analysis. The variation in the mean LS in individual lines of experiments 3, 5 and 6 varied from 0.53±0.18 cm to 2.09±0.15 cm. *S. habrochaites* LA1777 has a significant lower stem LS (0.71±0.15cm) than *S. lycopersicum* E6203 (1.31±0.11cm). Most of IL lines showed more or less the same susceptibility as the control (Fig. 9).Only three lines, LA3913, LA3915 and LA3920 were individually significantly different from the susceptible control (Fig. 10 and Fig. 11). Lines LA3913 and LA3915 were overlapped on the bottom of chromosome 1, and other six lines overlapped in this region (LA3914, LA3916, LA3971, LA3995 and LA3998) also presented a less LS. Hence one QTL was considered as *Rbchq*1b for reducing stem LS. However, QTL possibly existed in line LA3920 was not confirmed in other ILs with overlapping introgressions.

Table 5 Pearson correlation of different experiments with stem lesion size (LS) in *S. habrochaites* LA1777 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8
Exp1	1	-0.017	0.039	0.213	0.072	0.150	0.141	0.112
Exp2		1	0.065	0.009	0.198	-0.026	-0.012	-0.051
Exp3			1	-0.288**	0.352**	0.220*	0.021	-0.203
Exp4				1	-0.067	-0.084	0.084	0.227*
Exp5					1	0.220*	0.123	-0.209*
Exp6						1	0.195	0.133
Exp7							1	0.037
Exp8								1

*: significant at the 0.05 level; **: significant at the 0.01 level.

The mean DI of the different experiments varied from 48.5±1.0% to 84.6±1.0%. Significant correlations between experiments are shown in Table 6. Experiments 3, 6 and 8 were used for the final analysis of DI. The stem DI was in the parental lines 37.7±3.9**%** (*S. habrochaites* LA1777) and 71.1±4.0**%** (*S. lycopersicum* E6203). The stem DI in the IL population varied from 47.5±5.4% to 100.0%. For three lines a significant reduced level of stem DI was found (LA3953, LA3989 and LA4001). Two of these lines (LA3953 and LA3989) have overlapping introgressions in the mid part of chromosome 8 (Fig. 12), the third line LA3988 with an introgression in this region has also a somewhat lower DI. These results show that in the mid part of Chromosome 8 a QTL is present, *Rbchq8*, reducing DI and originating from *S. habrochaites* LA1777. Another QTL (LA4001) could not be confirmed by the results of other ILs (Fig. 13).
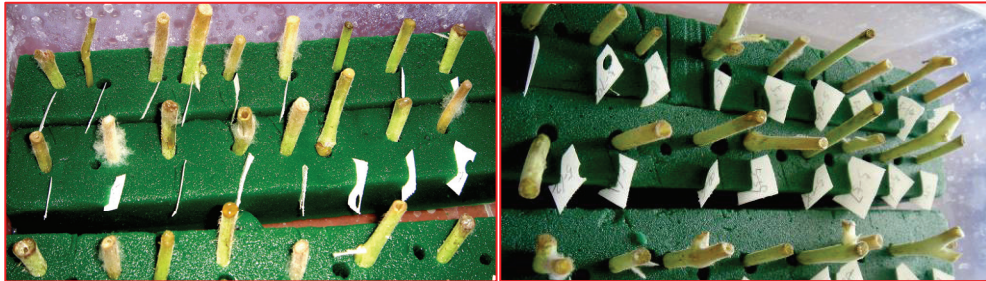
Fig. 9 Stem inoculation of *S. habrochaites* LA1777 IL population with *Botrytis cinerea*. Most IL lines were infected as the susceptible control E6203 and only a few lines showed less severe LS.
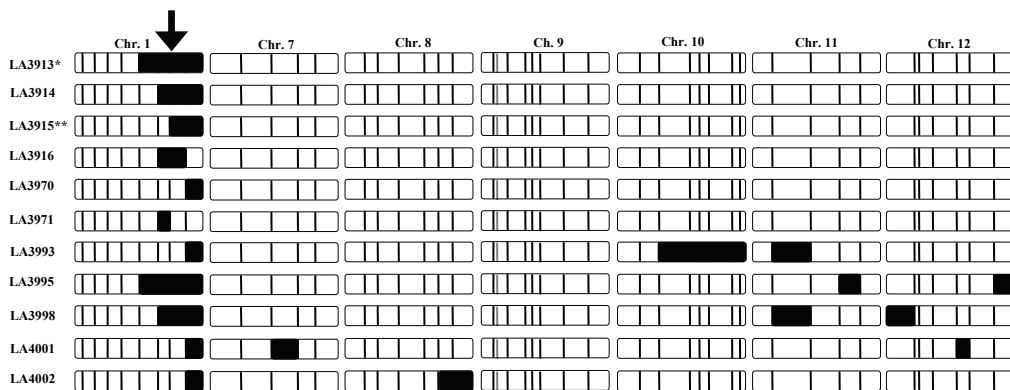
Lines	Stem LS of each experiment (cm^2)			N[a]	Mean LS(cm^2)	Mean DI (%)
	3	5	6			
LA3913	0.59	0.87	0.36	43	0.61 ±0.18[*]	86.7±6.6
LA3914	0.63	1.56	0.66	51	0.95 ±0.17(P=0.999)	67.1±5.9(P=0.958)
LA3915	0.34	0.73	0.52	54	0.53 ±0.18[**]	64.6±5.2(P=0.313)
LA3916	0.83	1.45	1.41	56	1.23±0.15	82.2±5.2
LA3970	0.84	0.60	1.97	56	1.13 ±0.16	88.0±5.4
LA3071	0.83	1.36	1.67	54	1.29±0.14	87.0±5.4
LA3993	0.89	2.36	1.79	54	1.68 ±0.14	81.3±5.5
LA3995	1.19	0.75	1.31	54	1.08 ±0.16	87.1±5.6
LA3998	1.11	0.99	1.66	58	1.25 ±0.14	94.6±5.4
LA4001	1.00	1.80	1.49	52	1.43 ±0.25	50.9±5.7[*]
LA4002	1.10	1.35	1.11	58	1.19 ±0.15	77.7±5.4
E6203	1.14	1.53	1.27	129	1.31 ±0.11	71.0±4.0
LA1777	0.72	0.91	0.77	101	0.80 ±0.15[*]	37.7±3.9

[a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 10 Eleven *S. habrochaites* LA1777 ILs containing the introgressions in chromosome 1 and their extra introgressions in other chromosomes. The map was based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent stem LS of each line in three independent experiments, and the mean LS and DI of the three experiments.
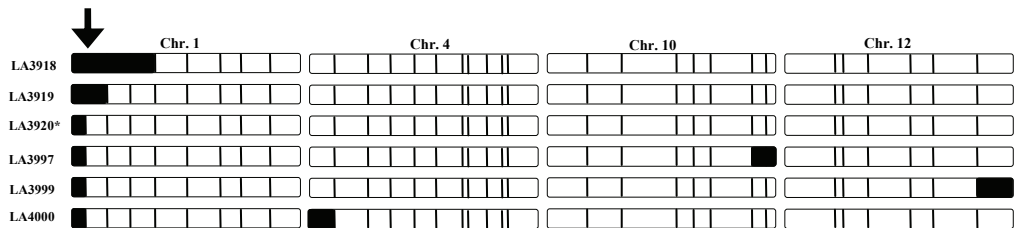
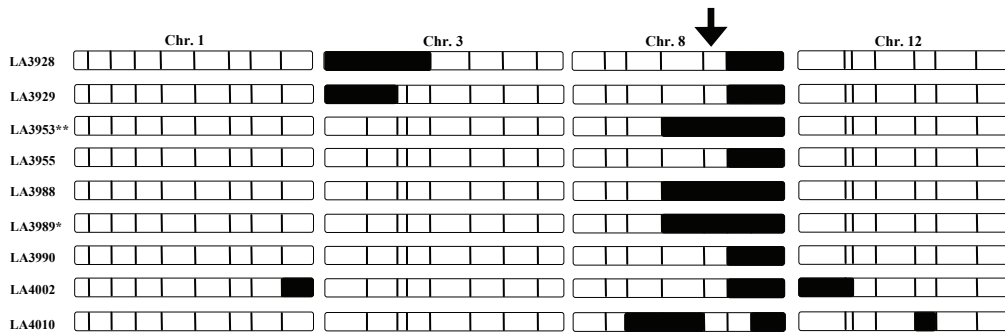| Lines	Stem LS of each experiment (cm^2)			N[a]	Mean LS (cm^2)	Mean DI (%)
	3	5	6			
LA3918	0.36	0.97	1.32	59	0.88 ±0.15(P=0.949)	84.4±5.2
LA3919	0.72	1.41	1.31	52	1.15 ±0.15	82.0±5.6
LA3920	0.60	0.73	0.75	55	0.69 ±0.15[*]	82.8±5.5
LA3997	1.26	1.73	2.00	55	1.66 ±0.16	81.8±5.5
LA3999	0.95	1.23	1.97	52	1.38 ±0.15	90.4±5.6
LA4000	1.44	1.27	0.72	54	1.14 ±0.14	88.3±5.7
E6203	1.14	1.53	1.27	129	1.31 ±0.11	71.0±4.0
LA1777	0.72	0.91	0.77	101	0.80 ±0.15[*]	37.7±3.9[**]

[a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 11 Six *S. habrochaites* LA1777 ILs, containing the introgressions in chromosome 1 and their extra introgressions in other chromosomes for each line. The map was based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent stem LS of each line in three independent experiments, and the mean LS and DI of the three experiments.

Table 6. Pearson correlation of different experiments with stem disease incidence (DI) in *S. habrochaites* LA1777 IL population

| Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8
Exp1		-0.085	0.279[**]	0.011	0.061	-0.087	0.073	0.008
Exp2			0.131	0.088	-0.016	-0.080	0.121	0.106
Exp3				0.056	0.155	0.339[**]	0.273[**]	0.335[**]
Exp4					-0.078	-0.074	-0.118	0.115
Exp5						0.138	0.002	0.141
Exp6							0.151	0.319[**]
Exp7								0.132
Exp8								

[*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

Lines	Stem DI of each experiment (%)			N^a	Mean LS (cm^2)	Mean DI (%)
	3	**6**	**8**			
LA3928	90.0	91.3	100.0	34	1.45 ±0.15	93.8±13.5
LA3929	60.0	83.3	72.0	59	1.00 ±0.18	71.8±5.4
LA3953	60.0	59.1	50.0	58	1.37 ±0.17	56.4±5.4**
LA3955	100.0	61.9	57.7	57	1.28 ±0.16	73.2±5.5
LA3988	50.0	91.3	56.5	56	1.42 ±0.17	65.9±5.5(*P*=0.961)
LA3989	20.0	83.3	39.1	57	1.28 ±0.24	47.5±5.4**
LA3990	90.0	81.0	50.0	55	1.45 ±0.16	73.7±5.5
LA4002	80.0	79.2	73.9	57	1.19 ±0.15	77.7±5.4
LA4010	80.0	90.9	95.8	56	1.27 ±0.15	88.9±5.5
E6203	88.8	88.0	36.4	127	1.31 ±0.11	71.0±4.0
LA1777	44.4	31.2	37.5	101	0.80 ±0.15*	37.7±3.9**

[a]:number of inoculation sites; * and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 12 Nine *S. habrochaites* LA1777 ILs containing introgressions on chromosome 8 and their extra introgressions on other chromosomes for each line. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent stem DI of each line in three independent experiments, and the mean LS and DI of the three experiments.
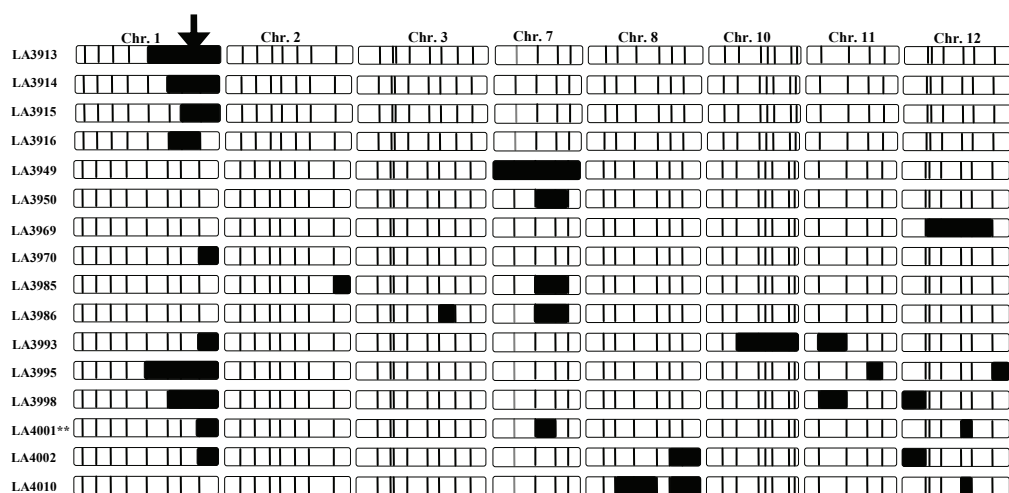
Lines	Stem DI of each experiment (%)			N^a	Mean LS (cm^2)	Mean DI (%)
	3	**6**	**8**			
LA3913	90.0	87.5	82.6	41	0.61 ±0.18[*]	86.7±6.6
LA3914	60.0	76.5	64.7	44	0.95 ±0.17	67.1±5.9(P=0.958)
LA3915	70.0	54.5	69.2	71	0.53±0.18[**]	64.6±5.2(P=0.313)
LA3916	70.0	87.0	89.7	72	1.23±0.15	82.2±5.2
LA3949	90.0	95.7	96.0	58	1.40 ±0.15	93.9±5.4
LA3950	80.0	95.2	100.0	32	1.74 ±0.15	91.7±13.5
LA3969	90.0	75.0	68.0	59	1.27 ±0.15	77.7±5.4
LA3970	100.0	100.0	64.0	58	1.13 ±0.16	88.0±5.4
LA3985	80.0	87.5	87.0	57	1.34 ±0.15	84.8±5.4
LA3986	100.0	83.3	90.9	56	1.71 ±0.14	91.4±5.5
LA3993	90.0	95.5	58.3	56	1.68 ±0.14	81.3±5.5
LA3995	70.0	91.3	100.0	44	1.08 ±0.16	87.1±5.6
LA3998	100.0	95.8	88.0	59	1.25 ±0.14	94.6±5.4
LA4001	20.0	55.6	77.3	50	1.43 ±0.25	50.9±5.7[**]
LA4002	80.0	79.2	73.9	57	1.19±0.15	77.7±5.4
LA4010	80.0	90.9	95.8	56	1.27 ±0.15	88.9±5.5
E6203	88.8	88.0	36.4	127	1.31 ±0.11	71.0±4.0
LA1777	44.4	31.2	37.5	101	0.80 ±0.15[*]	37.7±3.9[**]

[a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 13 Sixteen *S. habrochaites* LA1777 ILs containing introgressions on chromosome 1 and their extra introgressions on other chromosomes. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL. Values represent stem DI of each line in three independent experiments, and the mean LS and DI of the three experiments.

Fruit assay

Fruits were collected before breaking stage and inoculated to asses the level of resistance in nine independent experiments. Between experiments, the mean LS varied from 2.23 ± 0.47cm^2 to 7.56 ± 0.36cm^2 and the mean DI from $36.3\pm1.0\%$ to $97.5\pm3.4\%$. Significant correlations between experiments were found (Table 7) and experiments 5, 6 and 8 were used for the final analysis of LS. The mean LS was lower on fruits of the wild species (1.94 ± 0.48 cm^2) than on the susceptible control (2.90 ± 0.84 cm^2) (Fig. 14) but no significant differences for LS were found in the ILs. The LS on the fruits in the individual ILs varied from 1.95 cm^2 to 11.96 cm^2 and fourteen lines had significant larger LS.

Table 7 Pearson correlation of different experiments with green fruit lesion size (LS) in *S. habrochaites* LA1777 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8	Exp9
Exp1	1	0.242	0.124	0.250	0.199	0.256	0.436[**]	0.054	-0.007
Exp2		1	-0.037	-0.187	0.302[*]	-0.234	0.075	-0.222	0.013
Exp3			1	0.222	0.286[*]	0.150	0.084	0.285[*]	-0.092
Exp4				1	0.272[*]	0.408[**]	0.105	0.227[*]	0.016
Exp5					1	0.456[**]	0.064	0.227[*]	0.084
Exp6						1	0.159	0.085	-0.019
Exp7							1	0.182	-0.148
Exp8								1	0.192
Exp9									1

[*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

The mean DI on fruits varied between the experiments from $19.7\pm1.8\%$ to $73.5\pm1.4\%$. Experiments 1, 2 and 3 have been omitted since most of the lines had a 100% infection. Significant correlations resulted in the choice for further analysis using experiments 7, 8 and 9 (Table 8). The mean value of the individual ILs of these three experiments ranged from $13.6\pm5.6\%$ to $65.5\pm6.6\%$. The same values were observed for the wild accession LA1777 ($40.3\pm5.0\%$) and the control E6203 ($39.6\pm3.3\%$). Fifty one ILs had a lower DI than E6203. Five lines, LA3945, LA3984, LA3991, LA4002 and LA4008 showed a significant low DI but the results could not be confirmed in other ILs.

LS and DI were also determined in ripe fruits on a total of 100 fruits per line in seven independent experiments. The fruits of accession LA1777 were considered as ripe after the colour of the fruits changed into a deeper green color. The mean LS between experiments varied from 3.67 ± 0.28cm^2 to 6.46 ± 0.33cm^2, and the mean DI varied from $39.5\pm1.8\%$ to

98.2±4.0% for the complete IL population. Four experiments: 3, 5, 6 and 7 were used for further analysis (Table 9 and Table 10). The mean value of individual ILs ranged from 1.28±1.9cm^2 to 8.15±0.93cm^2. Sixty one lines out of ninety three lines showed significantly reduced LS and lines with overlapping introgressions were used to confirm QTLs. Twelve unambiguous QTLs *Rbchq1c*, *Rbchq2*, *Rbchq4a*, *Rbchq4b*, *Rbchq5*, *Rbchq6a*, *Rbchq7a*, *Rbchq7b*, *Rbchq9*, *Rbchq10a*, *Rbchq10b* and *Rbchq12*, located on chromosomes 1, 2, 4, 5, 6, 7, 9, 10 and 12 were identified. Other lines potentially harboring the QTLs had lower LS than the control (Fig. 15). This makes it possible to further pinpoint the location of a QTL. In addition, some lines likely harboring more than one QTL, for instance IL LA3964, presented a constant lower LS over all the experiments (Fig. 16).

Fig. 14 Response in green fruits of *S. habrochaites* LA1777 IL population, the susceptible parent *S. lycopersicum* E6203 and wild species LA1777 after inoculation with *Botrytis cinerea*.

Table 8 Pearson correlation of different experiments with green fruit disease incidence (DI) in *S. habrochaites* LA1777 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8	Exp9
Exp1	1	-0.012	-0.367[*]	0.072	0.268	0.043	-0.157	-0.345[*]	-0.220
Exp2		1	-0.168	0.07	-0.300[*]	0.100	0.060	-0.256	-0.253
Exp3			1	-0.069	-0.092	0.304	0.095	0.226	0.222
Exp4				1	0.178	0.125	0.023	0.048	0.095
Exp5					1	0.149	-0.093	-0.093	0.121
Exp6						1	0.190	-0.270	-0.258
Exp7							1	0.259[*]	0.234[*]
Exp8								1	0.460[**]
Exp9									1

[*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

Table 9 Pearson correlation of different experiments with ripe fruit lesion size (LS) in *S. habrochaites* LA1777 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7
Exp1	1	-0.105	0.087	0.024	0.473[**]	0.093	0.398[**]
Exp2		1	0.087	-0.212	0.126	0.063	0.137
Exp3			1	0.219[*]	0.264[*]	0.196	0.326[**]
Exp4				1	0.049	-0.041	0.036
Exp5					1	0.275[*]	0.479[**]
Exp6						1	0.273[*]
Exp7							1

[*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

Table 10 Pearson correlation of different experiments with ripe fruit disease incidence (DI) in *S. habrochaites* LA1777 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7
Exp1	1	0.288[*]	0.133	-0.299[*]	-0.049	0.259	0.175
Exp2		1	0.052	-0.271[*]	-0.064	0.211	0.236[*]
Exp3			1	0.072	0.247[*]	0.288[**]	0.286[**]
Exp4				1	0.060	0.031	0.015
Exp5					1	0.253[*]	0.430[**]
Exp6						1	0.364[**]
Exp7							1

[*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

Chr. 1

LA3913**
LA3914**
LA3915
LA3916**
LA3917**
LA3970
LA3971
LA3976**
LA3993**
LA3995**
LA3998**
LA4001
LA4002**

Chr. 2

LA3917**
LA3921
LA3922**
LA3923**
LA3924**
LA3965**
LA3972
LA3985**
LA4003**
LA4004**

Chr. 4

LA3932**
LA3933**
LA3934
LA3935**
LA3936
LA3937
LA3976**
LA3977**
LA3978**
LA4007**

Chr. 5

LA3938**
LA3939
LA3940
LA3980**
LA3981**
LA3984**

Chr. 6

LA3944**
LA3945**
LA3946**
LA3947
LA4004**
LA4005**
LA4008**
LA4009**

Chr. 7

LA3948**
LA3949**
LA3950**
LA3951**
LA3985**
LA3986
LA4001
LA4009**

Chr. 9

LA3956**
LA3957**
LA3958**
LA3959**
LA3960**
LA3991
LA4008**

Chr.10

LA3945**
LA3946**
LA3954**
LA3960**
LA3961**
LA3962**
LA3963**
LA3964**
LA3965**
LA3966
LA3993**
LA3994
LA3997*
LA4006*
LA4009**

Chr.12

LA3917**
LA3960**
LA3962**
LA3966
LA3968**
LA3969**
LA3995**
LA3998**
LA3999
LA4001
LA4002**
LA4007**
LA4010

Lines	QTLs	Linked markers	Ripe fruit LS in each experiment (cm^2)				N[a]	Mean LS (cm^2)	Mean DI (%)
			3	**5**	**6**	**7**			
LA3913	*Rbchq1b*	TG245-TG27	5.69	3.90	8.55	2.92	72	5.26±1.69**	75.5±9.8(*P*=0.143)
LA3914			4.24	2.51	-	5.95	75	4.23±0.82**	53.2±5.4**
LA3916			5.35	3.90	6.15	5.16	102	5.14±0.62**	81.6±3.6(*P*=0.347)
LA3995			3.61	2.09	6.30	2.79	90	3.70±1.47**	72.9±5.9(*P*=0.124)
LA3922	*Rbchq2*		4.68	2.70	5.93	3.84	94	4.29±0.63**	76.3±3.7**
LA3923			3.37	3.80	5.49	5.48	67	4.53±0.83**	77.8±4.8(*P*=0.160)
LA3924			2.23	2.79	2.47	4.19	59	2.92±0.82**	89.3±5.3
LA4003			3.47	5.55	4.42	4.69	42	4.53±1.38**	84.5±8.4
LA3932	*Rbchq4a*	TG370-TG264	3.31	2.16	1.20	2.89	64	2.39±1.45**	77.2±9.3*
LA3933			6.01	2.70	3.08	4.71	93	4.13±1.04**	89.2±6.8
LA3935	*Rbchq4b*	TG305-CD39	1.95	2.12	4.59	3.39	88	3.01±0.75**	77.5±4.3(*P*=0.06)

Lines	QTLs	Linked markers	Ripe fruit LS in each experiment (cm^2)				N[a]	Mean LS (cm^2)	Mean DI (%)
			3	5	6	7			
LA3977			4.45	3.01	6.06	3.85	83	4.34±0.69**	75.2±3.9**
LA3978			4.48	1.63	4.93	3.87	97	3.73±0.82**	74.9±4.5(*P*=0.240)
LA3938	*Rbchq5*	CT101-CD64	6.94	4.21	-	3.60	72	4.92±0.83**	76.0±4.7(*P*=0.143)
LA3980			5.90	2.78	3.49	5.42	88	4.40±0.76**	87.1±4.5
LA3981			4.49	2.18	2.17	3.40	73	3.06±0.99**	61.0±4.9**
LA3984			7.48	1.66	4.59	3.64	97	4.34±0.80**	67.2±4.1**
LA3944	*Rbchq6a*	TG164-TG292	5.74	0.89	-	1.86	72	2.83±1.02**	42.8±9.4**
LA4005			2.30	-	-	2.30	65	2.30±1.36**	28.9±12.6**
LA3948	*Rbchq7a*	CT195-TG216	3.67	6.30	4.13	3.41	94	4.38±0.84**	62.4±3.9**
LA3949	*Rbchq7a&7b*	TG202-TG61	3.81	3.89	7.91	3.88	88	4.87±0.71**	75.0±3.8(*P*=0.06)
LA3950	*Rbchq7b*		2.02	4.28	7.58	3.26	46	4.29±1.52**	76.2±5.9
LA3951			7.66	4.28	7.58	3.26	76	4.44±1.01**	84.4±9.9
LA3958	*Rbchq9*	CT196-CT112	5.05	5.71	5.56	4.04	71	5.09±1.29**	69.4±6.2(*P*=0.541)
LA3959			8.49	4.32	-	2.92	44	5.24±1.43**	66.7±11.0(*P*=0.999)
LA3954	*Rbchq10a*	TG241-TG233	6.90	2.52	2.02	3.07	77	3.63±1.5**	69.5±5.6(*P*=0.80)
LA4006			4.66	-	-	3.66	48	4.16±0.85*	82.1±5.1
LA3961	*Rbchq10a&10b*		4.39	2.09	-	3.42	52	3.30±1.00**	85.9±5.7
LA3963	*Rbchq10b*		5.29	3.05	-	2.89	57	3.74±1.07**	57.1±7.6**
LA3964			5.84	2.68	8.81	5.29	110	5.65±0.77**	75.0±4.3(*P*=0.549)
LA3997			6.28	2.77	4.94	5.70	86	4.92±0.93*	75.1±5.1
LA3968	*Rbchq12*	TG180-CT211	4.28	2.01	-	4.54	79	3.61±1.07**	55.4±6.7**
LA3969			4.54	4.86	5.54	4.81	112	4.94±0.58**	87.0±3.5
LA3917	*Rbchq1b&2&12*		1.85	1.50	2.71	7.25	32	3.33±1.30**	78.3±7.1
LA3945	*Rbchq6a&10b*		7.46	2.29	-	2.35	26	4.03±1.80**	43.8±10.8**
LA3946	*Rbchq6a&10b*		2.79	2.92	4.30	3.15	83	3.29±0.65**	82.1±3.9(*P*=0.564)
LA3960	*Rbchq9&10a&12*		3.49	4.56	-	2.41	30	3.48±1.94**	86.5±12.3
LA3962	*Rbchq10b&12*		4.17	3.50	5.45	4.02	108	4.28±0.69**	78.3±3.7(*P*=0.316)
LA3965	*Rbchq2&10b*		3.19	2.60	6.74	3.02	83	3.89±0.67**	80.1±3.7
LA3976	*Rbchq1b&4b*		2.53	2.83	6.74	5.09	89	4.30±1.03**	72.7±5.3(*P*=0.623)
LA3985	*Rbchq2&7b*		2.21	-	-	2.28	33	2.25±1.22**	71.3±6.6(*P*=0.148)

Lines	QTLs	Linked markers	Ripe fruit LS in each experiment (cm^2)				N[a]	Mean LS (cm^2)	Mean DI (%)
			3	5	6	7			
LA3993	*Rbchq1b&10b*		4.44	1.18	-	1.53	37	2.38±1.91[**]	85.2±12.2
LA3998	*Rbchq1b&12*		2.69	1.46	2.62	2.07	95	2.21±0.63[**]	87.5±3.8
LA4002	*Rbchq1b&12*		2.40	0.82	-	1.69	73	1.63±1.82[**]	59.6±12.5[**]
LA4004	*Rbchq2&6a*		2.81	3.63	8.02	4.32	50	4.70±1.91[**]	92.5±12.6
LA4007	*Rbchq1b&12*		3.23	5.14	7.22	3.22	66	4.70±1.63[**]	94.4±10.5
LA4008	*Rbchq6a&9*		6.21	-	-	1.93	75	4.07±0.84	44.4±8.7[**]
LA4009	*Rbchq6a&7a&10b*		1.56	-	-	1.00	17	1.28±1.90[**]	51.5±8.8[**]
E6203	-		8.80	4.59	8.76	7.06	172	7.30±0.48	91.2±2.9
LA1777	-		-	-	-	0.24	37	0.24±3.79[*]	3.4±6.9[**]

-: lines not tested in that particular experiment due to low number of seeds; [a]:number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 15 QTLs responsible for reducing LS in ripe fruit were identified on chromosome 1, 2, 4, 5, 6, 7, 9, 10 and 12 from *S. habrochaites* LA1777 IL populations. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL and the flanking markers were estimated. Values represent ripe fruit LS of each line in four independent experiments and the mean LS and DI of the four experiments.

Fig. 16 Comparison of IL line LA3964 harboring QTL *Rbchq6a* and *Rbchq10b* for resistance to *Botrytis cinerea* in red fruits and the susceptible control *S. lycopersicum* E6203.

S. habrochaites showed on average a low ripe fruit DI (3.4±6.9%) and *S. lycopersicum* a high ripe fruit DI (91.2±2.9%).The mean DI ranged from 28.9±12.6% to 93.8±4.5% in the individual ILs. Twenty lines (LA3914, LA3915, LA3922, LA3928,

LA3930, LA3932, LA3937, LA3944, LA3945, LA3948, LA3963, LA3068, LA3977, LA3981, LA3984, LA3994, LA4002, LA4005, LA4008 and LA4009) had a significant lower ripe fruit DI than the susceptible control. Three unambiguous QTLs *Rbchq3*, *Rbchq6b* and *Rbchq7c*, located on chromosomes 3, 6 and 7 were identified (Fig. 17). Most of these QTLs were involved in the reduction of both parameters (LS and DI).
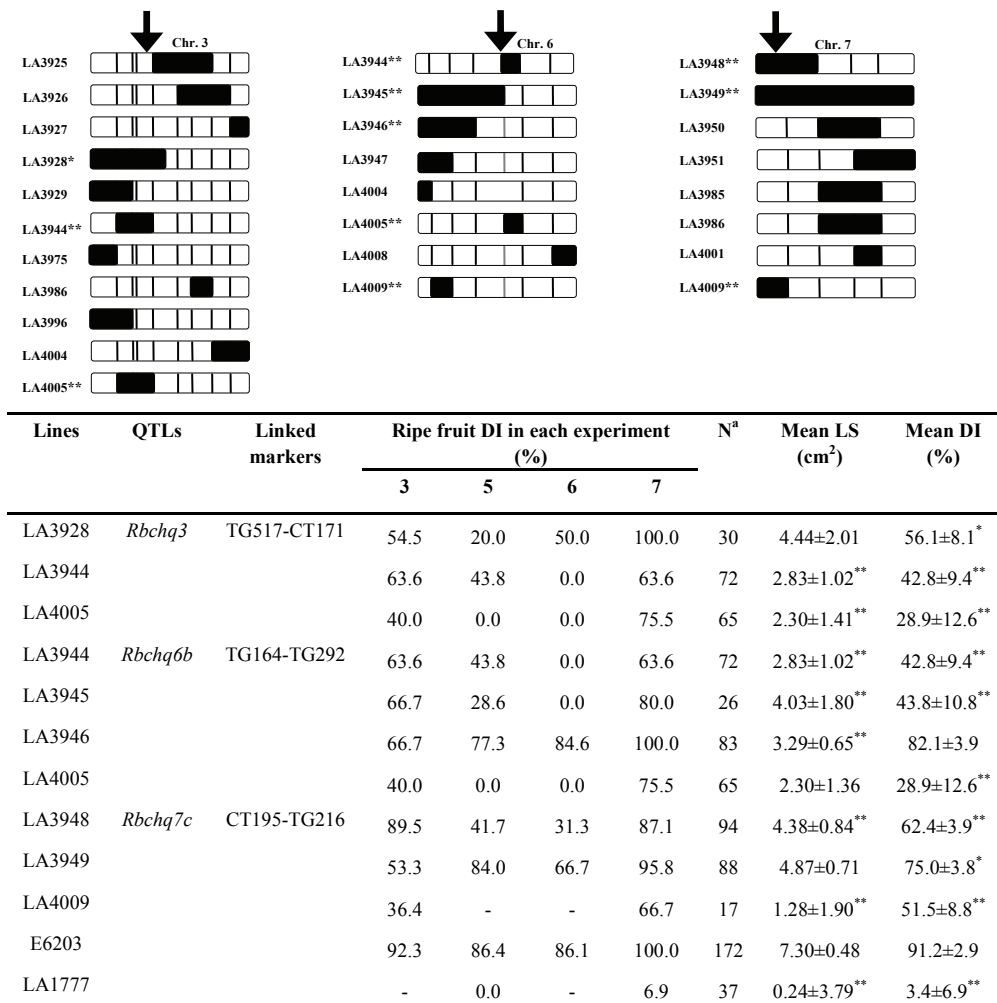
Lines	QTLs	Linked markers	Ripe fruit DI in each experiment (%)				N[a]	Mean LS (cm^2)	Mean DI (%)
			3	5	6	7			
LA3928	*Rbchq3*	TG517-CT171	54.5	20.0	50.0	100.0	30	4.44±2.01	56.1±8.1[*]
LA3944			63.6	43.8	0.0	63.6	72	2.83±1.02[**]	42.8±9.4[**]
LA4005			40.0	0.0	0.0	75.5	65	2.30±1.41[**]	28.9±12.6[**]
LA3944	*Rbchq6b*	TG164-TG292	63.6	43.8	0.0	63.6	72	2.83±1.02[**]	42.8±9.4[**]
LA3945			66.7	28.6	0.0	80.0	26	4.03±1.80[**]	43.8±10.8[**]
LA3946			66.7	77.3	84.6	100.0	83	3.29±0.65[**]	82.1±3.9
LA4005			40.0	0.0	0.0	75.5	65	2.30±1.36	28.9±12.6[**]
LA3948	*Rbchq7c*	CT195-TG216	89.5	41.7	31.3	87.1	94	4.38±0.84[**]	62.4±3.9[**]
LA3949			53.3	84.0	66.7	95.8	88	4.87±0.71	75.0±3.8[*]
LA4009			36.4	-	-	66.7	17	1.28±1.90[**]	51.5±8.8[**]
E6203			92.3	86.4	86.1	100.0	172	7.30±0.48	91.2±2.9
LA1777			-	0.0	-	6.9	37	0.24±3.79[**]	3.4±6.9[**]

-: lines not tested in that particular experiment due to low number of seeds; [a]: number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 17 QTLs responsible for reducing DI in ripe fruit were identified on chromosome 3, 6 and 7 from *S. habrochaites* LA1777 IL populations. The map is based on Monforte and Tanksley (2000). The arrow gives the most likely location of the suggested QTL and the flanking markers were estimated. Values represent ripe fruit LS of each line in four independent experiments, and the mean LS and DI of the four experiments.

Correlation of different parameters

Pearson correlation coefficients were calculated to asses the correlations between different parameters. The results (Table 11) show that low but significant correlations ($P<0.01$) were present between LS and DI in leaves, stems and ripe fruits. However, in general no correlations were detected between values measured in different tissues with some exceptions of low but significant correlations (leaf DI and stem LS/DI and ripe fruit LS/DI; stem DI and green fruit LS/ripe fruit DI; green fruit DI and ripe fruit DI).

Table 11 Correlation of different traits in *S. habrochaites* LA1777 IL population

Correlation	Leaf LS	Leaf DI	Stem LS	Stem DI	G-fruit[1] LS	G-fruit DI	R-fruit[2] LS	R-fruit DI
Leaf LS	1	0.429[**]	0.105	0.076	0.196	-0.248[*]	-0.070	0.081
Leaf DI		1	0.204[*]	0.315[*]	0.155[**]	-0.066	0.228[**]	0.421[**]
Stem LS			1	0.322[**]	0.124	0.155	-0.127	0.188
Stem DI				1	0.211[*]	0.190	0.090	0.210[*]
G-fruit LS					1	0.041	0.193	0.143
G-fruit DI						1	0.075	0.298[**]
R-fruit LS							1	0.529[**]
R-fruit DI								1

[1]G-fruit: green fruit; [2] R-fruit: ripe fruit; [*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

Susceptibility to *B. cinerea* in LA2951 IL population

Leaf assay

Eight independent experiments were conducted to measure leaf LS and DI in this IL population. The wild accession *S. lycopersicoides* LA2951 was not evaluated due to the difficulty in seed production. Between experiments, the mean value varied from 3.47±0.06 cm^2 to 5.87±0.06 cm^2 for LS and 59.6±0.70% to 97.8±0.72% for DI. Significant correlations between experiments were low (r=0.275, $P<0.05$) to middle (r=0.675, $P<0.01$) for LS in most of the experiments but for DI only experiment 1 and 4, experiment 4 and 5, and experiment 6 and 8 were significantly correlated (Tables 12 and Table 13). Experiments 2, 5, 7 and 8 were excluded for the final LS analysis because of the low correlations. Exclusion of these experiments only influenced the significance of the QTLs. *S. lycopersicum* VF36 has a mean leaf LS of 5.60±0.2 cm^2 and the mean leaf LS of IL individuals varied from 1.99±0.48 to 7.25±0.59 cm^2. Four lines (LA3883, LA4244, LA4277 and LA4278) showed a significantly reduced leaf LS, the reduction was 55.9%, 46.0%, 50.6% and 64.5% respectively. These lines

probably contain QTLs for reducing leaf LS originating from *S. lycopersicoides* LA2951. A complicating factor of this IL population compared to the *S. habrochaites* LA1777 IL population is that approximately 34% of the lines are sterile and need to be maintained with heterozygous introgressions (Canady et al. 2005). These heterozygous lines segregate in the progeny after selfing. Based on Canady et al. (2005) the above identified LA4278 is a heterozygous line, which contains two heterozygous fragments located on chromosomes 9 and 11. However, we found that homozygous progeny of this line can also be obtained after self-pollination (see materials and methods). LA3883 is a homozygous line which contains two fragments located on chromosome 7 and 11 respectively. LA4244 is a homozygous line which contains a single fragment located on the top of chromosome 4.

Table 12 Pearson correlation of different experiments with leaf lesion size (LS) in *S. lycopersicoides* LA2951 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8
Exp1	1	0.143	0.479**	0.538**	0.128	0.675**	0.310	0.377**
Exp2		1	0.302*	0.293*	0.147	0.072	0.319*	-0.004
Exp3			1	0.367**	-0.148	0.466**	0.503**	0.174
Exp4				1	0.237	0.391**	0.331*	0.287
Exp5					1	0.298	0.193	0.275*
Exp6						1	0.340**	0.484**
Exp7							1	0.193
Exp8								1

*: significant at the 0.05 level; **: significant at the 0.01 level.

Table 13 Pearson correlation of different experiments with leaf disease incidence (DI) in *S. lycopersicoides* LA2951 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8
Exp1	1	-0.038	0.025	0.463**	0.167	0.041	0.000	-0.078
Exp2		1	0.147	0.074	0.070	0.004	0.182	0.043
Exp3			1	0.067	0.075	0.129	0.263	-0.222
Exp4				1	0.526**	0.264	-0.091	0.130
Exp5					1	0.076	-0.084	0.232
Exp6						1	-0.147	0.386**
Exp7							1	-0.006
Exp8								1

*: significant at the 0.05 level; **: significant at the 0.01 level.

To confirm the resistance of these lines, lines with overlapping introgressions were also considered. Figure 18 shows that three lines (LA4270, LA4271 and LA4272) had

overlapping introgressions with LA4278 on chromosome 9 and four lines (LA3883, LA4234, LA4277 and LA4279) had overlapping introgressions on chromosome 11 (Canady et al. 2005). The first three lines had a LS similar to the control and indicate that the introgression on chromosome 9 does not contribute to the resistance level in LA4278 and hence that the QTL is present on chromosome 11. This is confirmed by the homozygous line LA3883 and the heterozygous line LA4277, which had significant lower LS. Both of them contain a short segment on the top of chromosome 11. Since LA4278 and LA3883 exhibited a low LS in all four experiments, we name this QTL *Rbclq11*. This introgression is flanked by markers TG557 and TG49. In the same manner, we identified another QTL (in introgression line LA4244) for reduced leaf LS; *Rbclq4*, which is located on Chromosome 4 and flanked by markers TG49 and TG146 (Fig. 18).

Experiments 1 and 4 were used for the final analysis for leaf DI. The mean DI value in the individuals varied from $28.6\pm5.6\%$ to 100%. The results showed that six ILs LA3870, LA3874, LA3886, LA3890, LA4233 and LA4244 had a significant lower leaf DI than the control. The homozygous ILs LA4233 and LA3874 have an overlap on the top of Chromosome 3. The heterozygous IL LA4263, with an introgression in this region, also had a lower DI. These results show that there is likely a QTL, *Rbclq3a*, located on the introgression flanked by markers TG479 and TG114 on Chromosome 3. In addition, line LA4244 is particularly interesting because it also showed significant lower LS. However, the possible QTLs involved in LA3870, LA3886 and LA3890 remain speculative.

Stem assay

Eight independent experiments were conducted to measure stem LS and DI. The mean value varied from 0.91 ± 0.04 cm^2 to 2.01 ± 0.04 cm^2 for LS and $66.3\pm2.1\%$ to $96.8\pm1.7\%$ for DI. No significant correlation was observed between the experiments and no QTLs were identified.

Fruit assay

Six independent experiments were used to evaluate resistance in the green fruit stage. Between experiments, the mean LS varied from 3.37 ± 0.73 cm^2 to 12.7 ± 0.39 cm^2, while the mean DI was almost always 100% with as exception experiment 6 (38.8%). A Pearson correlation was calculated for LS for the different experiments. Significant correlations were calculated between experiments 2, 3 and 5, and experiment 3, 4 and 5 (Table 14). Finally experiments 2, 3 and 5 were used for further analysis. Excluding experiments 1, 4 and 6, only influenced the significance of the QTLs. Based on experiments 2, 3 and 5 *S. lycopersicum* VF36 showed a mean LS of 8.43cm^2 and the values of the ILs varied from 3.27 ± 2.21 cm^2 to 18.55 ± 5.31 cm^2. The average LS of forty two individual lines was lower than the control

VF36 (Fig. 19) and of these lines the effect in only line LA4308 was significant but could not further be substantiated (Fig. 20). The reason is attributed to fewer samples from this IL population for green fruits.

Lines	QTLs	Flanking markers	Leaf LS of each experiment (cm^2)				N[a]	Mean LS (cm^2)	Mean DI (%)	Homo. line
			1	3	4	6				
LA3875	*Rbclq4*	TG49-TG146	-	-	4.91	-	17	4.91±0.54	100.0±9.9	Y
LA4244			3.50	-	-	2.55	77	3.02±0.34[*]	37.7±13.9	Y
LA4277	*Rbclq4/11*		-	-	-	2.77	47	2.77±0.42[*]	59.6±5.9	N
LA3883	*Rbclq11*	TG557-TG497	2.34	2.75	2.52	2.27	90	2.47±0.29[**]	79.2±4.7	Y
LA4278			1.54	2.34	1.60	2.46	56	1.99±0.48[**]	89.9±8.6	Y
LA3886			3.13	3.70	3.55	2.56	109	3.24±0.27	73.6±4.2	Y
LA4234			-	4.90	6.81	3.87	64	5.19±0.36	83.7±6.2	Y
LA4257			-	4.18	4.79	3.53	69	4.16±0.33	85.3±5.7	Y
LA4259			-	5.81	3.49	3.01	69	4.10±0.32	84.3±5.5	Y
LA4270			-	5.68	5.87	3.57	80	5.04±0.30	87.1±5.2	Y
LA4271			-	-	-	4.81	37	4.81±0.47	62.2±6.7	N
LA4272			-	-	-	3.91	44	3.91±0.41	68.2±6.1	N
LA4279			-	8.14	6.76	3.59	68	6.16±0.35	84.0±6.0	N
VF36			7.09	7.75	4.24	3.33	393	5.60±0.20	83.6±3.6	Y

-: lines not tested in that particular experiment due to low number of seeds; [a]: number of inoculation sites; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 18 QTLs responsible for reducing leaf LS were identified on chromosome 4 and 11 in *S. lycopersicoides* LA2951 IL population. The map was based on Canady (2005). The arrow gives the most likely location of the suggested QTL and flanking markers were estimated. Values represent leaf LS of each line in four independent experiments, and the mean LS and DI of the four experiments.

Table 14 Pearson correlation of different experiments with green fruit lesion size (LS) in *S. lycopersicoides* LA2951 IL population

Correlation	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6
Exp1	1	0.210	-0.017	-0.121	0.028	-0.111
Exp2		1	0.268*	0.010	0.310*	-0.090
Exp3			1	0.290*	0.295*	0.038
Exp4				1	-0.184	-0.145
Exp5					1	0.202
Exp6						1

*: significant at the 0.05 level; **: significant at the 0.01 level.

Fig. 19 Screening of green fruits of *S. lycopersicoides* lA2951 IL population after inoculation with *Botrytis cinerea*. IL lines LA3867, LA3890 and LA4253 showed a reduced LS and LA4232 and LA4251 presented the same LS as the susceptible control VF36.
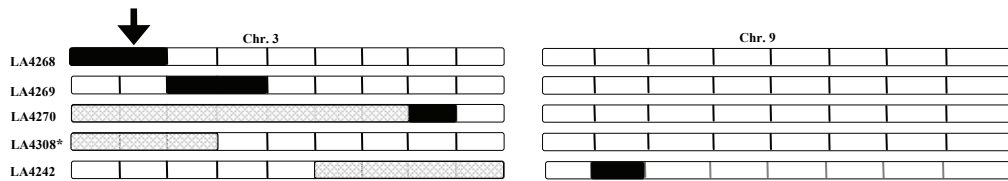
Lines	Green fruit LS of each experiment (cm^2)			N^a	Mean LS (cm^2)	Mean DI (%)
	2	**3**	**5**			
LA4268	26.89	9.34	9.89	8	15.37 ±3.26	91.7±9.4
LA4269	-	10.23	0.90	14	5.56 ±2.44	88.7±4.7
LA4270	12.33	5.81	0.94	15	6.36 ±2.77	90.0±4.7
LA4308	5.91	3.03	0.86	15	3.27 ±2.21*	87.2±5.2
LA4242	7.63	5.35	6.44	15	6.47 ±2.27	91.8±5.1
VF36	12.30	6.25	6.73	64	8.43 ±1.21	93.8±2.5

-: lines not tested in that particular experiment due to low number of seeds; ^{a}: number of inoculation sites; * and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 20 Five *S. lycopersicoides* LA2951 ILs containing introgressions in chromosome 3 and their extra introgressions in other chromosomes for each line. The map was based on Canady (2005). The arrow gives the most likely location of the suggested QTL. Values represent green fruit LS of each line in three independent experiments, and the mean LS and DI of the three experiments.

Four independent experiments were conducted on ripe fruits. The mean LS varied from 5.90±0.34 cm^2 to 12.0±0.36 cm^2 and the mean DI from 50.3±2.3% to 93.4±2.5% between experiments. Experiment 1, 2 and 3, and experiment 3 and 4 showed a significant correlation for LS; and experiment 1 and 2, experiment 2, 3 and 4 for DI (Tables 15 and 16). Experiments 1, 2 and 3 were further analyzed. The mean LS of *S. lycopersicum* VF36 was 12.0±0.58 cm^2 and the LS in the three IL populations ranged from 1.15±1.19 cm^2 to 23.24±0.77 cm^2. Out of sixty three lines with lower LS, eighteen lines presented a significantly lower value. Three clear QTLs, *Rbclq3b*, *Rbclq6*, and *Rbclq12*, were identified for reducing LS on ripe fruits (Fig. 21).

Table 15 Pearson correlation of different experiments with ripe fruit lesion size (LS) in *S. lycopersicoides* LA2951 IL population

Correlation	Exp1	Exp2	Exp3	Exp4
Exp1	1	0.349*	0.306*	0.213
Exp2		1	0.491**	0.202
Exp3			1	0.326*
Exp4				1

*: significant at the 0.05 level; **: significant at the 0.01 level.

Table 16 Pearson correlation of different experiments with ripe fruit disease incidence (DI) in *S. lycopersicoides* LA2951 IL population

Correlation	Exp1	Exp2	Exp3	Exp4
Exp1	1	0.607**	0.064	0.104
Exp2		1	0.426**	0.278*
Exp3			1	0.243*
Exp4				1

*: significant at the 0.05 level; **: significant at the 0.01 level.
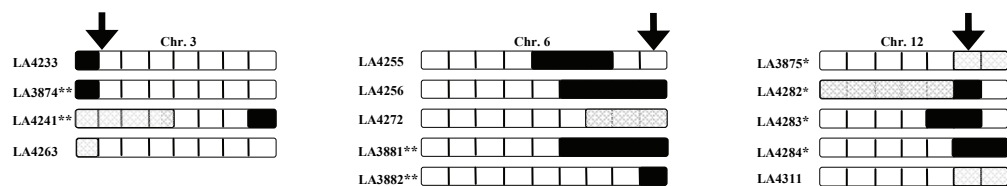
Lines	QTLs	Linked markers	Ripe fruit LS in each experiment (cm^2)			N[a]	Mean LS (cm^2)	Mean DI (%)	Homo. line
			1	2	3				
LA3874	*Rbclq3b*	TG479-TG114	-	1.61	4.55	5	3.08 ±2.20**	88.9±18.1	Y
LA4241				5.9	7.28	15	6.59 ±1.2**	100.0±11.0	Y
LA3881	*Rbclq6*	TG220-TG581		2.85		11	2.85 ±1.39**	55.5±10.8	Y
LA3882			8.58	4.37	6.90	72	6.62 ±0.60**	56.4±5.1*	Y
LA4256			2.33	6.55	-	3	4.44 ±2.70 (*P*=0.71)	66.7±18.3	Y
LA3875	*Rbchq12*	CT156-TG437	7.80	5.77	-	14	6.79 ±1.45*	90.9±12.1	Y
LA4282			-	3.80	7.10	21	5.45 ±1.12*	78.7±6.7	N
LA4283			11.16	6.92	7.05	89	8.38 ±0.52*	84.9±7.5	Y
LA4284			7.13	6.96	9.00	31	7.70 ±1.59*	64.6±7.0	Y
LA4311			10.42	5.84	6.99	24	7.75±1.45 (*P*=0.06)	77.3±10.1	N
VF36			16.26	7.89	11.93	46	12.02 ±0.77	86.8±6.6	Y

-: lines not tested in that particular experiment due to low number of seeds; [a]: number of inoculation sites; * and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 21 QTLs responsible for reducing ripe fruit LS were identified on chromosome 3, 6 and 12 in *S. lycopersicoides* LA2951 IL population. The map is based on Canady (2005). The arrow gives the most likely location of the suggested QTL and flanking markers were estimated. Values represent leaf LS of each line in three independent experiments, and the mean LS and DI of the three experiments.

Experiments 2, 3 and 4 were used for further analysis of DI. *S. lycopersicum* VF36 showed a mean DI of about 86.8±6.6% and the individual ILs ranged from 12.5±14.2% to 100%. Sixty ILs presented a lower DI than VF36, in ten lines this result was significant:

LA3867, LA3876, LA3877, LA3879, LA3883, LA4233, LA4238, LA3882, LA4254 and LA4251. However, these QTLs could not be validated.

Correlation of different parameters

Pearson correlation coefficients were also calculated to asses the correlations between different parameters. In general there is a significant correlation between LS and DI in the same tissue. But a very low correlation was found between leaf LS/DI, green fruit LS and ripe fruit LS/DI with the exception of between green fruit LS and ripe fruit DI (Table 17).

Table 17 Correlation of different traits in *S. lycopersicoides* LA2951 IL population

Correlation	Leaf LS	Leaf DI	G-fruit[1] LS	R-fruit[2] LS	R-fruit DI
Leaf LS	1	0.260[*]	-0.129	0.156	-0.113
Leaf DI		1	0.088	0.137	-0.020
G-fruit LS			1	0.163	0.252[*]
R-fruit LS				1	0.310[**]
R-fruit DI					1

[1]G-fruit: green fruit; [2] R-fruit: ripe fruit; [*]: significant at the 0.05 level; [**]: significant at the 0.01 level.

Discussion

Screening of wild species for *B. cinerea* resistance

Wild relatives of tomato have been screened to evaluate the resistance to *B. cinerea* in several research groups. Accessions of *S. chilense* (LA2747), *S. peruvianum* (LA2745), *S. habrochaites* (LA2314, LYC4), *S. pimpinellifolium* (LA1246), *S. neorickii* G1.1560, and *S. lycopersicoides* (LA2951) have shown partial resistance in leaf and/or stem (Davis et al. 2009;Decognet et al. 2009;Egashira et al. 2000;Finkers et al. 2007a;Guimarães et al. 2004). In this paper, twenty two wild accessions from nine different tomato species and three *S. lycopersicum* accessions were screened for leaf resistance. Two parameters for resistance were determined in a detached leaflet assay. Of these accessions, nine accessions including *S. pimpinellifolium* LA1246, *S. habrochaites* LA2314, PI126445, PI247087, LA1777, PI134417, *S. peruvianum* LA2745 and *S. lycopersicoides* LA2951, LA2408 have been previously screened and partial resistance in leaf and/or stem was observed. In our study, we confirmed that all these previously tested accessions showed some level of resistance. In three accessions: LA2951, PI134417 and LA1392, a great reduction of both leaf lesion size and of

disease incidence was identified. Our results are in agreement with previous research with *S. lycopersicoides* LA2951.

Resistance correlation of different experiments and tissues

In general low correlations were observed between different experiments and different tissues although we checked the quality of inoculum in all experiments by determining the germination efficiency. Variation was also observed within experiments. This is in agreement with the results obtained by other researchers (Davis et al. 2009;Decognet et al. 2009;Finkers et al. 2007a;Guimarães et al. 2004). The environment in which the plants were grown can vary (e.g. day length, hours of sun and temperature), affecting their physiologic condition and very small differences in inoculation conditions can cause relatively big differences in the response of the plants. Seasonal dependent resistance has been observed from the resistance resource *S. lycopersicoides* LA2951(Davis et al. 2009). The difficulties within and between experiments clearly show the need for a more robust bio-assay, especially in stem tests. Meanwhile low sample size is also a major reason for low correlation in the test because in some lines only few seeds or fruits were produced, especially in the *S. lycopersicoides* IL population. Some lines were frequently absent in several experiments.

Low correlations were found in the resistance levels between leaves, stems, green fruits and ripe fruits. This corresponds to earlier results where a low correlation was found between stem and leaf resistance. It indicates that different loci are responsible for resistance in different tissues. This and the large number of QTLs make the introgression of resistance to *B. cinerea* into tomato cultivars a great challenge. However, some significant correlations were seen for DI and LS in several tissues making it easier to transfer levels of resistance for both DI and LS into breeding lines.

In addition, our experiments were conducted in a sealed box. It apparently enhances infection by the zoospores causing a very high disease pressure (Decognet et al. 2009) and this makes the bioassay very vulnerable to small differences in the environmental conditions.

QTLs for leaf resistance

The screening of wild species showed that *S. lycopersicoides* LA2951, *S. habrochaites* PI134417, LA1392 and LA1777 have higher levels of leaf resistance. Quantitative resistance to *B. cinerea* was observed in two IL populations. After analysis of five independent experiments, thirty ILs of *S. habrochaites* LA1777 showed reduced LS. One QTL (*Rbchq1a*) was unambiguously identified. Other QTLs could not be confirmed. To identify more QTLs might be difficult because the single effects can be small and not significant in our screening

conditions. Moreover, interaction may also play a crucial role for resistance in this population and this is difficult to detect in ILs with single introgressions. Since we consider QTLs only if they are detected in several experiments and in more than one IL some QTLs might be valid but are not confirmed simply because no other ILs with this introgression was present in the population. Also in other studies it has been difficult or impossible to find QTLs for quantitative characters. Examples are the resistance to *Alternaria solani* and silver leaf whitefly which were not identified in this IL population (Graham et al. 2005; Momotaz et al. 2006). However, in a F_2 population of the same parental lines (*S. lycopersicum* E6203 and *S. habrochaites* LA1777) it was found that whitefly resistance is controlled by five to six recessive genes (Momotaz et al. 2006). In our research no QTLs for reducing leaf DI were found probably because a more robust bio-assay is needed.

Two unambiguous QTLs (*Rbclq4* and *Rbclq11*) for reduced leaf LS and one QTL (*Rbclq3b*) for leaf DI were identified in the *S. lycopersicoides* LA2951 IL population. Compared to *S. lycopersicum* VF36, leaf LS can be reduced by at least 46%. Recently four resistance loci for reduced DI were identified in the same wild species (*S. lycopersicoides* LA2951) with the *B. cinerea* isolate B05.10 on chromosomes 1, 2, 3 and 4 (Davis et al. 2009). These differences show that there might be isolate specific resistance in tomato. Isolate specific differences have been reported in *Arabidopsis thaliana* (Katherine et al. 2004; Rowe and Kliebenstein 2008). In contrast, we found that our QTL *Rbclq11* with a reduced LS co-localizes with a QTL for increased DI (Davis et al. 2009). IL11-C (LA4278) has a typical leaf phenotype with light yellow color in the true leaves and slow growth. The low LS measured in this line might be caused by its phenotype due to small leaf area. However, the consistently significantly low LS over several independent experiments strongly suggested that *Rbclq11* is real and located on the top of chromosome 11. This is substantiated by ILs with overlapping introgressions but without the typical leaf phenotype (LA3883). The identified ILs in this paper can be used to further study the underlying resistance mechanism. Guimaraes et al. (2004) have proven that *B. cinerea* resistance derived from LA2951 is dominant and causes induced hyphal death. Abscisic acid, ethylene and salicylate are known to be involved in the response to *B. cinerea* infection in tomato leaf (Diaz et al. 2002; Asselbergh et al. 2007b) and an ABA deficient tomato mutant had a high resistance level to *B. cinerea* (Asselbergh et al. 2007a).

QTLs for stem resistance
In our study, the wild species LA1777 had a significantly lower LS and DI than *S. lycopersicum* E6203. This is in accordance with the results of Nicot et al. (2002) and ten

Have et al. (2007). More than half of the *S. habrochaites* LA1777 ILs showed a reduced stem LS and DI. However, only few ILs showed a significant difference. Only two robust QTLs, *Rbchq1b* and *Rbchq8,* conferring stem DI resistance was identified. Furthermore, no significant correlation between the experiments from *S. lycopersicoides* LA2951 hinted that stem resistance in this wild species would be not existed. Recently, substantial progress also has been made in tomato in identifying and unraveling QTLs causing resistance to *B. cinerea* in *S. habrochaites* LYC4 and a total of ten QTLs have been detected in F_2 and IL populations (Finkers et al. 2008;Finkers et al. 2007b). These identified QTLs are located on tomato chromosome 1, 2, 3, 4, 6, 9, 11 and 12 respectively. QTL *Rbcqh8* is located on chromosome 8. Hence QTL *Rbcqh8* might be a novel locus for resistance to *B. cinerea*.

QTLs for fruit resistance

Pre- or post-harvest fruit decay caused by *B. cinerea* can be a severe loss for tomato production. In this study, twelve QTLs were identified for resistance on ripe fruit LS and three QTLs for DI, derived from *S. habrochaites* LA1777. However, it seems that in green fruits the same susceptibility or even larger susceptibility for *B. cinerea* is present than in *S. lycopersicum* E6203 because most ILs had a larger LS than E6203. Thirteen lines (LA3920, LA3926, LA3928, LA3939, LA3943, LA3949, LA3950, LA3951, LA3966, LA3972, LA3977, LA3981 and LA3996) showed significant larger LS than *S. lycopersicum* E6203. No QTL identification for green fruits in the *S. lycopersicoides* LA2951 IL population was mainly caused by low sample size. Three unambiguous QTLs, responsible for reducing ripe fruit LS, but no QTLs for a reduction of ripe fruit DI were found. Ripening-regulated susceptibility of tomato fruit to *B. cinerea* without ethylene requirement (Cantu et al. 2009) might hint to different infection mechanisms in leaf and fruit infection.

QTLs for different tissues using two IL populations

Two IL populations have been used for screening the possible loci for resistance to *B. cinerea* in different tissues and few or no QTLs were found for some tissues. This might be due to the experimental conditions and therefore a more robust bio-assay is needed. Comparing lines with overlapping introgressions, the results are not always consistent. Improved genotyping might solve this problem. Differences with other studies using the same accession and different crosses might be due to the fact that the *S. habrochaites* IL population is based on a single LA1777 plant and this plant might not have all of the genetic variation of the accession LA1777 (Momotaz et al. 2007). For example, *S. habrochaites* LA1777 has shown high resistance in stem (Nicot et al. 2002; ten Have et al. 2007) but we only identified two QTL.

Another explanation for differences is that the *S. habrochaites* and *S. lycopersicoides* IL populations do not cover the whole genome of the wild species. Hence some QTLs might not be present in the IL populations.

Comparative analysis of QTLs

In total twenty four QTLs, which are located over 12 chromosomes, were identified in this study from two IL populations. Among these identified QTLs, three QTLs are responsible for reducing leaf LS, one for leaf DI, two for stem DI, fifteen for ripe fruit LS, and three for ripe fruit DI. Meanwhile some QTLs are responsible for reducing both LS and DI in the same tissues. In a previous study, ten QTLs have been detected from a *S. habrochaites* derived F_2 and IL population, which confer resistance on stems and were located in tomato chromosome 1, 2, 3, 4, 6, 9, 11 and 12 respectively, three QTLs were identified from a *S. neorickii* derived population and located on chromosome 3, 4 and 9 (Finkers et al. 2008;Finkers et al. 2007b) and four QTLs for leaf resistance were located on chromosome 1, 2, 3 and 4 (Davis et al. 2009). Hence it is possible to compare the location from above four populations. Based on tomato high density map (Tanksley et al. 1992), all of the QTLs identified from these four populations were integrated in Figure 22 for leaf and stem resistance, and in Figure 23 for ripe fruit resistance. We found that some QTLs co-localize and that QTLs for DI and LS in different tissues seldom co-localize in the two populations we investigated with the exception of *Rbchq1a* and *Rbchq1b*, and *Rbclq3a* and *Rbclq3b*. This is also consistent with the result that there is a low correlation between different tissues.

Fig. 22 Overview of QTLs identified for leaf and stem resistance to *Botrytis cinerea*. The genetic map is based on the tomato high density map (Tanksley et al. 1992). Solid black vertical bars represent the suggested QTLs from *S. habrochaites* LA1777 and *S. lycopersicoides* LA2951 IL populations (this thesis). Gray vertical bars represent previously reported QTLs conferring stem resistance from *S. habrochaites* (*Rbcq*) and *S. neorickii* (*QTL*) (Finkers et al. 2008;Finkers et al. 2007a;Finkers et al. 2007b). Dashed vertical bars represent recently reported QTLs responsible for leaf resistance on chromosome 1 through 4, and susceptibility on chromosome 5 and 11 from *S. lycopersicoides* LA2951(Davis et al. 2009).

Fig. 23 Comparative analysis of QTLs conferring ripe fruit resistance to *Botrytis cinerea* respectively from *S. habrochaites* LA1777 (Solid black vertical bars) and *S. lycopersicoides* LA2951 (Gray vertical bars) IL populations. The genetic map is based on the tomato high density map (Tanksley et al. 1992).

Potential of pyramiding QTLs

In this paper, two populations have been used for QTL analysis with the resistance to *B. cinerea*. We proved that one and three QTLs respectively derived from *S. habrochaites* and *S. lycopersicoides* showed a good resistance on leaves, and several QTLs were involved in resistance on ripening fruit. In a previous study work, ten QTLs for stem resistance derived from an accession of *S. habrochaites* LYC4, three QTLs from *S. neorickii* (Finkers et al. 2008;Finkers et al. 2007a;Finkers et al. 2007b) and four QTLs from *S. lycopersicoides* (Davis et al. 2009) were identified. Hence QTLs respectively responsible for different tissues are now available for pyramiding QTLs in tomato breeding. However, low correlation between different tissues for resistance to *B. cinerea* and isolate specific resistance would make this difficult and complicated. Interestingly, we found that lines LA3913, LA3914, LA3915 and LA3916 from *S. habrochaites* LA1777 IL population are involved in the resistance for several traits. For example, LA3913 significantly reduces leaf LS, stem LS and ripe fruit LS. Therefore these lines are very interesting for tomato breeding. In addition, most of the IL lines with multiple introgressions showed greatly reduced LS on ripe fruits (Fig. 15). For example, LA3998 and LA4002, which might harbor QTL *Rbch1b* and *Rbch12*, showed lower LS than the ones only harboring a single QTL. This result shows the prospective of pyramiding QTLs for *B. cinerea* resistance in tomato.

CHAPTER 3

Identification and mapping of quantitative resistance to late blight (*Phytophthora infestans*) in *Solanum habrochaites* LA1777

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Abstract

Most of the commercial cultivars of tomato, *Solanum lycopersicum*, are susceptible to late blight (*Phytophthora infestans*). Qualitative and quantitative resistance has been described in wild relatives of tomato. Screening of whole plants of three *S. habrochaites* accessions (LA1033, LA2099 and LA1777), showed that accession LA1777 had a good level of resistance to several isolates of *P. infestans*. Introgression line populations of *S. habrochaites* LA1777 were used to screen individual chromosome regions of the wild species. Two major isolates were used and two parameters were measured: lesion size (LS), and disease incidence (DI). Substantial variation was observed between the individual lines. QTLs were identified for Lesion Size. The presence of five QTLs derived from LA1777 (*Rlbhq4a*, *Rlbhq4b*, *Rlbhq7*, *Rlbhq8* and *Rlbh1q12*) result in unambiguous higher levels of resistance. All QTLs co-localized with previously described QTLs from *S. habrochaites* LA2099 except QTL *Rlbq4b*, which is therefore a novel QTL.

Key Words: tomato, late blight, *Phytophthora infestans*, quantitative resistance, *Solanum habrochaites*, introgression lines

Introduction

The oomycete *Phytophthora infestans* (Mont.) de Bary, the causal agent of late blight, is one of the most destructive pathogens of potato and tomato. Late blight causes serious yield and economic losses especially under favorable conditions for the pathogen (wet and cool temperatures) both in the open field as well as in non-heated greenhouses. The responsible

pathogen is heterothallic and forms oospores with A1 and A2 mating types and has been found in different areas of the world (Gotoh et al. 2005). The co-existence of mating types and the sexual reproduction increase the chance of developing resistance to fungicides such as metalaxyl (Goodwin et al. 1998;Gotoh et al. 2005). In addition, the spread of the disease may be also initiated from spores present in the soil (Widmark et al. 2007). The genetic diversity, rapid evolution and the broader range of virulence factors have made this pathogen more and more aggressive (Drenth et al. 1995;Gotoh et al. 2005). Tomato plants can be completely destroyed in a few weeks despite the use of chemicals to control the infections (Jones et al. 1991).

The most effective and environmentally favorable way to prevent devastation of tomato plants by this pathogen is to incorporate natural resistance into cultivars. Two kinds of host plant resistance to *P. infestans* have been described in tomato (Labate et al. 2007). Firstly there is the qualitative or race-specific resistance. This resistance is based on "R" genes, examples are *Ph-1*, *Ph-2*, *Ph-3*，*Ph-4* and *Ph-5* which originate from the wild species *S. pimpinellifolium* and the position of these R-genes has been determined on chromosomes 7, 10, 9, 2 and 1 respectively (Chunwongse et al. 2002;Conver and Walter 1953;Foolad et al. 2006;Labate et al. 2007;Moreau et al. 1998). However, these qualitative resistances are not durable due to the rapid evolution of compatible races of the pathogen. The first three genes have already been broken by newly evolved races of *P. infestans* (Labate et al. 2007) and the *Ph-4* and *Ph-5* genes still need to be tested in the field. Moreover, combining or pyramiding several genes could provide a more durable resistance than deploying just a single one (Foolad et al. 2006).

The second type of resistance is quantitative and non race-specific, and often partial. More genes are involved and this kind of resistance is considered to be more durable. Since 1970 potato research has been focused on introducing quantitative resistance to late blight (Wastie 1991), in spite of the fact that already eleven single R genes were known. For tomato, *S. habrochaites* is believed to be a potential donor for high levels of partial resistance (Brouwer et al. 2004). Five to six consistent QTLs have been identified in two BC_1 populations, where *S. habrochaites* LA2099 was the source of resistance (Brouwer et al. 2004). Although lines with all four QTLs introgressed were more resistant to *P. infestans* in different environments, the linkage drag resulted in poor horticultural performance. Hence, fine mapping has been conducted for three QTLs in order to make it possible to reduce the linkage drag (Brouwer and St Clair 2004). One QTL was identified from a wild desert species *S. pennellii*(Smart et al. 2007). Mapping and fine mapping facilitate researchers and breeders to identify desirable QTLs and to use them in marker assisted selection (MAS).

An introgression line population (IL) has several advantages over segregating populations such as F_2 and BC_1. Such a population is advantageous for QTL mapping because it can be phenotyped with many replicates and in different environments, which makes it possible to detect QTLs with smaller effects and allows also an estimation of the Genotype × Environment (G x E) interaction (Chaïb et al. 2006;Eshed and Zamir 1996b;Gur and Zamir 2004;Lecomte et al. 2004;Monforte et al. 2001). At least five IL populations have been developed in tomato, they are derived from *S. pennellii* LA716 (Eshed and Zamir 1994a), *S. habrochaites* LA1777 (Monforte and Tanksley 2000), *S. habrochaites* LA407 (Francis et al. 2001), *S. habrochaites* LYC4 (Finkers et al. 2007) and *S. lycopersicoides* LA2951(Canady et al. 2005). The *S. pennellii* IL library has been extensively explored to identify QTLs for several traits including disease resistances (Astua-Monge et al. 2000;Smart et al. 2007), fruit quality (Rousseaux et al. 2005;Tieman et al. 2006) and yield (Eshed and Zamir 1996b). Recently, an introgression line of *S. habrochaites* LA1777 has been identified with a significant contribution on marketable fruit yield (Hanson et al. 2007).

In this paper we describe the screening of three *S. habrochaites* accessions (LA1777, LA2099 and LA1033), which have shown high levels of resistance to *P. infestans* (Brouwer et al. 2004), and we found that of the three accessions LA1777 gave the highest resistance levels to several races of late blight originating from China, especially to *P. infestans* race $T_{1,2,3,4}$ which already overcame the *Ph-1*, *Ph-2*, *Ph-3* and *Ph-4* genes. Since the genetic distance between LA2099 and LA1777 is substantial, LA1777 might harbor other QTLs for late blight resistance as LA2099. In this paper, the IL population derived from *S. habrochaites* LA1777 (Monforte and Tanksley 2000) has been screened. Results and comparisons with earlier studies are presented and discussed.

Material and methods

Plant material
A total of six accessions including three *S. habrochaites* (LA1777, LA2099 and LA1033), a susceptible *S. lycopersicum* control (the inbred line 99165) and two commercial *S. lycopersicum* hybrids (HZ14 and HZ18) were used to evaluate resistance levels for *P. infestans* with race $T_{1,2,4}$ and $T_{1,2,3,4}$. Disease testing was always performed after the sixth true leaf had developed.

The introgression lines (IL) used in this study were derived from *S. habrochaites* accession LA1777 (a self-fertile, homozygous green fruited, indeterminate accession) in the background of *S. lycopersicum* E6203 (a red fruited, determinate, processing-type tomato). In

total the introgression lines cover at least 85% of the wild species genome (Monforte and Tanksley 2000). In total 93 of the 98 available lines of the *S. habrochaites* library were screened. Seeds were kindly provided by the Tomato Genetic Resource Center (TGRC, Davis USA). In order to get new seeds fruits of the individual introgression lines were collected after self pollination in the greenhouse.

The seeds were germinated in an incubator at 25C° and then transferred to 10 cm pots containing a medium of peat–vermiculite with organic fertilizer. Greenhouse temperature ranged from 15-18 °C at night and from 20-25 °C at day time.

Inoculum preparation

Two *P. infestans* isolates from China were used in the resistance assays: $T_{1,2}$ and $T_{1,2,4}$ races (A1 mating type, metalaxyl-resistant). Among them, race $T_{1,2}$ is the most epidemic isolate in China and present in eighteen provinces (Dr. Feng IVF,CAAS Beijing China and Dr. Tian AVRDC Taiwan personal communication). Isolates $T_{1,2}$, and $T_{1,2,4}$ are virulent on tomato genotypes containing the resistance genes *Ph*-2 or *Ph*-3 respectively. For the accessions one extra *P. infestans* race $T_{1,2,3,4}$ (the most virulent race found in China up to now collected in a greenhouse in Beijing, China by the department of Pathology CAAS) was used. Cultures of *P. infestans* were grown at 17°C on Rye B agar (Caten and Jinks 1968) and transferred to new plates monthly. Isolates were periodically grown on leaves of susceptible control tomato cv Zaofeng no.2 to maintain pathogenicity and profuse sporulation. Inoculum for disease assays was prepared by washing 8-day-old sporulating lesions with sterile distilled water. Spore concentrations were determined using a hemocytometer and diluted to the desired concentration (1×10^4 spores ml^{-1}).

Detached-leaflet assay

The *S. habrochaites* IL population was evaluated by a droplet method using $T_{1,2}$ races in five independent experiments. Five to fifteen plants of each genotype were used for each experiment. From each individual plant the sixth true leaf was detached with a razor blade and immediately inserted in moist florist foam. The abaxial surface of three of the top leaflets was inoculated with a drop of 20µl of sporangial suspension (1×10^4 ml^{-1} spores). Leaves were transferred to transparent plastic boxes, sealed with a transparent plastic membrane, covered by the lids and randomly placed in a growth cabinet at 16°C without light. After 24 h, the regime was changed to 16°C with 12 h light and 12 h dark. Late blight resistance was assessed 6 days post inoculation (dpi). The largest length and width (perpendicular to the length) of each lesion was measured resulting in the Lesion Size (LS) and the ellipse area was calculated following the formula LS = (length×width×π)/4. No lesion or a lesion remaining

within the size of the inoculum droplet ($\leq$0.3 cm^2) was considered as no infection or as arrested lesion (Vleeshouwers et al. 1999). For each genotype, the percentage of infected leaflets was calculated as disease incidence (DI).

Whole plant assay in growth cabinets

Wild species were tested for resistance using a whole plant assay with race $T_{1,2}$. Greenhouse-grown plants with fully stretched six true leaves were moved to cabinets. Thirty plants of each genotype were evaluated in three blocks using a randomized complete block design.

For whole plant inoculations, each plant was spray inoculated until the water started to drip off. The dew cabinets had an air temperature of 17-18°C. The first 24 hours after inoculation no light was used, after this a regime of 12 h light (18°C): 12 h dark (16°C) was used. After seven days the plants were scored individually for disease severity on a scale of 0-6, where 0 = no symptoms; 1 = <5% leaf area affected and small (<2 mm) lesions; 2 = 6-15% leaf area affected and restricted (<4 mm) lesions; 3 = 16-30% leaf area affected and/or few superficial small stem lesions; 4 = 31-60% leaf area affected and/or few small penetrating stem lesions; 5 = 61-90% leaf area affected and/or deep expanding stem lesions; 6 = 91-100% leaf area affected, extensive stem damage, or plant death (Chunwongse et al. 2002). Percentage disease index (PDI) was calculated with the following formula: PDI = sum of all ratings×100/total no. of observations × maximum rating grade (Chaerani et al. 2007).

Statistical analysis

All statistical analyses were performed using SPSS 13.0. Differences in *P. infestans* resistance in the *S. habrochaites* IL population were analyzed using the procedure of general linear model (GLM). LS data was transformed by square root to meet a normal distribution. Mean estimates for each line were calculated using the following models: PDI=constant + genotype + block + genotype × block. LS= constant + genotype + experiment + genotype × experiment. DI= constant + genotype + experiment + genotype × experiment. The correlation between traits was calculated by Pearson correlation coefficients. Trait data for experiments were tested for homogeneity of variance using a Levene test. Significance of QTL was determined by comparing mean values of individual ILs to the control *S. lycopersicum* E6203 at the 0.05 level by Dunnett test.

Results

Comparison of three accessions of *S. habrochaites*

From an earlier experiment it was clear that *S. habrochaites* accession LA1777 was resistant to *P. infestans* race $T_{1,2}$ (Fig. 1). Analysis of different accessions and controls with race $T_{1,2,4}$ of *P. infestans* show that all three accessions of the wild species *S. habrochaites* gave a good

resistance level to that particular race. However, the two accessions LA1777 and LA2099 with a mean PDI of 20.0 and 21.7 were more resistant than the accession LA1033 (mean PDI ~34), but all were significantly more resistant than the susceptible controls (mean PDI ~ 86.1). The same two accessions of *S. habrochaites* (LA1777 and LA2099) also showed enhanced resistance levels to the most virulent race: $T_{1,2,3,4}$ (Fig. 2).

Fig.1 Screening of tomato wild species for resistance to *P. infestans* race T1,2. Top left: *S. habrochaites* LA1777. Top right: the susceptible control *S. lycopersicum* 99165. Bottom left: the susceptible control *S. lycopersicum* HZ14. Bottom right: the resistant control *S. lycopersicum* CLN2037B with *Ph*-3 gene (provided by AVRDC).
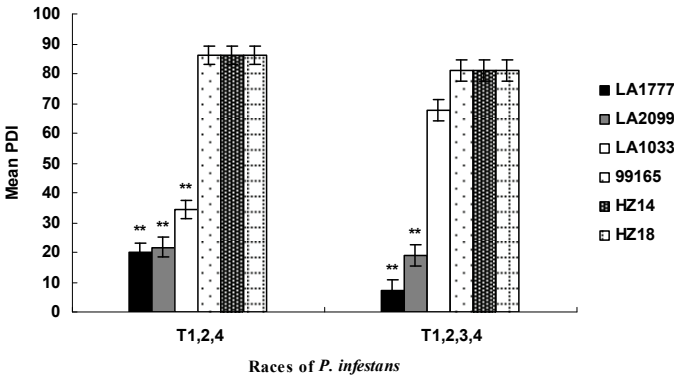
Fig. 2 Comparison of three accessions of wild species *S. habrochaites* for resistance to isolates $T_{1,2,4}$ and $T_{1,2,3,4}$. The bar indicates the standard error. ** indicates values in the lines that are significantly different from the susceptible control (*S. lycopersicum* 99165) at the 0.01 level.

Detached leaflet assay in LA1777 IL population with race $T_{1,2}$

The IL population was analyzed with the detached leaflet assay using isolate $T_{1,2}$ in five independent experiments over two years. Two traits were evaluated for each individual introgression line: lesion size (LS) expressed as the mean size of *P. infestans* lesions of infected leaves, and disease incidence (DI) expressed as the percentage of inoculated leaves that were successfully infected.

Between experiments, the mean LS varied from 2.34 ± 0.06 cm^2 to 5.00 ± 0.05 cm^2, while the mean DI varied from $75.6\pm0.9\%$ to $84.0\pm0.6\%$ for the IL population (Table 1). The disease scorings of LS were higher in 2006 than in 2007. However, the mean DI remained more or less the same over all five experiments. Significant correlations were observed for LS between experiments 1, 2, 4 and 5 but no significant correlation was present with the LS of experiment 3 (Table 2). Experiment 3 was excluded for the analysis of the results, but this only influenced the level of significance. Significant correlations for DI were only observed between experiments 1 and 4, and between experiments 3 and 5. The data of the experiments with significant correlation were analyzed. There is a significant difference between *S. habrochaites* LA1777 (2.33 ± 0.31cm^2) and *S. lycopersicum* E6203 (4.05 ± 0.16 cm^2) and the mean LS ranged from 2.89 ± 0.19cm^2 to 6.28 ± 0.48cm^2 among the ILs. A total of fifty four lines showed smaller LS (0.30%-28.5%) and this was significant in thirty one lines (Table 3 and Fig. 3). Thirty one lines identified herein can harbor a number of QTLs conferring resistance to *P. infestans* as determined by Lesion Size. We designated the identified QTLs as Resistance to Late Blight QTL (*Rlbq*) followed by the number of the chromosome on which they are located.

Table 1 Mean lesion size (LS) and mean disease incidence (DI) estimated over 93 ILs as indication for disease progress in each experiment

Experiment	Year	LS (cm^2)	N[a]	DI (%)	N[b]
1	2006	5.00±0.05	1280	77.2±1.1	1659
2	2006	4.63±0.05	1516	75.6±0.9	2005
3	2007	2.34±0.06	1067	77.0±1.1	1386
4	2007	2.78±0.03	3195	84.0±0.6	3801
5	2007	3.44±0.046	2796	76.3±0.7	3665

[a] number of leaflets that had lesion growth, [b] number of leaflets that were tested in five experiments.

Table 2 Pearson correlation of different experiments

	Exp1	Exp2	Exp3	Exp4	Exp5
Exp1	1	0.23[*]	-0.17	0.21[*]	0.14
Exp2		1	0.06	0.37[**]	0.23[*]
Exp3			1	0.06	-0.10
Exp4				1	0.33[**]
Exp5					1

[*] significant at 0.05 level; [**] significant at 0.01 level.

Table 3 Estimated mean of lesion size (LS) and disease incidence (DI) in introgression lines (IL) and two parent control lines. Means of each trait for each IL were compared to the mean of *S. lycopersicum* cv. E6203 using a Dunnett test by GLM mode and significant differences are marked with [*] ($P<0.05$) or [**] ($P<0.01$)

ILs	LS (cm^2)	N[a]	DI (%)	N[b]
LA3915	3.91±0.21[*]	98	83.8±4.6	117
LA3916	3.76±0.19[*]	107	86.3±3.9	124
LA3918	3.95±0.21[*]	97	82.9±4.2	117
LA3919	3.72±0.24[**]	86	72.9±4.5	118
LA3921	3.75±0.20[**]	101	82.1±4.2	123
LA3922	3.92±0.20[**]	97	85.1±4.3	114
LA3923	3.66±0.22[**]	83	76.6±4.4	109
LA3925	3.52±0.21[**]	88	82.2±4.3	107
LA3929	3.48±0.19[**]	101	82.8±4.0	122
LA3931	3.82±0.19[*]	106	82.2±3.9	129
LA3932	3.61±0.22[**]	82	73.9±4.0	111
LA3934	3.49±0.21[**]	95	76.6±4.3	124
LA3935	3.59±0.22[**]	93	76.9±4.6	121
LA3937	3.20±0.21[**]	85	72.0±3.9	118
LA3941	3.19±0.26[**]	64	66.0±4.8[*]	97
LA3948	3.67±0.21[*]	92	74.8±3.9	123
LA3949	2.89±0.19[**]	102	77.3±3.7	132
LA3959	3.39±0.19[**]	102	82.3±3.8	124
LA3961	3.72±0.21[*]	96	76.2±3.9	126
LA3963	3.67±0.19[*]	100	84.0±3.8	119
LA3964	3.92±0.21[*]	84	83.2±4.4	101
LA3965	3.56±0.18[**]	107	80.9±3.6	132
LA3967	3.40±0.20[*]	101	82.3±3.9	122
LA3969	3.46±0.19[**]	111	88.8±4.0	125
LA3976	3.45±0.19[**]	108	87.1±3.9	124
LA3979	3.75±0.22[**]	86	76.1±4.3	113
LA3988	3.73±0.22[**]	88	80.7±4.7	109
LA3989	3.75±0.19[**]	113	88.3±3.9	128
LA3990	3.95±0.20[*]	85	70.8±3.8	120
LA4006	3.59±0.17[**]	119	86.2±3.6	138
LA4007	3.74±0.19[*]	104	83.9±3.9	124
LA3954	3.78±0.21	84	65.6±3.7[*]	128
E6203	4.05±0.16	141	83.4±3.3	169
LA1777	2.33±0.31**	51	38.9±5.7[**]	131

[a] number of leaflets that had lesion growth. [b] number of leaflets that were tested in four experiments.
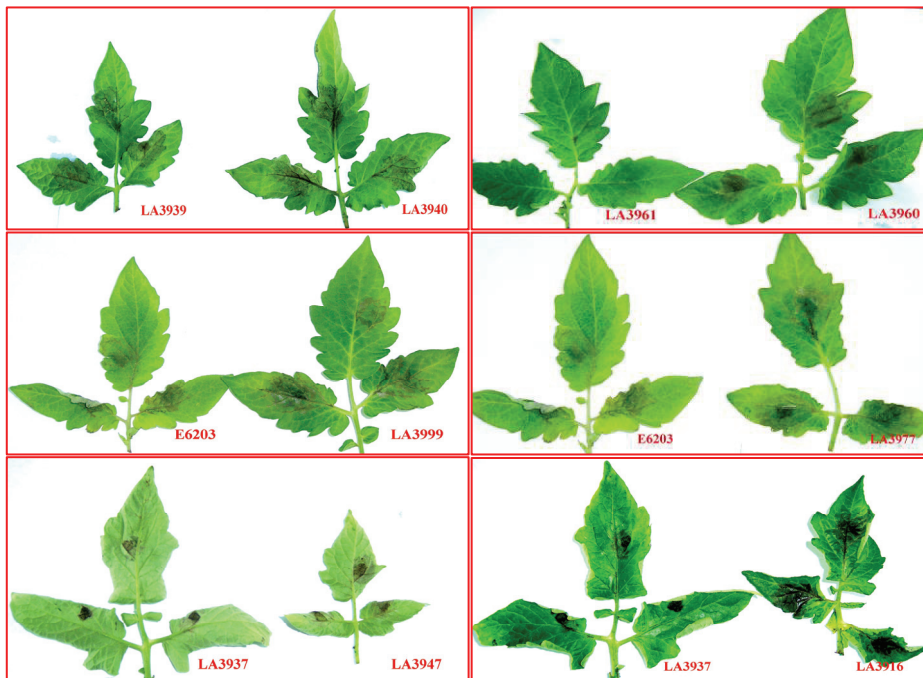
Fig. 3 Screening of *S. habrochaites* LA1777 IL population for resistance to *Phytophthora infestans*. Lines LA3916, LA3937 and LA3961 showed clearly reduced leaf LS. Lines LA3939, LA3940, LA3960, LA3999 and LA3977 showed the same susceptibility as the control *S. lycopersicum* E6203.

Based on the IL map constructed by Monforte and Tanksley (2000), the introgressions of these thirty one lines derived from wild species *S. habrochaites* LA1777 were distributed on 11 of the 12 chromosomes. We will focus in this paper only on the five most significant and substantial QTLs, which were located on chromosomes 4 (2 QTLs), 7, 8 and 12.

Rlbq4a and *Rlbq4b*. Sixteen of the 93 lines contain an introgression of chromosome 4. Ten of these lines were significantly more resistant than the control (Fig. 4 and Table 4). The significant effects in lines LA3931, LA3959, LA3979 and LA4006 show the presence of a QTL (*Rlbq4a*) at the top of Chromosome 4. More markers will have to be determined to pinpoint the QTL more precisely. The fact that lines LA3930 and LA4000 are not resistant will make it likely that the position can be determined rather precisely. However, also some lines with other introgressions on Chromosome 4 had higher resistance levels, the significant higher resistance levels of ILs LA3934, LA3935, LA3937, LA3976 and LA4007 shows that there must be a second QTL (*Rlbq4b*) towards the bottom of Chromosome 4. Again

additional markers are needed to explain why lines such as LA3936, LA3977 and LA3978 do not show resistance.
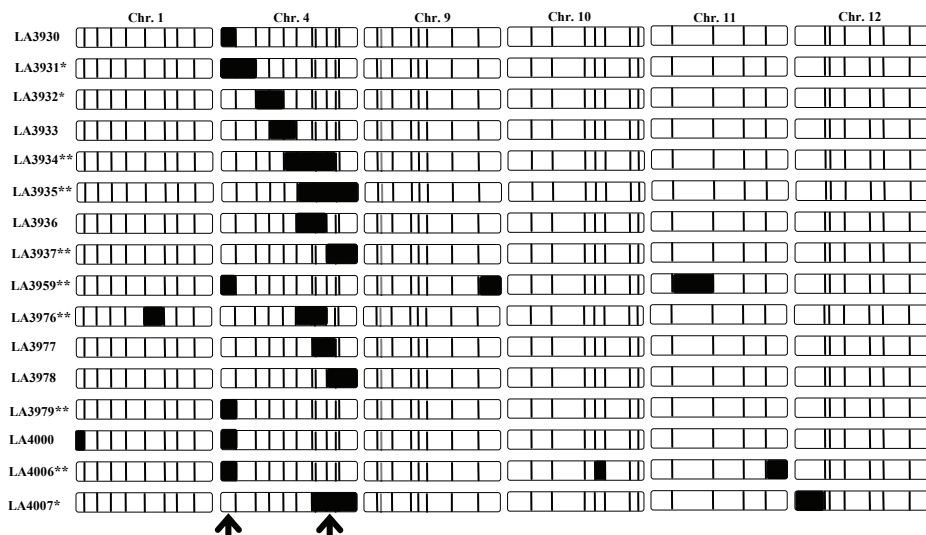
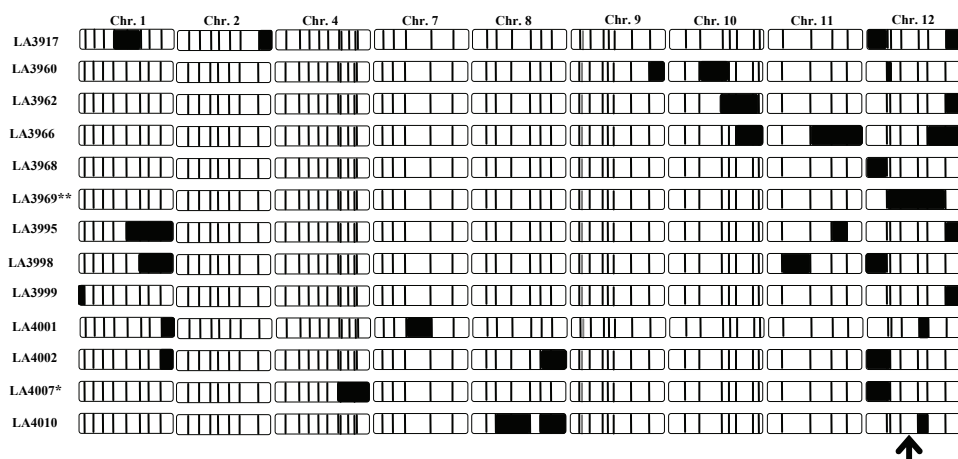**Fig. 4** The introgression lines (ILs) of *S. habrochaites* LA1777 containing the introgressions which are located on chromosome 4. The map was drawn based on the originally published reference (Monforte and Tanksley 2000). Some lines with extra introgressions located in other chromosome regions are also indicated. * and ** indicate values in the lines that are significantly different from the susceptible control at 0.05 and 0.01 level respectively. The arrows indicate the most likely location of the suggested QTLs.

Table 4 Leaf lesion size (LS) assayed in four independent experiments, and means of LS and disease incidence (DI) of sixteen lines containing the introgressions on chromosome 4 and two parent lines.

ILs	LS (cm^2) in different experiments				LS (cm^2)	DI (%)
	1	**2**	**4**	**5**		
LA3930	4.62	5.84	2.28	3.49	4.06±0.20	76.8±3.8
LA3931	4.81	5.11	2.28	3.09	3.82±0.19[*]	83.4±3.9
LA3932	4.56	4.40	2.43	3.04	3.61±0.23[**]	72.0±4.0
LA3933	5.19	5.50	3.06	4.27	4.50±0.18	86.7±3.9
LA3934	4.62	4.75	2.18	2.39	3.49±0.21[**]	78.9±4.3
LA3935	5.51	3.73	2.58	2.54	3.59±0.22[**]	81.0±4.6
LA3936	4.53	4.33	2.84	3.38	3.78±0.19	83.7±3.9
LA3937	3.83	3.29	2.34	3.34	3.20±0.21[**]	70.5±3.9
LA3959	4.66	3.84	2.41	2.64	3.39±0.19[**]	81.1±3.8
LA3976	4.33	3.96	2.77	2.73	3.45±0.19[**]	86.1±3.9
LA3977	6.38	4.80	2.76	3.16	4.27±0.24	71.9±4.7
LA3978	4.77	3.65	3.18	3.84	3.86±0.21	71.0±4.1
LA3979	3.71	5.13	2.70	3.46	3.75±0.22[**]	73.8±4.3
LA4000	5.85	5.29	2.70	3.34	4.30±0.20	76.5±4.0
LA4006	4.38	4.19	2.58	3.20	3.59±0.17[**]	85.3±3.6
LA4007	4.96	3.79	2.58	3.57	3.73±0.19[*]	82.9±3.9
E6203	6.10	4.05	3.10	2.94	4.05±0.16	83.3±3.3
LA1777	2.41	2.17	2.25	2.51	2.33±0.31[**]	55.6±5.7[**]

* and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

In the same manner, we identified three other QTLs for smaller LS: on top of Chromosome 7: *Rlbq7* (Fig. 5 and Table 5), and bottom of Chromosome 8: *Rlbq8* (Fig. 6 and Table 6), and the middle of Chromosome 12: *Rlbq12*, (Fig. 7 and Table 7).
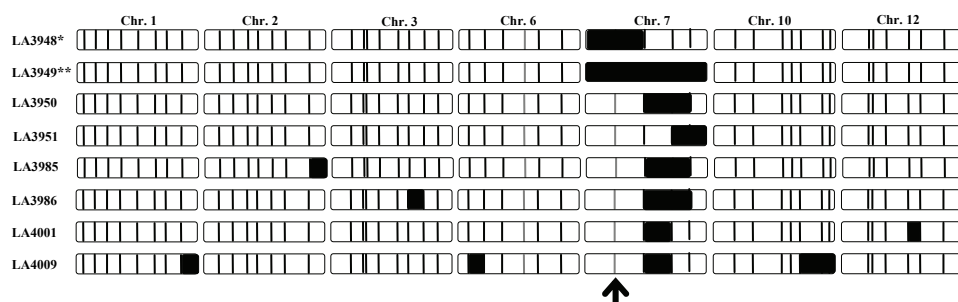
Fig. 5 The ILs of *S. habrochaites* LA1777 containing the introgressions which are located on chromosome 7. The map was drawn based on the originally published reference (Monforte and Tanksley 2000). Some lines with extra introgressions located in other chromosome regions are also indicated. * and ** indicate values in the lines that are significantly different from the susceptible control at 0.05 and 0.01 level respectively. The arrow indicates the mostly likely location of the suggested QTL.

Table 5 Leaf lesion size (LS) assed in five independent experiments, and mean value of leaf LS and disease incidence (DI) of eight lines containing the introgressions on chromosome 7 and two parent lines.

ILs	LS (cm^2) in four experiments				LS (cm^2)	DI (%)
	1	**2**	**4**	**5**		
LA3948	4.36	4.24	2.74	3.35	3.67±0.21*	73.1±3.9
LA3949	3.66	3.39	1.90	2.62	2.89±0.19**	77.4±3.7
LA3950	5.24	6.34	-	7.28	6.28±0.48	65.8±8.9
LA3951	5.93	5.06	2.60	3.98	4.39±0.18	82.1±3.7
LA3985	4.42	5.85	3.39	4.96	4.66±0.21	87.3±4.6
LA3986	4.16	2.19	2.99	4.41	3.44±0.21	78.0±4.3
LA4001	5.46	4.21	3.18	3.45	4.08±0.22	85.5±4.6
LA4009	5.72	4.65	-	-	5.19±0.42	69.0±7.9
E6203	6.10	4.05	3.10	2.94	4.05±0.16	83.3±3.3
LA1777	2.41	2.17	2.25	2.51	2.33±0.31**	55.6±5.7**

-: lines not tested in that particular experiment due to low number of seeds; * and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.
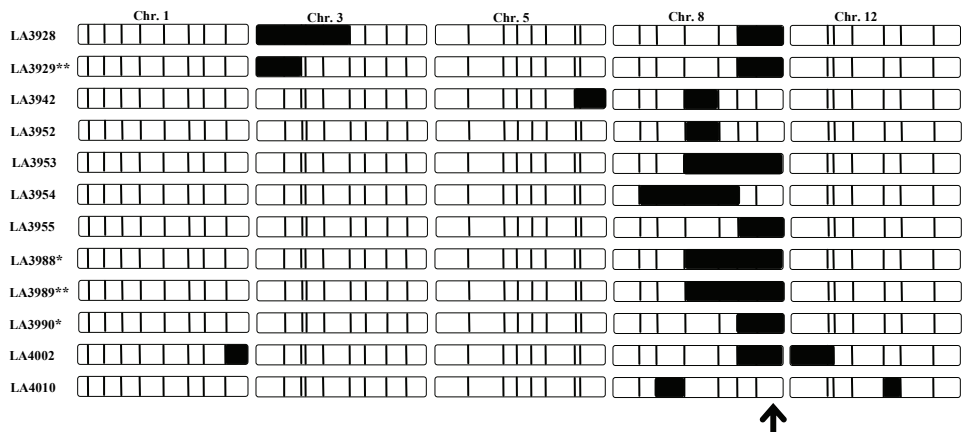
Fig. 6 The ILs of *S. habrochaites* LA1777 containing the introgressions which are located on chromosome 8. The map was drawn based on the originally published reference (Monforte and Tanksley 2000). Some lines with extra introgressions located in other chromosome regions are also indicated. [*] and [**] indicate values in the lines that are significantly different from the susceptible control at 0.05 and 0.01 level respectively. The arrow indicates the most likely location of the suggested QTLs.

Table 6 Leaf lesion size (LS) assed in four independent experiments, and mean value of leaf LS and disease incidence (DI) of twelve lines containing the introgressions on chromosome 8 and two parent lines.

ILs	LS (cm^2) in four experiments				LS (cm^2)	DI (%)
	1	2	4	5		
LA3928	3.66	5.31	-	-	4.49±0.34	76.2±6.4
LA3929	4.43	4.61	2.06	2.80	3.48±0.19[**]	82.3±4.0
LA3942	4.39	3.81	3.22	3.61	3.76±0.20	70.6±3.8
LA3952	5.24	4.26	2.59	3.595	3.92±0.18	78.8±3.7
LA3953	4.65	4.57	2.79	3.10	3.78±0.20	84.1±4.0
LA3954	4.97	4.15	2.85	3.16	3.78±0.20	65.6±3.7[**]
LA3955	4.19	5.09	3.54	2.71	3.88±0.19	80.6±3.8
LA3988	5.52	3.55	3.19	2.66	3.73±0.22[**]	85.2±4.7
LA3989	5.36	4.06	2.31	3.28	3.75±0.19[**]	86.0±3.9
LA3990	5.14	5.09	2.32	3.27	3.95±0.20[*]	69.9±3.8[**]
LA4002	5.09	4.38	3.64	4.05	4.29±0.22	78.2±4.4
LA4010	6.57	4.67	2.82	3.88	4.48±0.19	84.2±3.9
E6203	6.10	4.05	3.10	2.94	4.05±0.16	83.3±3.3
LA1777	2.41	2.17	2.25	2.51	2.33±0.31[**]	55.6±5.7[**]

-: lines not tested in that particular experiment due to low number of seeds; [*] and [**] indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

Fig. 7 The ILs of *S. habrochaites* LA1777 contain the introgressions which are located on chromosome 12. The map was drawn base on the originally published reference by (Monforte and Tanksley 2000). Some lines with extra introgressions located in other chromosome regions were also indicates. * and ** indicate values in the lines that are significantly different from the susceptible control at 0.05 and 0.01 level respectively. The arrow indicates the mostly likely location of the suggested QTLs.

Table 7 Leaf lesion size (LS) assed in four independent experiments, and mean value of leaf LS and disease incidence (DI) for thirteen lines containing the introgressions on chromosome 12 and two parent lines.

ILs	LS (cm^2) in four experiments				LS (cm^2)	DI (%)
	1	2	4	5		
LA3917	5.55	5.53	2.92	3.38	4.35±0.28	67.6±5.5*
LA3960	4.79	4.52	2.45	3.47	3.81±0.19	81.3±3.5
LA3962	5.40	5.56	2.74	3.48	4.29±0.18	81.0±3.5
LA3966	6.23	4.90	3.18	3.57	4.47±0.22	88.8±3.8
LA3968	5.45	5.75	3.28	3.24	4.43±0.19	72.5±3.5
LA3969	4.28	4.09	2.36	3.12	3.46±0.19**	88.4±3.7
LA3995	4.61	5.19	3.03	3.56	4.10±0.20	86.9±3.6
LA3998	5.11	6.16	3.76	4.02	4.76±0.20	81.8±3.8
LA3999	3.97	6.24	3.64	2.55	4.10±0.18	79.2±3.3
LA4001	5.46	4.21	3.18	3.45	4.08±0.22	83.1±4.1
LA4002	5.09	4.38	3.64	4.05	4.29±0.22	78.6±4.0
LA4007	4.96	3.79	2.58	3.77	3.73±0.29*	83.6±3.6
LA4010	6.57	4.67	2.82	3.88	4.48±0.29	84.7±3.6
E6203	6.23	4.05	3.10	2.94	4.05±0.16	83.4±3.3
LA1777	2.75	2.17	2.25	2.51	2.33±0.31**	55.9±5.7

* and ** indicate that lines presented significant difference when compared to the susceptible control at 0.05 and 0.01 level, respectively.

The mean DI of *S. habrochaites* LA1777 was 55.9%, a significant lower value than in the susceptible parent *S. lycopersicum* E6203 with 83.4%. The values of the individual lines of the IL population varied from 65.6% to 90.8%. Two lines, LA3941 (Chr. 5) and LA3954 (Chr. 8), showed a significantly decreased DI. However, this was not confirmed in other lines with overlapping introgressions. Hence, the identified QTLs must be further confirmed in additional experiments.

Detached leaflet assay in LA1777 IL population with race $T_{1,2,,4}$

In order to see whether the identified resistance is real and consistent, the more virulent race $T_{1,2,4}$ was used in one experiment. The difference in LS between the parental lines *S. habrochaites* LA1777 (0.91cm^2) and *S. lycopersicum* E6203 (3.20cm^2) was highly significant. For the individuals of the IL population LS varied from 1.22 cm^2 (line LA3918) to 6.39 cm^2 (LA3969). Of the 93 IL lines, fifty three lines had a reduced LS ranging from 0.67%-61.85%. Twelve lines gave a significant difference; these were LA3914, LA3918, LA3920, LA3922, LA3923, LA3928, LA3937, LA3948, LA3981, LA3999, LA4004 and LA4005. Five lines, LA3918, LA3922, LA3923, LA3937 and LA3948 were also identified after inoculation with race T$_{1,2}$. All lines containing QTL *Rlbq4b* or *Rlbcq7* showed lower LS than the control.

The mean DI of *S. habrochaites* LA1777 (63.4%) was significantly different from the DI of *S. lycopersicum* E6203 (83.4%). The DI ranged among the individuals of the IL population from 66.0% to 92.7% (data not shown), but where not significantly different from the control.

Discussion

Wild species conferring resistance to *P. infestans*

Three wild species, *S. pimpinellifolium*, *S. pennellii* and *S. habrochaites*, have been reported to give qualitative and quantitative resistance to *P. infestans* (Chunwongse et al. 2002;Conver and Walter 1953;Moreau et al. 1998;Smart et al. 2007;Turkensteen 1973). Quantitative resistance in plants has been suggested to be more durable because qualitative genes are easily overcome in rapidly evolving strains of the pathogen. In *S. habrochaites* a high level of quantitative resistance to several isolates was found (Brouwer et al. 2004). In our study, three wild accessions of *S. habrochaites* were evaluated for resistance to different races of *P. infestans*. The results agree with previous research showing that in accessions of *S. habrochaites* quantitative genes are present which give relatively high levels of late blight resistance (Brouwer et al. 2004;Chunwongse et al. 2002). However, the resistance level of the three accessions is quite different for two races of *P. infestans*. Accessions LA1777 and

LA2099 had a higher level of resistance to race $T_{1,2,4}$ than accession LA1033. Accession LA1033 was almost completely susceptible to race $T_{1,2,3,4}$ maybe because it only has late-bight-resistance alleles complementary to the *Ph-3* gene, a gene which can be overcome by race $T_{1,2,3}$ (Chunwongse et al. 2002). However, both LA1777 and LA2099 showed a very good resistance to the most virulent race $T_{1,2,3,4}$. Brouwer et al. (2004) have shown that LA2099 has a very good resistance to USA isolates 7629 and 9175, which are virulent on tomato genotypes containing the *Ph1* and *Ph2* gene (Brouwer et al. 2004). We found that LA1777 and LA2099 have potential resistances to several other races of *P. infestans* with LA1777 as the most resistant. Recently, two new resistance genes, *Ph-4* and Ph-5, have been identified from *S. pimpinellifolium* (Foolad et al. 2006;Labate et al. 2007) and another gene located on chromosome 6 of *S. pennellii* was described by (Smart et al. 2007). We also screened a nightshade *S. lycopersicoides* LA2951 IL population and it seems that this population also harbors resistance to *P. infestans* (data not shown). Hence, it might be that more wild species can serve as potential sources for resistance genes to *P. infestans*. These wild species provide a rich resource for breeding tomatoes with resistance to late blight.

QTLs identified by different experiments

We have made an effort to explore introgression lines of *S. habrochaites* LA1777 for resistance loci against *P. infestans*. A major race ($T_{1,2}$) of *P. infestans* was used in five independent detached leaf experiments over two years. The mean LS in these two years varied greatly which might be caused by inoculum quality or differences in individual lines under different experimental conditions (Vleeshouwers et al. 1999). Low to moderately correlations were also reported between different assay methods (Brouwer et al. 2004). To identify introgressions with resistance genes only the experiments with significant correlation between experiments were used. While analyzing the data of IL populations, the five QTLs could only be identified after combining the data of four independent experiments because not all of them were significant in single experiments. Some of the identified QTLs were not detected when another inoculum (race $T_{1,2,4}$) was used. In conclusion independent experiments are needed in search for quantitative resistance to *P. infestans*. Brouwer et al. (2004) reported that neither a detached leaflet nor a whole-plant assays can entirely substitute *P. infestans* screenings in tomato. Hence, field or greenhouse tests should add more evidence to prove the true nature of the identified QTLs (Brouwer et al. 2004).

DI as a measure for infection efficiency was also evaluated in our study. For DI low or no correlation between experiments was found and QTLs responsible for DI could not be

identified in this study. Vleeshouwers et al. (1999) found that a highly constant humidity in closed trays apparently enhances infection by the zoospores causing a very high disease pressure. In the detached leaf assay some well known resistant wild *Solanum* genotypes were partially infected. A high amount of successful infections reduces the change to find QTLs for disease incidence but makes the chance higher to find QTLs for LS (more data points). Vleeshouwers et al. (1999) suggested when the DI is to be used as a parameter for resistance, a different screening methodology must be chosen, e.g., incubation of detached leaves in open trays, or intact plants in climate chamber or field. Another complicating factor in a bioassay can be that the percent infection is negatively correlated to plant height (Brouwer et al. 2004) and that some leaves become rotten during the 5 days that the evaluation is carried out. We tried not to include these rotten leaves in the data analysis, but it is sometimes difficult to distinguish between infected and rotten leaves. This can be the reason that introgression lines appear to be more susceptible than the control. The leaves of *S. habrochaites* LA1777 rot more easily than the leaves of the ILs.

In this study we only focused on lines with larger effect on resistance to *P. infestans*. Only QTLs identified in several lines with overlapping introgressions are considered as reliable. The IL population we used covered about 85% of the genome of LA1777 population. Some QTLs for resistance to *P. infestans* might be missed therefore. Moreover, the exact location of each QTL still needs to be confirmed due to lack of precise flanking markers in each introgression line.

Comparative analysis of QTLs from different populations

On all 12 tomato chromosomes QTLs for resistance to *P. infestans* have been detected in another *S. habrochaites* accession namely LA2099 (Brouwer et al. 2004). A total of eight QTLs showed consistent resistance over experiments (Brouwer et al. 2004). We found that *Rlbq4a*, *Rlbq7*, *Rlbq8b* and *Rlbhq12* co-localize with previously identified *lb4a*, *lb7a*, *lb8b* and *lb12b* respectively on chromosome 4, 7, 8 and 12 (Brouwer et al. 2004). Because *Rlbq4b* was not detected in the previous study and the fact that it showed a good resistance against two different races of late blight, we think that this novel QTL from LA1777 is important and worthwhile introgressing in tomato varieties. A next step could be to clone the genes underlying these QTLs.

Potential of pyramiding QTLs

Up to now, both qualitative and quantitative genes have been identified in several different wild tomato species. The interaction between these QTLs and single genes is still unknown

but lines with all four QTLs, from *S. habrochaites* LA2099, have shown a high level of resistance to *P. infestans* under different environments (Brouwer et al. 2004;Brouwer and St Clair 2004). In our study, five QTLs have been identified and two of them give also resistance to the most virulent race. Not all QTLs were identified in each experiment and not all were found in at least two lines with overlapping introgressions. Most QTLs have a limited effect and QTL interaction might be a key factor to get very high levels of resistance. Therefore we suggest developing combinations of several QTLs, not only from one wild species but also from different wild species. Such as a combination of QTL *Rlb4b* derived from *S. habrochaites* LA1777, one QTL derived from *S. pennellii* LA716(Smart et al. 2007) and some QTLs derived from *S. habrochaites* LA2099(Brouwer et al. 2004). In this way pyramiding of these or similar effective QTLs might pave the way for durable resistance to *P. infestans*.

CHAPTER 4

Investigating seedling salt tolerance in two tomato introgression libraries

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Abstract

Soils with higher concentrations of salt are becoming more and more a constraint for many crops to obtain high yields. Wild tomato species, adapted to adverse environments, are a potential reservoir for genes underlying quantitative trait loci related to salt tolerance in tomato. In this study two introgression line (IL) populations derived from two different wild species, *S. pennellii* LA716 and *S. lycopersicoides* LA2951, were used to identify QTLs for salt tolerance in the seedling stage. On chromosomes 2, 6, 7, 10, 11 and 12, in total ten major QTLs were identified in the *S. pennellii* introgression lines. Additionally, five major QTLs were identified in the *S. lycopersicoides* introgression lines, which are located on chromosomes 4, 6, 9 and 12. Three of the in total 15 QTLs co-localize in the two IL populations. Three *S. pennellii* ILs (IL6-2, IL7-1 and IL7-5) harboring QTLs on chromosome 6 and 7 were crossed and complete dominance, resulting in the highest tolerance to salt, was found. Moreover, less-than-additive interactions between the studied QTLs were observed.

Key words: Tomato, seedling, Salt tolerance, *Solanum pennellii, Solanum lycopersicoides*

Introduction

Salt tolerance of crops has recently received a lot of attention due to the increase of salinised cultivated lands throughout the world. This increase is caused by both natural phenomena and human activities (Ghassemi et al. 1995). Secondary salinisation of cultivated lands is caused

by improper agricultural practices such as the use of too much chemical fertilization and/or inadequate irrigation management. Furthermore, land degradation caused by secondary salinisation is getting more and more a problem (Ghassemi et al. 1995; Zhang and Zhang 2007).

In addition, the competition for the available fresh water resources have resulted in development of irrigation with saline water (Ghassemi et al. 1995). Tomato, which is a worldwide economic important crop and adapted to various climates, is also suffering from salinised soils. Too much salt in the soil results in a reduced plant development and growth and subsequently in a lower yield. Most modern tomato cultivars are already sensitive to moderate levels of salt in the soil (Rush and Epstein 1976; Costa et al. 1990; Hassan et al. 1990; Saranga et al. 1992; Foolad and Lin 1997), although a large proportion of tomatoes are cultivated in saline areas (Burns et al. 1990; Foolad 1997). China is one of the largest producers for both the fresh and the processing tomato market (http://www.fas.usda.gov) and in China the majority of processing tomatoes is grown in salinised soils (Mao et al. 2002). Sixty percent of the processing tomatoes in China are transplanted seedlings and forty percent is sown directly. Salinity slows down tomato shoot growth and the growth of younger seedlings; the higher the saline concentration the larger the reduction in shoot growth (Cuartero and Fernández-Muñoz 1999; Flowers 2004; Cuartero et al. 2006). The tomato response to salt stress is differently regulated in different development stages (Costa et al. 1990; Saranga et al. 1992). This has also been reported in other crop species (Greenway and Munns 1980; Shannon 1985; Maas 1986; Lauchli and Epstein 1990; Johnson et al. 1992; Foolad et al. 1999). During flowering and fruit setting, tomato plants are able to withstand NaCl concentrations which are sufficient to kill them in the seedling stage (Elshourbagy and Ahmed 1975). This makes it also important that tomatoes are more salt tolerant in their seedling stage. Transplanting of seedlings with higher salt tolerance guarantees a better performance and a faster growth.

Accessions of wild species adapted to dry or seashore regions have been evaluated for salt tolerance and some accessions of *S. pimpinellifolium, S. peruvianum*, *S. cheesmaniae, S. habrochaites*, *S. chmielewskii* and *S. pennellii,* showed certain levels of salt tolerance (Rush and Epstein 1976; Costa et al. 1990; Hassan et al. 1990; Saranga et al. 1992; Foolad and Lin 1997). To identify the chromosomal regions associated with a stable salt tolerance, molecular markers and quantitative trait loci (QTL) analyses have been used. Seven QTLs, on chromosomes 1, 2, 3, 7, 8, 9 and 12, for better seed germination under saline conditions were identified in various segregating populations derived from *S. pennellii* LA716 and *S. pimpinellifolium* LA722. Three stable QTLs causing salt tolerance during the vegetative growth were identified on chromosomes 3, 5 and 9, which were originated from the wild

species LA722 (see review Foolad 2004). Other QTLs were identified for fruit-related traits under salt stress (Monforte et al. 1996, 1997a, 1997b, 1999). Limited research has been done to identify QTLs for salt tolerance in terms of yield, but some QTLs were identified (Bretó et al. 1994; Villalta et al. 2007).

Introgression lines (IL) are produced by crossing a well known cultivar with an exotic species followed by repeated backcrossing and marker selection. Ideally, a derived line has only a single introgression and the complete library of introgression lines represents the entire genome of the wild parent. A big advantage of IL populations is that they can be evaluated in different environments and laboratories since they are genetically homozygous and in principle an unlimited number of seeds can be obtained (Eshed and Zamir 1995; Gur and Zamir 2004). The effect of a single QTL as well as of interactions between QTLs can be efficiently studied (Anbinder et al. 2009). Currently five IL populations have been described in tomato (Eshed and Zamir 1995; Canady et al. 2005; Yang and Francis 2005; Finkers et al. 2007). Among these five populations, the *S. pennellii* LA716 IL library has been extensively explored to identify QTLs for several traits related to biotic stress (Eshed and Zamir 1995; Astua-Monge et al. 2000; Chunwongse et al. 2002; Rousseaux et al. 2005; Tieman et al. 2006). However, this *S. pennellii* accession also has a lot of potential for studies on abiotic stress but only few reports have been published. In one of them, five loci, conferring salt tolerance during seed germination, have been identified using a F_2 population (Foolad et al. 1997) but salt tolerance in the seedling stage was not studied. Two IL populations, derived from *S. pennellii* LA716 and *S. lycopersicoides* LA2951 have been screened for salt tolerance in seedlings in this study. A number of QTLs have been identified in both populations and the value of these QTLs results is being discussed in this chapter.

Materials and methods

Plant material

Two introgression libraries were used for screening seedlings under saline conditions. One of them was the *S. pennellii* IL library LA716 in the background of *S. lycopersicum* cv. M82 (Eshed and Zamir 1995). LA716 is a self-fertile, homozygous green fruited, indeterminate accession and M82 is a red fruited, determinate, processing-type tomato. The population is composed of a primary set of 52 ILs with a representation of the *S. pennellii* genome in as few as possible lines. A further 26 sub-lines are available for certain regions. All ILs were screened together with the parental line M82 in six independent experiments. The second IL population is the *S. lycopersicoides* LA2951 in the background of *S. lycopersicum* VF36

(Canady et al. 2005). The primary set is 56 ILs and a secondary set of 34 sub-lines can be used for improving map resolution in certain regions. LA2951 is a tomato-like nightshade species and is a self-fertile, homozygous green fruited, indeterminate accession. VF36 is a red fruited, determinate, beef-type tomato. Of the total library of 90 lines only 77 lines were available and screened in four independent experiments, some of these lines were sterile or did not produce enough seeds. Sterility caused by homozygous introgressions make some lines of the *S. lycopersicoides* IL population difficult to maintain. This applies especially for three lines (LA4242, LA4277 and LA4282) and in a lesser extend for another thirteen lines where only very few seeds are produced (LA4231, LA4234, LA4263, LA4266, LA4236, LA3875, LA4253, LA4300, LA4260, LA4270, LA4276, LA4278 and LA4282)(Canady et al. 2005). When enough seeds could be obtained after self pollination in these fifteen ILs the lines with homozygous introgressions were screened although they have been originally categorized as heterozygous introgressions (Canady et al. 2005).

Three lines (IL6-2, IL7-1 and IL7-5) from the IL population of *S. pennellii* LA716 with a higher salt tolerance in seedlings were crossed to M82 and pair wise crossed to generate introgression line hybrids (ILHs).

Evaluation of salt tolerance in the seedling stage

Salt tolerance in seedlings was measured according to a slightly modified method of Foolad and Chen (1999). Seeds were surface sterilized with 0.5% NaOCl solution, rinsed with water and sown in pots containing a 1:1:1 peat–perlite–vermiculite (v/v) medium. Three to ten seedlings with four fully developed true leaves were transferred into hydroponic tanks after their roots were washed to remove attached growing medium. Each tank ($68 \times 40 \times 28$ cm) contained 20L of half-strength modified Hoagland solution (Epstein 1972) and plants were grown in a greenhouse with average day and night temperatures of approximately 20-25°C and 15-18°C. The hydroponic solutions were continuously and vigorously aerated. The first increase of salts (50 mM NaCl + 5 mM $CaCl_2$) was added four days after transplanting and every day the concentration was enhanced with 50mM NaCl + 5 mM $CaCl_2$ to achieve a final concentration of 700 mM NaCl + 70 mM $CaCl_2$. The solution with the final concentration was changed weekly. From the third week on, after the final salt concentration was reached, the plants were evaluated.

Each plant was visually evaluated using a scale of 0 to 9 (Table 1). The data were transformed into percentage performance (i.e., values were multiplied by 11) and used for analysis. The performance value of each genotype was determined as the average of the performance values of individuals within the genotype.

Table 1 Evaluation parameters of plants under salt stress.

Score	Phenotypes of plant
0	Dead plant with all leaves and stems damaged
1	Almost all of the leaves damaged
2	Most of the leaves damaged with obvious drying of leaves
3	Complete curled and severely damaged dry leaves
4	Complete curled and moderate damaged dry leaves
5	Complete curled leaves and slight damages of some of the leaves
6	Complete curled leaves
7	Green plants with moderate inward curled leaves
8	Normal green plants with slight inward curled leaves
9	Healthy plant with no visible symptoms of salt damage (e.g., chlorosis, necrosis, wilting).

Nomenclature

We name the identified QTLs as follows: Salt Tolerance from *S. pennellii* QTL (*Stpq*) or Salt Tolerance from *S. lycopersicoides* QTL (*Stlq*) followed by the number of the chromosome. If QTLs are located on the same chromosome, we used the letters a, b, c.

Statistic analysis

All statistical analyses were performed using SPSS 13.0. Phenotypic data were analyzed using the general linear model (GLM). The data were transformed into percentage performance (i.e. values were multiplied by 100 and divided by 11 because of the classes). The mean value of performance percentage (%) was calculated using the following models: percentage performance (%) =constant + genotype + experiment + genotype × experiment. For QTL mapping, each IL was compared to the parental control M82 or VF36. If there was a significant difference from the reference genotype M82 or VF36, a QTL was assumed to be present in the introgression line.

For further studies the genetics of certain identified QTLs from *S. pennellii* LA716, ILs were crossed and the analytical method described previously by Gur and Zamir (2004) and Semel et al. (2006) was followed. If an IL was significantly different from M82 and the ILH (the product of the cross) had a score in between the IL and M82, there are three possibilities: (i) If the score of the ILH was significantly different from the IL but not from M82, it was considered recessive; (ii) If the ILH differed from both parents or did not differ from either of them, it was considered as additive; and (iii) If the ILH differed from M82 but not from IL, the QTL was considered as dominant with a further refinement that when the ILH was significantly higher or lower than both its parents, it was considered as overdominant.

To estimate the interactions between QTLs, a modified method (Eshed and Zamir 1995) was followed. The lines in each test were M82, ILHa (IL(a) × M82), ILHb (IL(b) × M82) and ILHab (IL(a) × IL(b)). The mean value of performance percentage of introgressed segment was calculated by the general linear model (GLM). The interaction effect was estimated as ((M82 + ILHab) - (ILHa + ILHb)) and its significance was determined by an *F* test. The complete additivity of the QTL was estimated as ((ILHa-M82) + (ILHb-M82)). The expected values were tested against the observed values and the regression line was tested against a null hypothesis of complete additivity (expected = observed or H0: β =1 vs. H1: β< >1).

Results

Evaluation of *S. pennellii* LA716 IL population

The performance value was used to evaluate salt tolerance in seedlings over six independent experiments in two years. During the gradual increase of the salt concentration leaf chlorosis and wilting became visible. The mean percentage performance (%) varied from 14.5% to 53.1% (Table 2). The correlations between experiments were low and only significant correlations were present between experiments 1, 2 and 6, as well as between experiments 3 and 4 (Table 3). Only experiments 1, 2 and 6 were further used, since experiment 3 and 4 were executed in the rainfall season which might have had an influence on the conditions in the greenhouse. The performance rate of the different introgression lines in the three experiments, varied from 15.3% to 64.6% and the control M82 had a value of 31.9%. Leaving out experiments 3 and 4 made the significance of the QTLs higher but the direction of the effects remained the same. The performance rate of nine lines was lower than M82, with IL6-3 as the lowest (15.3%). Eighteen lines (IL1-1-3, IL1-4-18, IL2-1, IL4-3-2, IL6-2, IL7-1, IL7-4-1, IL7-5, IL7-5-5, IL8-2-1, IL8-3-1, IL9-1-2, IL10-1-1, IL11-1, IL11-2, IL11-4, IL12-2 and IL12-3) performed significantly better than M82 (Table 4 and Fig.1).

Table 2 Mean value of percentage performance (%) in the *S. pennellii* LA716 IL population.

Experiment	Year	Percentage performance (%)	N[a]
1	2006	33.9 ± 1.3	209
2	2007	34.6 ± 1.2	210
3	2007	14.5 ± 0.9	449
4	2007	35.8 ± 0.9	526
5	2007	47.9 ±0.7	594
6	2007	53.1 ± 0.7	625

[a] number of individuals in each experiment varying from 3 to 10 plants per line per experiment depending on the number of available seeds.

Table 3 Pearson correlation of different experiments for the *S. pennellii* LA716 IL population

	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6
Exp1	1	0.250[*]	0.144	0.185	0.221	0.341[**]
Exp2		1	-0.086	-0.110	0.200	0.367[**]
Exp3			1	0.273[*]	0.122	0.086
Exp4				1	0.183	0.100
Exp5					1	0.035
Exp6						1

[*] significant at 0.05 level; [**] significant at 0.01 level.

Table 4 The percentage performance (%) of lines from the *S. pennellii* LA716 IL population with a significant difference as compared to the control under salt stress. The average is calculated over three experiments using a GLM procedure and Dunnett test. Highlighted in grey are the 10 confirmed QTLs.
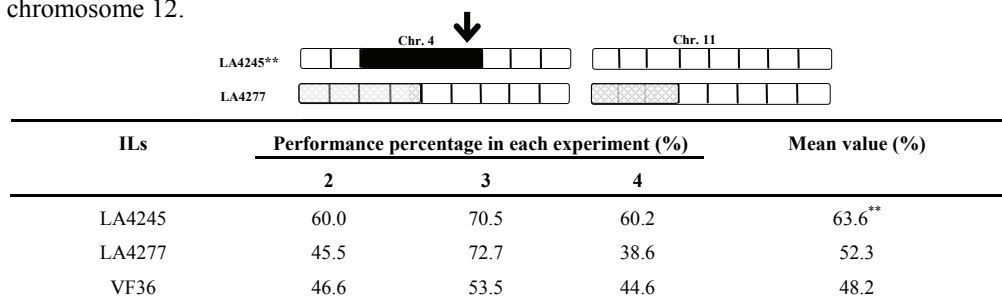
IL line	QTL	Consistent in three experiments	Flanking markers	Percentage performance in each experiment (%)			Mean value (%)
				1	2	6	
IL1-1-3		No	TG24-CT233	33.33	30.30	67.05	43.56[*]
IL1-4-18		No	TG258-TG27	42.42	39.39	65.91	49.24[**]
IL2-1	*Stpq*2	Yes	CT205-TG304	42.42	33.33	63.64	46.47[**]
IL4-3-2		No	TG182-CD55	-	-	63.64	63.64[**]
IL6-2	*Stpq*6	Yes	TG365-TG292	72.72	51.52	69.42	64.56[**]
IL7-1	*Stpq*7a	Yes	TG438-TG499	54.55	45.45	57.85	52.65[*]
IL7-4-1	*Stpq*7b	Yes	CT52-CT158	51.52	39.39	61.36	50.76[**]
IL7-5		Yes	TG418A-TG61	54.54	45.45	53.79	51.26[*]
IL7-5-5		Yes	TG418A-TG272A	-	-	57.95	57.95[**]
IL9-1-2		No	GP39-TG9	18.18	36.36	69.32	41.29[*]
IL10-1-1	*Stpq*10	Yes	TG230-TG303	27.27	51.52	61.36	46.72[*]
IL11-1	*Stpq*11a	Yes	TG557-TG523	39.39	39.39	69.32	49.37[**]
IL11-2	*Stpq*11b	Yes	CT651-TG400	30.30	48.48	64.77	47.85[*]
IL11-4	*Stpq*11c	Yes	CT105A-TG393	42.42	36.36	62.5	47.10[*]
IL12-2	*Stpq*12a	Yes	TG180-TG111	45.45	27.27	62.5	45.08[*]
IL12-3	*Stpq*12b	Yes	CT211A-CT80B	42.42	33.33	60.23	45.33[*]
M82	-	-	-	27.27	27.27	41.13	31.89

-: lines not tested in that particular experiment due to low number of seeds; [*] and [**] indicate that lines presented significant difference when compared to the control at 0.05 and 0.01 level, respectively. Flanking markers are according to Eshed and Zamir (1995).

Fig. 1 Identification of salt tolerance within the *S. pennellii* LA716 IL population. Top left: IL6-2 showed the highest tolerance to salt. Top right: IL7-5 also showed a high tolerance. Bottom: individual performance with a final concentration of 700mM NaCl + 70mM CaCl$_2$.

The *S. pennellii* LA716 IL population consists of primary lines and sub-lines. Some lines have completely or partly overlapping introgressions and can thus be considered as repetitions in the experiment. Potentially they also allow a more precise map position determination (Table 4 and Fig. 2).

IL2-1 had a better performance under salt stress; its border markers are markers TG304 and CT205. This result was confirmed in the sub line IL2-1-1 with also a good salt tolerance and almost reached the significance level (*P*=0.154). We refer to this QTL as

*Stpq*2a. Another QTL (*Stpq*7a) was detected with IL7-1 in all three experiments; line IL7-2, which is overlapping with IL7-1 is also salt tolerant. IL7-4-1, IL7-5 and IL7-5-5 have overlapping introgressions in the region flanked by the markers TG418A and TG272A and no overlap with IL7-1. All of these three lines have a significant better performance under salt stress and it was concluded that there must be another QTL located on chromosome 7, *Stpq*7b.
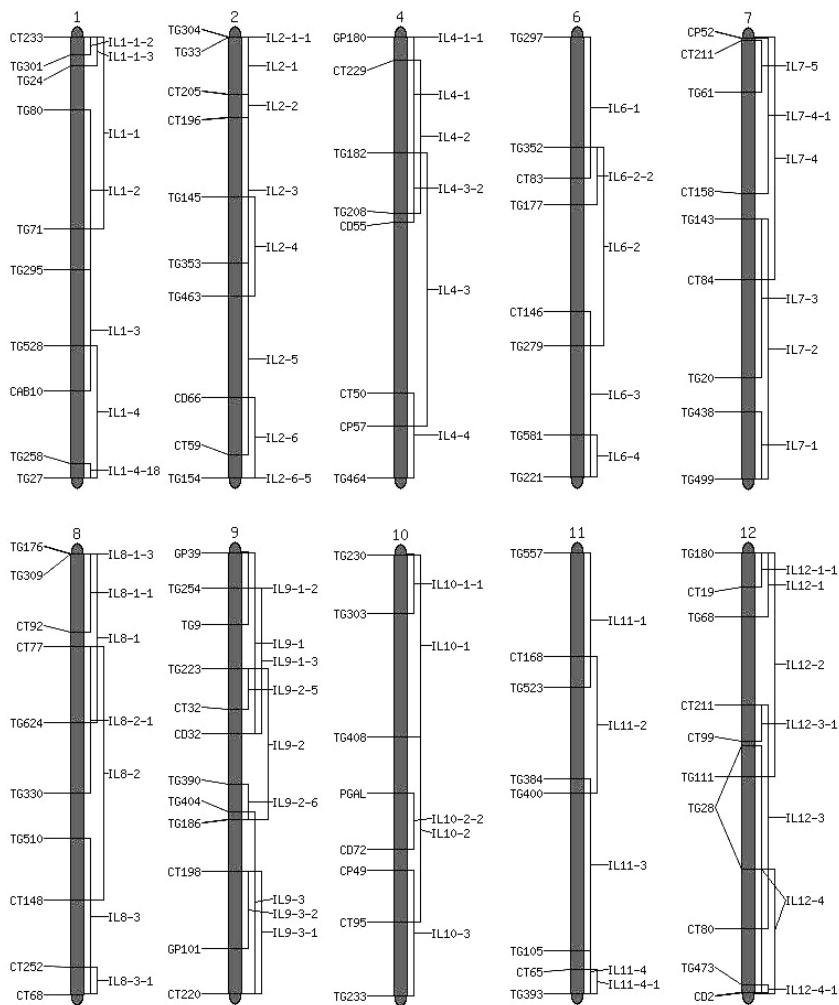
Fig. 2 Location of introgressions of *S. pennellii* LA716 on chromosomes 1, 2, 4, 6, 7, 8, 11 and 12; The eighteen salt tolerance lines (IL1-1-3, IL1-4-18, IL2-1, IL4-3-2, IL6-2, IL7-1, IL7-4-1, IL7-5, IL7-5-5, IL8-2-1, IL8-3-1, IL9-1-2, IL10-1-1, IL11-1, IL11-2, IL11-4, IL12-2 and IL12-3) are shown in the map. The map was copied from http://solgenomics.net/.

A total of ten major QTLs (see Table 4) located respectively on chromosome 2, 6, 7, 10, 11 and 12 were identified. The other putative QTLs on chromosomes 1, 4, 7 and 9 are questionable since their effect are observed in only one experiment and were not seen in lines with overlapping introgressions. For example we can look at the results obtained in line IL4-3-2 and IL4-3; IL4-3-2 had a better performance under salt stress (Table 4) but IL4-3 performed worse under salt stress than M82, although it was the parental line of IL4-3-2 (Fig. 2). The same phenomenon was observed for the QTL on Chromosome 9. More detailed marker studies are needed to know the exact locations and number of introgressions.

Evaluation of the *S. lycopersicoides* LA2951 IL population

Four independent experiments over two years were done to measure salt tolerance in the *S. lycopersicoides* LA2951 IL population (Table 5). The correlations between the different experiments were not high (Table 6) and finally only the three experiments with a significant correlation were used for the analysis (experiments 2, 3 and 4). Leaving out one experiment only changed the significance of the QTLs like in the *S. pennellii* LA716 population. The cultivar VF36 had a performance percentage of 48.2%. The response to the salt treatment varied among the different ILs in the range from 32.9% to 69.7%.

Table 5 Mean value of percentage performance (%) under salt stress of the *S. lycopersicoides* LA2951 IL population

Experiment	Year	Mean value of performance percentage (%)	N[a]
1	2006	24.16	338
2	2007	52.15	497
3	2007	58.54	455
4	2007	40.29	495

[a] number of individuals that were used in each experiment, varying from 4 to 8 plants per line per experiment depending on the number of available seeds.

Table 6 Pearson correlation of four independent experiments for the *S. lycopersicoides* LA2951 IL population

	Exp1	Exp2	Exp3	Exp4
Exp1	1	0.351[**]	0.332[**]	0.061
Exp2		1	0.503[**]	0.377[**]
Exp3			1	0.408[**]
Exp4				1

[*]: significantly different at 0.05 level; [**]: significantly different at 0.01 level.

Exceptions were the two ILs, LA4244 and LA4314, with an extremely low performance rate of 19.8% respectively 6.1%. Thirteen lines had a significant higher salt tolerance. After analyzing the results five QTLs (*Stlq*4, *Stlq*6, *Stlq*9b, *Stlq*12a and *Stlq*12b) were robust (Table 7). Either their effects were confirmed in lines with overlapping introgressions or the effect was clearly present in the three independent experiments.

Table 7 Percentage performance (%) of lines with a significant effect under salt stress. The average is calculated over three experiments using GLM procedure and Dunnett test. Highlighted in grey are the 5 confirmed QTLs

IL	QTL	Consistent in three experiments	Chr.	Flanking markers	Percentage performance in each experiment (%)			Mean value (%)	Homo. line
					2	3	4		
LA4236		No	2	TG33-TG554	70.0	69.3	-	69.7**	Yes
LA4245	*Stlq*4	Yes	4	TG180-TG68	60.0	70.5	60.2	63. 6**	Yes
LA4253	*Stlq*6	Yes	6	TG297-Adh-2	65.0	70.5	59.1	64.8**	Yes
LA4257		No	7	TG438-TG499	65.9	65.9	53.4	61.7**	Yes
LA4306		No	8	TG176-TG510	61.2	64.8	69.3	65.1**	No
LA4242		Yes	3, 9	TG42-TG244	64.5	68.2	40.9	57.8*	No
LA4268		Yes	9	TG42-TG244	-	-	68.2	68.2*	Yes
LA4270		No	9	TG105B-TG424	54.6	64.8	64.6	61.0**	Yes
LA4271	*Stlq*9b	Yes	9	TG186-CT220	63.6	68.2	59.1	63.6**	Yes
LA4279		No	11	TG180-TG68	50.0	67.1	67.1	61.4**	No
LA4313	*Stlq*12a	Yes	12	TG180-TG68	55.8	70.4	63.6	63.3**	Yes
LA4282	*Stlq*12a	Yes	12	TG180-TG111	75.8	60.2	50.0	61.0*	Yes
LA4284	*Stlq*12b	Yes	12	CT156-TG473	63.6	70.5	59.1	64.4**	Yes
VF36	-	-	-	-	46.6	53.5	44.6	48.2	Yes

- lines not tested in that particular experiment due to low number of seeds; * and ** indicate that lines presented significant difference when compared to the control at 0.05 and 0.01 level, respectively. Flanking markers were based on Canady et al. (2005) and TGRC (http://tgrc.ucdavis.edu/).
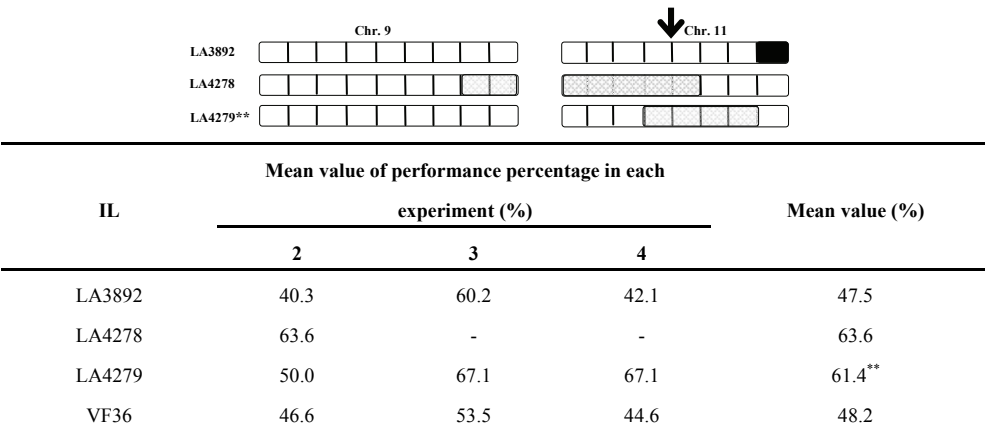
Some examples

Line LA4245 contains a homozygous introgression on chromosome 4 and was persistent salt tolerant in the three experiments. Line LA4277 has a partly overlapping heterozygous introgression on chromosome 4 and was not salt tolerant in two out of three experiments (Fig. 3). In case of one dominant QTL it must be located in the middle of chromosome 4 (*Stlq*4). Another example is LA4253, with a homozygous introgression on chromosome 6, this IL showed salt tolerance in three experiments indicating a single QTL (*Stlq*6). The homozygous introgression on the top of chromosome 9 in LA4242 is also present in lines LA4268 and LA4270. LA4270 showed a significant higher salt tolerance over three independent experiments and LA4268 had a high performance in the only experiment where it was evaluated (Fig. 4). Hence it is likely that QTL *Stlq*9a is located in the overlap of the introgressions of LA4242 and LA4268 (or LA4270), located near the top of Chromosome 9. However LA4271 is also salt tolerant and we must presume another QTL (*Stlq*9b) located in

the overlap of the introgressions of LA4270 and LA4271 but not in the introgression of
LA4272. This means that two QTLs are present in line LA4270 but their effects are not
additive. In a similar way two QTLs *Stlq*12a and *Stlq*12b (Fig. 5) could be identified on
chromosome 12.

ILs	Performance percentage in each experiment (%)			Mean value (%)
	2	3	4	
LA4245	60.0	70.5	60.2	63.6[**]
LA4277	45.5	72.7	38.6	52.3
VF36	46.6	53.5	44.6	48.2

[*] and [**] indicate that lines presented significant difference when compared to the control at 0.05 and 0.01 level,
respectively.

Fig. 3 Two *S. lycopersicoides* LA2951 ILs, containing overlapping introgressions of chromosome 4. and the extra
introgression on chromosome 11; solid segments are homozygous introgressions; shaded segments indicate
heterozygous introgressions. The map was based on (Canady et al. 2005). The arrow indicates the most likely
location of the suggested QTL. Values represent percentage performance (%) of each line in three independent
experiments and average of the three experiments.
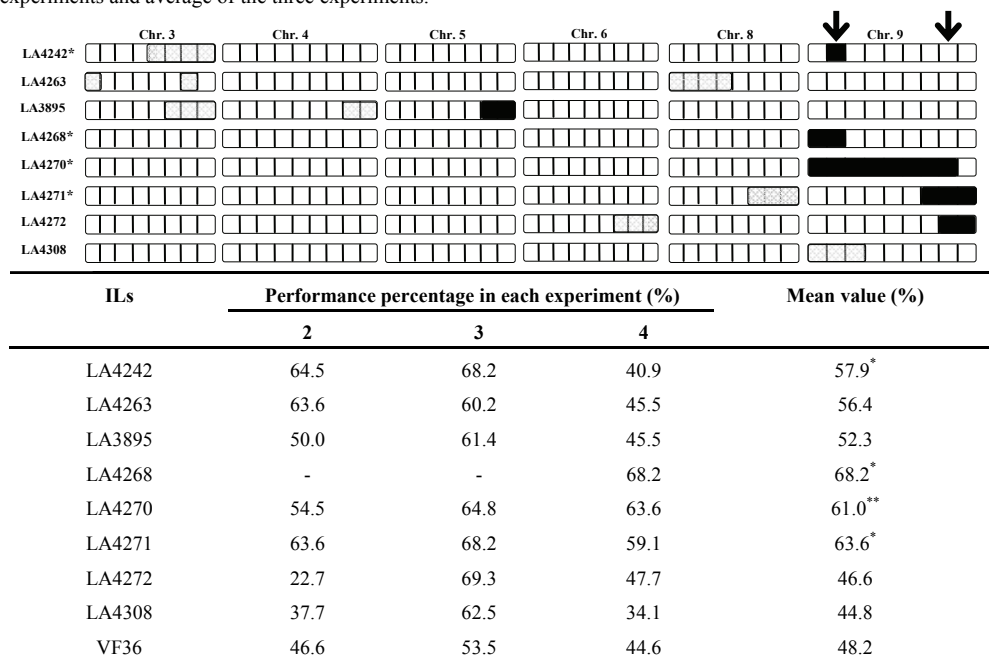
ILs	Performance percentage in each experiment (%)			Mean value (%)
	2	3	4	
LA4242	64.5	68.2	40.9	57.9[*]
LA4263	63.6	60.2	45.5	56.4
LA3895	50.0	61.4	45.5	52.3
LA4268	-	-	68.2	68.2[*]
LA4270	54.5	64.8	63.6	61.0[**]
LA4271	63.6	68.2	59.1	63.6[*]
LA4272	22.7	69.3	47.7	46.6
LA4308	37.7	62.5	34.1	44.8
VF36	46.6	53.5	44.6	48.2

-: lines not tested in that particular experiment due to low number of seeds; [*] and [**] indicate that lines presented
significant difference when compared to 0.05 and 0.01 level, respectively.

Fig. 4 Eight *S. lycopersicoides* LA2951 ILs, containing introgressions on chromosomes 3, 4, 5, 6, 8 and 9. Solid
segments are homozygous introgressions; shaded segments are heterozygous introgressions. The map was based on
(Canady et al. 2005). The arrows give the most likely location of the QTL. Values represent percentage
performance (%) of each line in three independent experiments and the average of the three experiments.
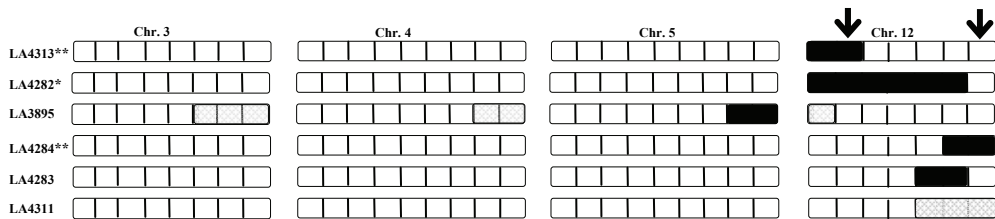
ILs	Mean value of performance percentage in each experiment (%)			Mean value (%)
	2	3	4	
LA4313	55.8	70.5	63.6	63.3[**]
LA4282	75.8	60.2	50.0	62.0[*]
LA3895	50.0	61.4	45.5	52.3
LA4284	63.6	70.5	59.1	64.4[**]
LA4283	22.7	52.3	26.1	33.7
LA4311	56.6	67.0	47.7	57.1
VF36	46.6	53.5	44.6	48.2

[*] and [**] indicate that lines presented significant difference when compared to the control at 0.05 and 0.01 level, respectively.

Fig. 5 Six *S. lycopersicoides* LA2951 ILs, containing introgressions on chromosomes 3, 4, 5, and 12. Solid segments are homozygous introgressions; shaded segments are heterozygous introgressions. The map was based on (Canady et al. 2005). The arrows give the most likely location of the QTL. Mean value of percentage performance (%) assessed in three independent experiments, and total mean value of percentage performance (%).

More experiments are needed to confirm the three putative QTLs in lines LA4236 (Chr. 2), LA4257 (Chr. 7), LA4306 (Chr. 8), and LA4279 (Chr. 11). For example, line LA4236 with a homozygous introgression on chromosome 1 was more salt tolerant but was only evaluated in two out of three experiments and no lines with overlapping introgressions were evaluated. LA4257 has a homozygous introgression on chromosome 7 and was more salt tolerant but five other lines with introgressions in this region were not salt tolerant (Fig. 6). The introgression on chromosome 8 of LA4306 might be responsible for the higher salt tolerance but five other lines with partly or completely overlapping introgressions did not show any effect. Another putative QTL is located on chromosome 11 (Fig. 7).
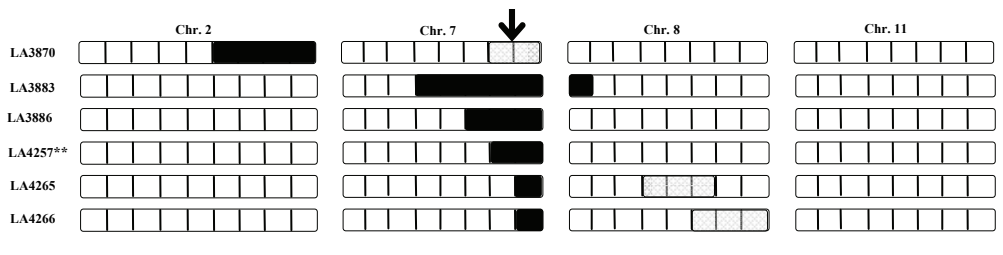
ILs	Performance percentage in each experiment (%)			Mean value (%)
	2	3	4	
LA3870	38.6	51.1	28.4	39.4
LA3883	62.9	40.9	34.1	45.0
LA3886	44.2	56.8	38.6	46.5
LA4257	65.9	65.9	53.4	61.7[**]
LA4265	32.5	61.4	35.2	43.0
LA4266	31.2	70.5	32.0	44.9
VF36	46.6	53.5	44.6	48.2

[**] indicates that lines presented significant difference when compared to the control at 0.01 level.

Fig. 6 Six *S. lycopersicoides* LA2951 ILs, containing introgressions on chromosomes 2, 7, 8 and 11. Solid segments are homozygous introgressions; shaded segments are heterozygous introgressions. The map was based on (Canady et al. 2005). The arrow gives the most likely location of the QTL. Values indicate percentage performance (%) of each line in three independent experiments and the mean value of the three experiments.

IL	Mean value of performance percentage in each experiment (%)			Mean value (%)
	2	3	4	
LA3892	40.3	60.2	42.1	47.5
LA4278	63.6	-	-	63.6
LA4279	50.0	67.1	67.1	61.4[**]
VF36	46.6	53.5	44.6	48.2

-: lines not tested in that particular experiment due to low number of seeds; [**] indicates that lines presented significant difference when compared to the control at 0.01 level.

Fig. 7 Three *S. lycopersicoides* LA2951 ILs, containing introgressions on chromosomes 9 and 11. Solid segments are homozygous introgressions; shaded segments are heterozygous introgressions. The map was based on (Canady et al. 2005). The arrow gives the most likely location of the QTL. Values indicate percentage performance (%) of each line in three independent experiments and the average of the three experiments.

Genetic analysis of some QTLs derived from *S. pennellii* IL population

Three ILs (IL6-2/*Stlq6*, IL7-1/*Stlq7a* and IL7-5/*Stlq7b*) with introgressions of *S. pennellii* LA716, were used to study dominance and interactions of these QTLs with relatively large effects. This was done in four independent experiments (Table8). Three significantly correlated experiments (experiment 2, 3 and 4) were used for the final evaluation of salt tolerance in these lines (Table 9). The results showed that all 3 ILs and ILHs with M82 (hybrid of IL and M82) had a significantly better performance under salt stress (Fig. 8). The effects were semi-dominant since there were no significant differences between ILH and IL. Interactions of QTLs were detected by combining ILs in total of three combinations (IL6-2×IL7-1, IL6-2×IL7-5 and IL7-1×IL7-5). The results show that in the combinations of QTLs tested there is a clear non-additive or even epistatic effect because the effect of the double heterozygotes was smaller than the sum of the effects of the corresponding single heterozygotes (Fig. 9).

Table 8 Mean value of percentage performance (%) estimated for double-heterozygous ILHs as estimation for salt tolerance in four experiments

Experiment	Year	Mean value of performance percentage (%)	N[a]
1	2007	54.97±2.36	64
2	2007	62.93±1.69	64
3	2007	65.06±1.21	64
4	2007	63.49±2.11	64

[a] number of individuals that were used in each experiment.

Table 9 Pearson correlation of four independent experiments for double-heterozygous ILHs

	Exp1	Exp2	Exp3	Rxp4
Exp1	1	0.419[*]	0.228	0.371
Exp2		1	0.559[**]	0.474[*]
Exp3			1	0.474[*]
Exp4				1

[*]: significant at 0.05 level. [**]: significant at 0.01 level.
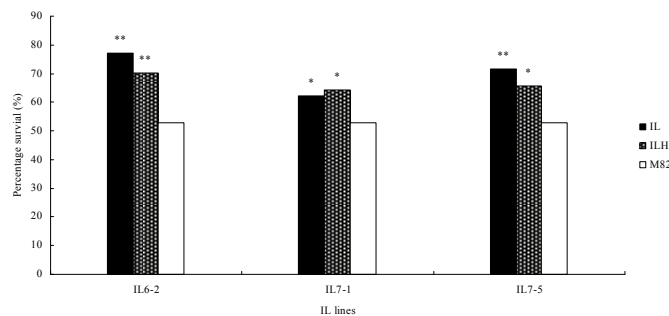
Fig. 8 Mean value of percentage performance (%) of the lines IL6-2, IL7-1 and IL7-5 with a homozygous introgression, the recurrent parent and their hybrid [*]: significantly different at 0.05 level; [**]: significantly different at 0.01 level.
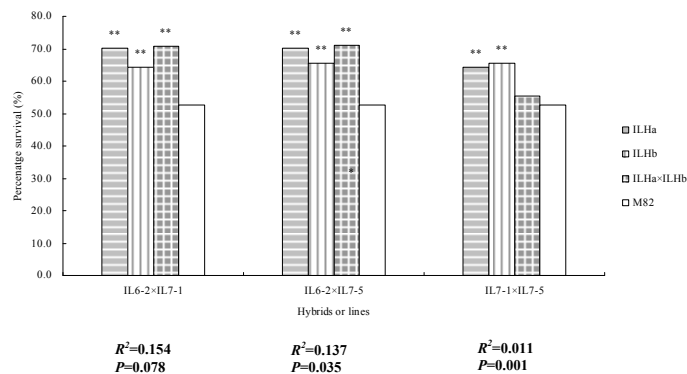
Fig. 9 (Semi)dominant effects in hybrids with wildtype and non additive or epistatic effects in hybrids between two IL lines. Mean value of percentage performance (%) of ILHa (M82×ILa) (first bar from the left), ILHb (M82×ILb)(second bar) and the lines with both introgressions (ILa x ILb)(third bar), and the recurrent parent M82 is the fourth column. R^2 value was calculated by linear regression analysis; P value was calculated by F test; [**]: significantly different at 0.01 level.

Discussion

Identification of QTLs for salt tolerance in the seedling stage

Two IL populations have been used to reveal quantitative trait loci for conferring salt tolerance in the seedling stage of tomato in this study. Six and four independent experiments were conducted on the *S. pennellii* LA716 and *S. lycopersicoides* LA2951 IL populations. Although each experiment was done in a similar way low correlations between all experiments were observed, this was especially the case in the *S. pennellii* introgression library. This is probably due to differences in the plant physiology caused by the changing

environmental conditions in the greenhouse. For example, experiment 3 and 4 for the *S. pennellii* LA716 population were conducted in the rainfall season, which affects the plant growth and development. Therefore we only used the experiments which were correlated to analyze further for the detection of QTLs. The genetic background of the recurrent parent of the IL population is of importance to get significant differences, in our studies *S. lycopersicum* VF36 is more salt tolerant than M82. The large variation in circumstances makes that not all QTLs could be identified in all experiments and therefore repeated experiments were necessary. However the most robust QTLs, which make a stable and large contribution for salt tolerance, always showed a consistent performance for salt tolerance. For example, line IL6-2 harboring QTL *Stpq*6 showed a robust performance over all experiments.

Ten and five major QTLs respectively from the two IL populations showed an unambiguous level of tolerance because lines harboring these QTLs presented a consistent salt tolerance in all analyzed experiments. However, some other QTLs were not confirmed by overlapping introgressions or by repetitions. To confirm these QTLs, more experiments need to be done. Also in the *S. lycopersicoides* IL population, there are questionable QTLs but in this population there is the problem that some lines only have heterozygous introgressions meaning that a quarter of the offspring plants are without introgression. Identification with markers of the lines lacking an introgression is a possibility to circumvent this problem. A limited seed production also limits the possibility of doing many experiments. Furthermore, some QTLs for salt tolerance might not be detected because the IL populations do not represent the entire genome. The most important QTLs can be fine mapped via recombinant screenings.

In our study with the two different introgression populations chromosomes 6 and 12 were found to contain QTLs important for salt tolerance during the seedling stage. Also two other chromosomes (4 and 9) were showing QTLs although not in all experiments in a repeatable fashion. However the fact that they pop up in different experiments indicates that they do contain regions of interest which merit further research in the future.

Comparative analysis of QTLs for abiotic stress

This study has revealed ten unambiguous regions on chromosome 2, 6, 7, 10, 11 and 12 from *S. pennellii* LA716 and five unambiguous regions on chromosome 4, 6, 9 and 12 from *S. lycopersicoides* LA2951 with a significant effect on tomato salt tolerance in the seedling stage. In previous studies, eight QTLs for salt tolerance in the seedling stage were placed on chromosomes 1, 3, 5, 6, 9 and 11 in an interspecific backcross between a salt-sensitive *S. lycopersicum* NC84173 and a salt-tolerant *S. pimpinellifolium* accession LA722 (Foolad and

Chen 1999). *S. pennellii*, *S. lycopersicoides* and *S. pimpinellifolium* are quite distantly related wild species. Molecular markers make it possible to compare map positions of QTLs coming from different species. QTLs located on chromosome 6 are present in all the three populations. *Stpq*6 derived from *S. pennellii* is flanked by markers TG365 and TG292, *Stlq*6 from *S. lycopersicoides* is flanked by markers TG297 and Adh-2, and one QTL from *S. pimpinellifolium* by markers CT285 and TG477. Based on the tomato high density map (Tanksley et al. 1992), the region flanked by these markers could be limited to markers CT285 and Adh-2, in which they might be co-localized together. By the same manner, QTLs *Stpq*11a (TG557 and TG523) and *Stpq*11b (CT651 and TG400) from *S. pennellii* and one QTL (TG497 and CT107) from *S. pimpinellifolium*) co-localize on chromosome 11 and might be restricted between markers TG651 and TG523. It is also possible that two QTLs (*Stpq*11a and *Stpq*11b) identified in *S. pennellii* are in fact one locus.

In addition, eight QTLs conferring salt tolerance during seed germination have been found with a F_2 population derived from *S. pennellii* LA716, which were located on chromosome 1, 2, 3, 7, 8, 9 and 12 (Foolad et al. 1997). The four QTLs originating from *S. pennellii* LA716 were on chromosome 1, 3, 9 and 12. Additional seven QTLs were from *S. pimpinellifolium* located on chromosome 1, 2, 5, 7, 9 and 12. Five regions on chromosome 2, 7, 8, 9 and 12 are shared by the possibly co-localized QTLs. QTLs located on chromosome 2 are presented in the above three populations. *Stpq*2 from *S. pennellii* for seedling stage is flanked by markers TG304 and CT205. One QTL from *S. pennellii* F_2 for seed germination is flanked by markers TG31 and Prx-2. Both of these QTLs are co-localized in the region between markers TG31 and CT205 but not with the QTL from *S. pimpinellifolium* flanked by markers CT59 and TG104. On chromosome 7, *Stpq*7b (TG418A and TG272A) and one QTL (CT52 and CT113) from *S. pimpinellifolium* might be restricted to markers TG113 and CT52. On chromosome 8, *Stpq*8a (CT77 and TG330) and one QTL (TG45 and Aps-2) from *S. pennellii* might be between markers TG77 and Aps-2. On chromosome 9, *Stlq*9b (TG186 and CT220) and one QTL (TG35 and Est-2) from *S. pennellii* might be between markers TG35 and Est-2. On chromosome 12, *Stpq*12a (TG180 and TG111) or/and *Stpq*12b (CT211A and CT80B) and one QTL (Pgi-1 and TG311B) from *S. pennellii* F_2 might be co-localized between markers CT211A and Pgi-1. Salt tolerance during seed germination is independent of that during vegetative growth (Foolad 1999). Co-localization of some QTLs for salt tolerance during seed germination and the seedling stage would strengthen the evidence that the same genes might control the rate of seed germination and seedling growth, and crosstalk for response to adverse abiotic and biotic stressors may share the common pathway, in which secondary metabolisms make a significant contribution (Chinnusamy et al. 2004; Glombitza

et al. 2004). Salt tolerance QTLs might also influence cold tolerance during seed germination. One introgression (IL7-5-5) from *S. pennellii* LA716 causing drought tolerance (Gur and Zamir 2004) has shown a higher salt tolerance in the seedling stage in our study. In addition, more precise map positions will make it possible to speculate about co-localization of QTLs identified in different studies. For instance to look for co-localisation with the 49 QTLs for nineteen traits under salt stress in two populations derived from *S. pimpinellifolium* and *S. cheesmaniae*. These QTLs were distributed over eleven chromosomes with the exception of chromosome 9 (Villalta et al. 2007).

Genetic effects of QTLs for salt tolerance

Phenotypic variation caused by QTLs is similar to variation for simple Mendelian inherited loci (Anbinder et al. 2009). Three ILs with QTLs (*Stpq*2, *Stpq*6 and *Stpq*7) have been used to make crosses in order to study dominance and additivity. All three QTLs were found to be semi-dominant. Combinations of introgression hybrids were made and the effect of the double heterozygotes was smaller than the sum of the effects of the corresponding single heterozygotes. Hence the detected effect was non-additive or even epistatic. This result was in agreement with previous studies, in which QTLs also showed less-than-additive effects (Eshed and Zamir 1995). The dominant nature of QTLs for salt tolerance in the seedling stage is positive for tomato hybrid breeding. In studies on the effects of QTLs on yield improvement under drought condition also dominant and even over-dominant effects were found (Gur and Zamir 2004).

Potential of pyramiding of QTLs for tomato salt tolerance

Pyramiding three independent yield-promoting segments (IL7-5-5, IL8-3 and IL9-2-5) from the drought tolerant *S. pennellii* LA716 has led to novel varieties with dramatically increased productivity under normal cultivation conditions but also in the presence of drought stress (Gur and Zamir 2004). We identified ten and five QTLs for salt tolerance respectively from two wild species for the seedling stage. Combinations of QTLs are possible but for this it is necessary to reduce the introgression size because the linkage drag would also be larger and will most probably mask the effects. For example, IL6-2 showed the highest tolerance to salt in *S. pennellii* IL population, however it presented vigorous growth with very poor fruit setting as we observed in the field. Furthermore, combining QTLs in a single genetic background can lead to unexpected results. For example, less additive and overdominant effects have been found for yield and other quality traits (Eshed and Zamir 1995). Generally these types of complex traits are affected by epistasis, locus heterogeneity, pleiotropy and

their interaction with environments (Glazier et al. 2002; Coaker and Francis 2004; Semel et al. 2006; Causse et al. 2007).

In spite of all the research that has been conducted on tomato salt tolerance, it seems that the development of a salt tolerant cultivar is still far away even if transgenic strategies are being deployed (Cuartero et al. 2006). The main reason for this is the genetic and physiological complexity of salt tolerance (Cuartero and Fernández-Muñoz 1999; Flowers 2004; Cuartero et al. 2006). However the present study shows that improvement can be achieved albeit maybe at smaller steps and less fast then hoped for.

CHAPTER 5

General discussion

Introduction

Tomato growth and yield are greatly influenced by abiotic and biotic stresses. Through the continuous selection by humans for a long time, tomatoes have most of the required (edible) characters of the fruits, but no selection has found place to preserve their potential to withstand changing environments. Wild relatives of tomato (e.g. *S. pennellii, S. habrochaites*, *S. pimpinellifolium, S. lycopersicoides*) are still adapted to natural adversity and have not lost their potential for responding to various abiotic and biotic stresses. In this thesis we explore the potential resistance in some of these crossable wild relatives against *Botrytis cinerea* (Chapter 2) and *Phytophthora infestans* (Chapter 3), both of which can be very destructive biotic factors for tomato production. We used for this research two IL populations based on the wild species *S. habrochaites* and *S. lycopersicoides*. Meanwhile we also explored the potential tolerance to salt stress (Chapter 4), an abiotic stress factor which is becoming a more severe problem in open field and in protected greenhouse production. Two IL populations based on the wild relatives *S. lycopersicoides* and *S. pennellii* were analyzed. The main aim of this thesis was to find leads to further improve the success in tomato breeding against biotic and abiotic stresses.

Wild species for modern tomato breeding

Although tomato wild relatives harbor many valuable traits to be used for increasing yield, the emphasis for tomato breeding in the past was mainly on the exploration and introgression of disease resistance from these wild species. This is due to the fact that for most of these traits only one or a few genes are involved. Species like *S. chilense*, *S. peruvianum*, *S. habrochaites* and *S. pimpinellifolium* are rich resources of interesting traits (Rick and Chetelat 1995). Since the last decade, also quantitative trait loci (QTL) for tomato improvement have been explored and used in breeding programs with the aid of DNA based technologies

(Tanksley et al. 1989). Further QTL identification from wild species was conducted using advanced backcross populations (Tanksley and Nelson 1996) and introgression lines (Eshed and Zamir 1995), both of them could directly be used for further breeding because the lines in such populations possess a tomato background. For example, the three IL populations used in this thesis were produced in the genetic background of three elite cultivars M82, E6203 and VF36. However, as summarized in Chapter 1, most of the QTLs identified in these IL populations are related to yield or fruit quality. In this thesis, we show that *S. habrochaites* LA1777, *S. pennellii* LA716 and *S. lycopersicoides* LA2951 are a source for tolerance to *P. infestans*, *B. cinerea* and a source for improved salt tolerance. In many cases where quantitative trait variation was dissected single genes were responsible for the QTL (Price 2006;Tanksley and Fulton 2007). This suggests that introgression of QTLs from wild species to modern breeding lines is rather straight forward.

QTL detection using introgression lines

Three IL libraries were used in this thesis to identify QTLs by quantitative assay methods. The advantages of IL as a permanent source have been reviewed in Chapter 1 and the power of IL for detecting QTLs, especially minor QTLs, was proven by comparing the IL to an F2 population in previous work (Finkers et al. 2007b;Jeuken et al. 2008). The obvious advantage of the IL population is that epistatic interactions or less-than-additive effects do not complicate the interpretation and functionally redundant QTLs can also be easily detected (Finkers et al. 2007b). Another advantage of an IL population is the possibility to repeat experiments in time and space. However，the disadvantage of this type of population is the long time required for their development and not all necessary genes have been captured due to a single plant used routinely for the outcrossing. In our studies only low to medium correlations were observed among the independent bioassays for *B. cinerea* and *P. infestans* resistance and also for salt tolerance. In other words large variation was observed between and within the different experiments and within one experiment for each genotype. The interaction between QTLs and environments is present and unpredictable resulting in the fact that minor QTLs often can not be detected in only one experiment. We believe that main reasons for this are:

(1) Each genotype from an IL population has a different phenotype and can adapt in a different way to the environment.

(2) All plants were grown in a greenhouse under natural light conditions. Therefore growth and development can be quite different between independent experiments.

For example, experiments 3 and 4 for salt tolerance (Chapter 4) were performed in the rainy season (July and August); these two experiments were not correlated to the other 4 experiments executed in the non-rainy season. Davis et al. (2009) showed that the resistance to *B. cinerea* of *S. lycopersicoides* LA2951 is weaker during the winter months and in doing so demonstrated the interaction between QTLs and environment.

The fact that a clear trait in the original accession can not be recovered in the IL population can be caused by:

(1) An IL population is derived from a single F1 after crossing the wild species and tomato. Heterozygosity in the original accession can result in the loss of favorable alleles.

(2) IL populations do not represent the entire genome of the wild species due to various reasons such as lethality and limited marker analysis during selection.

(3) Only combinations of genes result in resistance or tolerance. These genes do not necessarily   have to be located on the same introgressions.

(4) Heterozygous lines existed especially in *S. lycopersicoides* IL population hide the possibility for QTL identification, special for these with recessive character.

For example, *S. habrochaites* LA1777 is resistant to TYLCV and YoMoV but all IL lines were susceptible for both diseases (Momotaz et al. 2007). In our experiments (Chapter 3), *S. habrochaites* LA1777 showed a high resistance to *Phytophthora infestans* but only few QTLs with a relatively small effect were identified.

The fact that a clear effect in one introgression line is not seen in another introgression line with overlapping introgressions can be due to:

(1) The exact size of the introgressions still needs to be addressed more accurately. For example, line LA4278 showed a clear phenotype with slow growth and light yellow leaves, which should partly overlap with LA4279 and LA4277. However, the phenotype was absent in LA4279 and LA4277.

(2) Genes with a negative effect might be present in the same introgression fragment interacting with genes with positive effects. In some cases, one particular IL showed a certain level of resistance or tolerance but another larger IL completely overlapping the first one did not show these effects. This might be a quite general feature as we observed this for all traits analyzed in this thesis. The combined effect of chromosomal regions acting in the opposite direction from that of one or both individual chromosomal regions have been found in tomato using IL lines when several traits, including plant height and leaf number at five weeks, and specific leaf area and time to witling under drought stresss, were analyzed (Christopher and Leonie 2009).

(3) Sample size plays an important role to get significant effects. Limited sample size due to poor fertility occurred especially in *S. lycopersicoides* population.

In conclusion, we believe that

(1) Independent experiments over several years are necessary to identify reliable QTLs, especially when QTLs have minor effects. (2) Most QTLs identified in this thesis need to be further confirmed e,g. in segregating recombinant progenies despite the fact that some of the QTLs (for *Phytophthora infestans* and *Botrytis cinerea* resistance) have been described on similar chromosomal locations in other studies (Brouwer et al. 2004). (3) ILs provide a starting point to study additivity, dominance and interactions of QTLs. This can be done by crossing individual QTLs and if needed selfing and selection for homozygous introgressions (Chapter 4). (4) SubNILs are favorable to be developed for the substitution of some heterozygous ILs and ILs with larger introgressions.

Botrytis cinerea resistance in different tomato plant tissues

There are almost no cultivars with an acceptable resistance to *B. cinerea*. Accessions of wild tomato species such as *S. chilense* LA1932 (Chetelat and Stamova 1999), *S. peruvianum* LA2745, *S. habrochaites* LA2314, *S. pimpinellifolium* LA1246 (Egashira et al. 2000) and *S. lycopersicoides* LA2951(Guimarães et al. 2004) have been described to contain useful resistance to *B. cinerea*. Especially *S. habrochaites* accessions seem to be a good source for exploring resistance to this destructive disease (Egashira et al. 2000;Finkers et al. 2007a;Finkers et al. 2007b;Nicot and Moretti 2002). We confirmed this and extended the number of accessions with resistance in Chapter 2 by conducting the assay in leaves, stems and fruits using two introgression line populations. Leaf assay tests showed that the QTLs from *S. lycopersicoides* LA2951 were responsible for a reduced leaf lesion size and that these QTLs were more effective than the QTLs from *S. habrochaites* LA1777. It is consistent with the results of the screening of these two accessions. *Rbclq4* and *Rbclq11* showed the potential for leaf resistance (Lesion Size) using one isolate collected in a greenhouse in China. Recently QTLs from *S. lycopersicoides LA2951* involved in leaf resistance were described on chromosomes 1, 2, 3 and 4 using another strain (B01.10; (Davis et al. 2009). The QTL on chromosome 4 identified in this thesis and the one identified in the work of Davis et al. (2009) do not co-localize in the same region of chromosome 4. Quantitative resistance to *Botrytis cinerea* is a complex phenomenon (Katherine et al. 2004) and in Arabidopsis different QTLs can be effective against different isolates of *B. cinerea* (Katherine et al. 2004;Rowe and Kliebenstein 2008). There is a large genetic variation between different isolates of this necrotrophic fungus with a wide host range (Williamson et al. 2007). For example, different

requirements for conidia germination of the isolates have been observed (Cotoras et al. 2009). Differences in the bioassay can also cause differences in the number and nature of the QTLs (Katherine et al. 2004). Especially the resistance in *S. lycopersicoides* LA2951 seems to be more easily affected by changing circumstances (Davis et al. 2009). This was confirmed in this thesis (Chapter 2). Seasonal differences have an effect on tomato plant physiology resulting in somewhat different leaves which potentially can increase or decrease the leaf susceptibility in *S. lycopersicoides*. Accumulation of camalexin in Arabidopsis has been shown to occur in response to *B. cinerea* infection of leaves (Katherine et al. 2004;Rowe and Kliebenstein 2008). A chemical analysis of those ILs with a QTL is a possibility for further research understanding partial resistance to *B. cinerea.*

Compared to *S. habrochaites* LYC4 and *S. neorickii* G1.1601(Finkers et al. 2008;Finkers et al. 2007a;Finkers et al. 2007b) only a few QTLs from *S. habrochaites* LA1777 and no QTLs from *S. lycopersicoides* LA2951 were found for stem resistance. Nevertheless, the identified stem resistance seems to be stable in different seasons and therefore interesting for tomato cultivars meant for greenhouse production since there is less infection after pruning of the side shoots.

The increased susceptibility of ripe fruits to all kinds of pathogens may be an inherent outcome of ripening, which is likely to facilitate the dispersal of mature seed (Gillaspy et al. 1993). This is not beneficial for tomatoes and we showed that some introgressions resulted in a reduced lesion size in ripe fruits. In green fruits both positive and negative effects were observed; *S. habrochaites* LA1777 ILs were identified resulting in an increased susceptibility whereas introgressions from *S. lycopersicoides* result in the opposite effect. Up to now, the mechanism in regulating ripening associated pathogen susceptibility is still not completely clear. Simultaneous suppression of *LePG* (for polygalacturonase) and *LeExp1* (for expansin) in ripening fruits delays or decreases ripening associated susceptibility to *B. cinerea*, which concurrently reduces wall disassembly and slows fruit softening (Cantu et al. 2008a). Meanwhile, absence of the endo-β-1,4-glucanases Cel1 and Cel2 in ripening tomato fruit also reduces susceptibility to *Botrytis cinerea* (Victor et al. 2007). However, cell wall disassembly may also generate signals including pectin-derived oligosaccharides that activate antipathogen responses (Cantu et al. 2008b;Cervone et al. 1989;Cote and Hahn 1994;Vorwerk et al. 2004). The results from this thesis provide an alternative way to understand the interaction of tomato fruit with this necrotrophic microorganism because ILs with QTLs are the starting points for the development of Nearly Isogenic Lines, which can be used for further studies.

The low correlation for the resistance between leaves and stems seen in Chapter 2 is a common phenomenon (Finkers et al. 2007a;Nicot and Moretti 2002;ten Have et al. 2007). Also there is a low correlation of the resistance between fruits and leaves respectively stems. This obviously complicates breeding for general resistance to *B. cinerea*. But some ILs were identified with simultaneously lower LS or DI for leaf, stem and fruit. If only one locus is responsible in these ILs this locus can be potentially important and useful in tomato breeding.

Phytophthora infestans resistance in tomato

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) De Bary, is a highly destructive disease and one of the most severe problems of tomato production. Qualitative and quantitative resistance has been found in accessions of wild species. Qualitative genes were identified in *S. pimpinellifolium* (Brouwer et al. 2004;Brouwer and St Clair 2004;Chunwongse et al. 2002;Conover and Walter 1953;Foolad et al. 2008;Moreau et al. 1998) and quantitative resistance in other accessions of wild relatives including *S. habrochaites* and *S. pennellii* (Abreu et al. 2008;Brouwer et al. 2004;Brouwer and St Clair 2004;Smart et al. 2007). These results show the rich resource for introgressing resistance in commercial cultivars. Especially *S. habrochaites* is a broad source for resistance to this fungus, which might be due to its adaption to low temperature and high humidity environments. In chapter 3, the accession of this wild species LA1777 has been proved the potential resistance to several isolates as compared to LA1033 and LA2099, which has been used previously to explore the potential quantitative loci (Brouwer et al. 2004;Lough 2003). Five unambiguous QTLs were found using an IL population developed from it. QTL *Rlbq4b*, not co-localized with the previously identified QTLs, might be a novel QTL. In addition, *S. lycopersicoides* LA2951 was also collected from an area with similar environmental conditions. The primary results from the screening of the IL population of this accession in two correlated independent experiments (r=0.399, *P*<0.01) showed that seventy six out of seventy eight ILs had a lower LS than the susceptible control VF36, of which thirty eight ILs were significantly lower. Three clear QTLs (*Rlblq2*, *Rlblq4* and *Rlblq9*) were identified (Table 1). Although fifty four ILs had a lower DI only nine were significant, however the putative QTLs involved in these lines could not be further confirmed as what happened in Chapter 3 with LA1777 IL population. The main reason might caused by a highly constant humidity in closed trays which apparently enhances infection by the zoospores causing a very high disease pressure (Vleeshouwers et al. 1999). More experiments need to be executed to find the true value of these QTLs. However, all the presented evidences indicate the possibility to obtain durable resistance in tomato especially since breaking resistance by *P.*

infestans on tomato seems not to occur as fast as in potato where the more than 11 specificities (*R1–R11*) which were introgressed into cultivars were rapidly overcome by new strains of *P. infestans* (Park et al. 2009;Pel et al. 2009).

Up to now, slow progress has been made concerning the unraveling of the mechanism of *P. infestans* infection in tomato. There is evidence that partial resistance in tomato to *P. infestans* is independent of ethylene, jasmonic acid and salicylic acid signaling pathways (Smart et al. 2003). Generally, resistance (*R*) gene transcript levels appear to be correlated to disease resistance however differences in disease-resistant phenotypes associated with plant age in potato is not caused by *R*-gene transcript abundance (Millett et al. 2009). The developed ILs identified in this thesis add tools for further understanding the interaction between tomato and late blight.

Table 1 QTLs for responsible for reducing Lesion size (LS) and disease incidence (DI) from *S. lycopersicoides* LA2951 IL lines against *P. infestans*.

Lines	QTLs	Linked markers	Leaf LS in each experiment (cm^2)		N^a	Mean LS (cm^2)	Mean DI (%)	Homo. line
			1	2				
LA3869	*Rlblq2*	TG191-TG507	1.87	5.69	57	3.78 ±0.44**	54.4±6.3%	Y
LA3870		Fdh-TG507	3.90	4.97	97	4.44 ±0.37**	60.3±5.7%	Y
LA3871		Fdh-TG507	4.89	5.14	104	4.89 ±0.38*	43.4±4.7%	Y
LA4239		Fdh-TG507	4.76	5.18	98	4.97 ±0.32**	59.4±4.8%	Y
LA3877	*Rlblq4*	TG22-TG464	2.45	4.97	51	3.71 ±0.46**	55.1±6.6%	Y
LA4246		CT50-TG464	4.55	4.58	54	4.57 ±0.49**	47.1±6.5%	Y
LA4247		TG22-TG464	3.33	6.15	87	4.74 ±0.31**	71.1±5.1%	Y
LA4314		TG22-TG464	4.38	5.14	52	4.76 ±0.36**	92.9±6.6%	N
LA4268	*Rlblq9*	TG105B-TG9	3.86	5.92	89	4.89 ±0.34**	60.6±5.1%	Y
LA4269		TG9-TG10	4.32	6.02	118	5.17 ±0.29*	61.0±4.4%	Y
LA4270		TG105B-TG424	5.11	5.30	80	5.20 ±0.35*	63.3±5.3%	Y
LA4308		TG105B-CT143	4.90	4.45	81	4.68 ±0.36**	59.7±5.3%	N
VF36		-	7.48	5.74	218	6.61 ±0.21	65.2±3.2%	Y

*and ** indicate that lines presented significant difference when compared to the control at 0.01 level.

Salt tolerance improvement in tomato

Most modern tomato cultivars are sensitive to moderate levels of salt. The genetic and physiological complexity of salt tolerance makes improvement difficult in tomato. In Chapter 4, we evaluated salt tolerance in seedlings in two IL populations (*S. pennellii* LA716 and *S. lycopersicoides* LA2951). For screening, the phenotype under salt stress was evaluated three times at different times. Pearson correlation coefficients were calculated to asses the correlation between these three evaluations in time. In both IL populations a high correlation between the experiments (r=0.85-0.95 for *S. pennellii* population and r=0.78-0.95 for *S. lycopersicoides* population) was observed. In total forty three QTLs were identified in both populations. Most ILs with a QTL from *S. lycopersicoides* performed better than the ILs with QTLs of *S. pennellii*; the two populations were screened simultaneously. However, it is discussable whether it is allowed to compare the effects in this way because the different genetic background and different level of salt tolerance of the respective tomato parent in the two populations. M82 (of the *L. pennellii* population) showed a lower value (31.89%) as VF36 (48.2%). Four of the QTLs for salt tolerance co-localize in the two IL populations. Our results and previous studies (Foolad et al. 2001), indicate that one loci located on chromosome 6 might co-localized in three populations. QTLs might be conserved among tomato species as is also seen in other crops (Hamwieh and Xu 2008). This might provide a clue for the evolution of salt tolerance in tomato species. The *S. pennellii* IL with the highest survival percentage under salt stress showed a very vigorous growth but a poor fruit production. Another interesting QTL is located in chromosome 7 here salt and drought tolerance seem to co-localize because IL7-5-5 has a good drought tolerance (Gur and Zamir 2004). We also observed that IL7-5-5 had a high fruit setting in the field resulting in a very good yield. Two other lines (IL8-3 and IL9-2-5) showing drought tolerance did not show positive effects for salt tolerance.

Previous studies have indicated that tomato salt tolerance, as in other crops, also depends on the developmental stage (Foolad 2004). Based on this we also primarily tested both IL populations for salt tolerance during seed germination (data not shown). We found that some *S. pennellii* ILs (LA7-1, IL7-4-1, IL7-5, IL7-5-5, IL11-1, IL11-2, IL12-2 and IL12-3) contributed to salt tolerance in both stages. No *S. lycopersicoides* ILs contributed to salt tolerance in both stages though some ILs (LA4238, LA4239 and LA4249) were more tolerant to salt during the seed germination stage.

Phenotypic variation caused by QTLs is similar to variation for simple Mendelian inherited loci (Tanksley and Fulton 2007). Heterozygous and double introgression lines with

the QTLs (*Stpq2*, *Stpq6* and *Stpq7*) have proved that all three QTLs were considered as dominant and the interaction between QTLs was less-than-additive. The dominant nature of QTLs for salt tolerance in the seedling stage is positive for tomato hybrid breeding. Many evidences have been documented to know the mechanism of salt tolerance and quantitative and qualitative analysis of the differentially expressed proteins of tomato under salt stress is an important step towards further elucidation of mechanisms of salt stress resistance (Chen et al. 2009).

Co-evolution of QTLs for abiotic and biotic resistance

In total, we identified five QTLs for resistance to *P. infestans*, twenty three QTLs to *B. cinerea* and fifteen for salt tolerance. We put these QTLs conferring biotic and abiotic stress on the tomato map together with other previously identified QTLs. It can be seen that most of these QTLs are located close to the telomeres and further away from the centromere. Most of these QTLs also co-localized with qualitative loci. A coordination of plant responses to pathogens and abiotic stresses, including the expression of overlapping sets of genes in response to infection and abiotic stresses, has been suggested (AbuQamar et al. 2009;Fujita et al. 2006;Synan AbuQamar 2009). The plant hormones ethylene (ET), salicylate (SA), jasmonate (JA) and abscisic acid (ABA) act synergistically or antagonistically to regulate plant responses to pathogens and abiotic stress factors. Abscisic acid regulates the plant response to drought, low temperature and osmotic stress. Recently, ABA has emerged as a positive or negative regulator of disease resistance, depending on the nature of the host–pathogen interaction (Anderson et al. 2004;Lorenzo et al. 2004;Mauch-Mani and Mauch 2005). ABA deficiency in tomato and impaired ABA responses in Arabidopsis result in increased resistance to *B. cinerea*, and other necrotrophic pathogens, as a result of the reduced ABA signaling but increased JA- or ET responsive gene expression (Anderson et al. 2004). An abscisic acid-induced myb1 (SlAIM1) gene encoding an R2R3MYB transcription factor has been proved to be induced by pathogens, plant hormones, salinity and oxidative stress (AbuQamar et al. 2009). The results from this thesis add to a clustering on chromosomes 6, 7, 9, 11 and 12. For example, genes *Mi-3*, *Gpa2*, *Rx* and some QTLs *Rbchq12*, *Stpq12a*, *Stlq12a*, *Rbchq12* are all co-localized on chromosome 12 and might be involved in the coordinating pathways for abiotic and biotic stress. In addition, specific loci exist for specific pathways involved in either abiotic or biotic resistance. The results from this thesis add to a clustering of genes involved in biotic stress on chromosomes 1, 3, 4, 5, 7, 8 and 10. For example, *Py-1*, *Gro1.4 Pi_QTL*, *Rbclq3a*, *Rbclq3b*, *Rbchq3* and A-17 are clustered on chromosome 3 and they are involved only in resistance to different biotic stresses.

Combination for abiotic and biotic tolerance for tomato breeding

The exploration of wild species has been proven to be an effective strategy to overcome the small gene pool in cultivated tomato. Abiotic stress tolerance in crops, is mainly controlled by QTLs that behave in a quantitative manner. In the last decade, DNA based tools have become indispensable for combining genetic factors, controlling abiotic and biotic stress, in cultivars. A combination of three fragments from *S. pennellii* for drought tolerance have produced a tomato with a good yield in a dry climate (Gur and Zamir 2004). In this paper, forty four QTLs were identified in three IL populations. As indicated in Chapter 1, QTLs in ILs have several advantages for tomato improvement. Firstly, the genetic background is based on an elite line. Secondly the genome fragment from the wild species is flanked by known markers. Once QTLs are identified they can be used directly for introgression by MAS. Thirdly, some QTLs may be co-localized or some loci might simultaneously control both an abiotic as well as a biotic tolerance. Hence once a fragment is introgressed into an inbred line it possesses multiple characteristics. Fourth, if there is linkage drag, it can more easily be manipulated in ILs because enough marker information is known to make smaller introgressions. For example, QTL *Stpq*6 showed a potential for salt tolerance in Chapter 3 but is probably linked to a poor yield production. An obstacle might be that co-localized loci come from different species and that low recombination frequencies make it difficult or impossible to combine the loci in one genotype. Especially if recessive genes or more than two genes are involved in the same region. One example of this: both *Pto* and *Rx3* are located on chromosome 5 and originate from different species. Both have been successfully incorporated in one genotype via MAS (Yang and Francis 2005). Once the combination has succeeded it will be convenient to select for in a breeding program due to the tight linkage. However, QTL pyramiding is not always optimal since effects don't have to be additive. Epistasis seems to play a key role as seen in tomato yield (Eshed and Zamir 1995) and the less-than-additive effect for salt tolerance in Chapter 3. Interaction between QTLs and the environment might reduce QTL effects as observed in tomato with antagonistic epistasis for specific leaf area and time to wilting under drought stress (Christopher and Leonie 2009) and for *B. cinerea* resistance in chapter 4. Although many QTLs have been identified in tomato as listed in Chapter 1, only the most important ones have been used even though marker assisted selection (MAS) provides an effective tool. It is still a challenge for effective introgression of dozens of QTLs at the same time.

Future research

In this thesis, we identified QTLs for several abiotic and biotic stress using three IL populations derived from *S. habrochaites*, *S. pennellii* and *S. lycopersicoides*, which are distantly related wild species. These wild species also have other beneficial characteristics due to the evolution after adaptation to various environments. For example, accessions of *S. habrochaites* and *S. lycopersicoides* also showed tolerance to cold, the virus CMV and early blight resistance as shown in Chapter 5 and other studies (Zhao et al. 2005). *S. habrochaites*, *S. pennellii* and *S. lycopersicoides* also possess the potential for tolerance to several insects. Hence these IL populations can be and are used for the identification of QTLs responsible for other stresses.

All QTLs in this thesis were identified by replicated independent experiments. The effect of single QTLs and the genetic stability still need to be studied in the progeny. QTLs with large effects should be confirmed in segregating recombinant populations or in heterozygous ILs. Heterozygous ILs provide a convenient way to predict the genetic effect. Recently, 105 double-introgression lines (DILs) derived from *S. habrochaites* IL population have been used to explore ecophysiological traits differences between *S. habrochaites* and *S. lycopersicum* (Christopher and Leonie 2009). Double-introgression lines from different species can be used to understand the interaction of QTLs between these different wild species. Qualitative genes can be introgressed into the same genetic background as IL populations, and the interaction between qualitative and quantitative traits can be further studied. For example, several qualitative genes (*Ph-2*, *Ph-3*, *Ph-4* and *Ph-5*) for resistance to *P. infestans* might be introgressed into the genetic background E6203 and/or M82 and these established NILs will be available for the study of qualitative and quantitative interaction.

Our results provide material for comparative study of stress tolerance between species together with other accumulated data in the future. For example, metabolite data of *S. habrochaites* and *S. pennellii* IL population are available (http://tomet.bti.cornell.edu/), and it provides a tool for further understanding of the mechanism of stress tolerance. Also the tomato sequence project will be finished soon providing another tool for genetic studies in tomato and especially of the QTLs identified in this study.
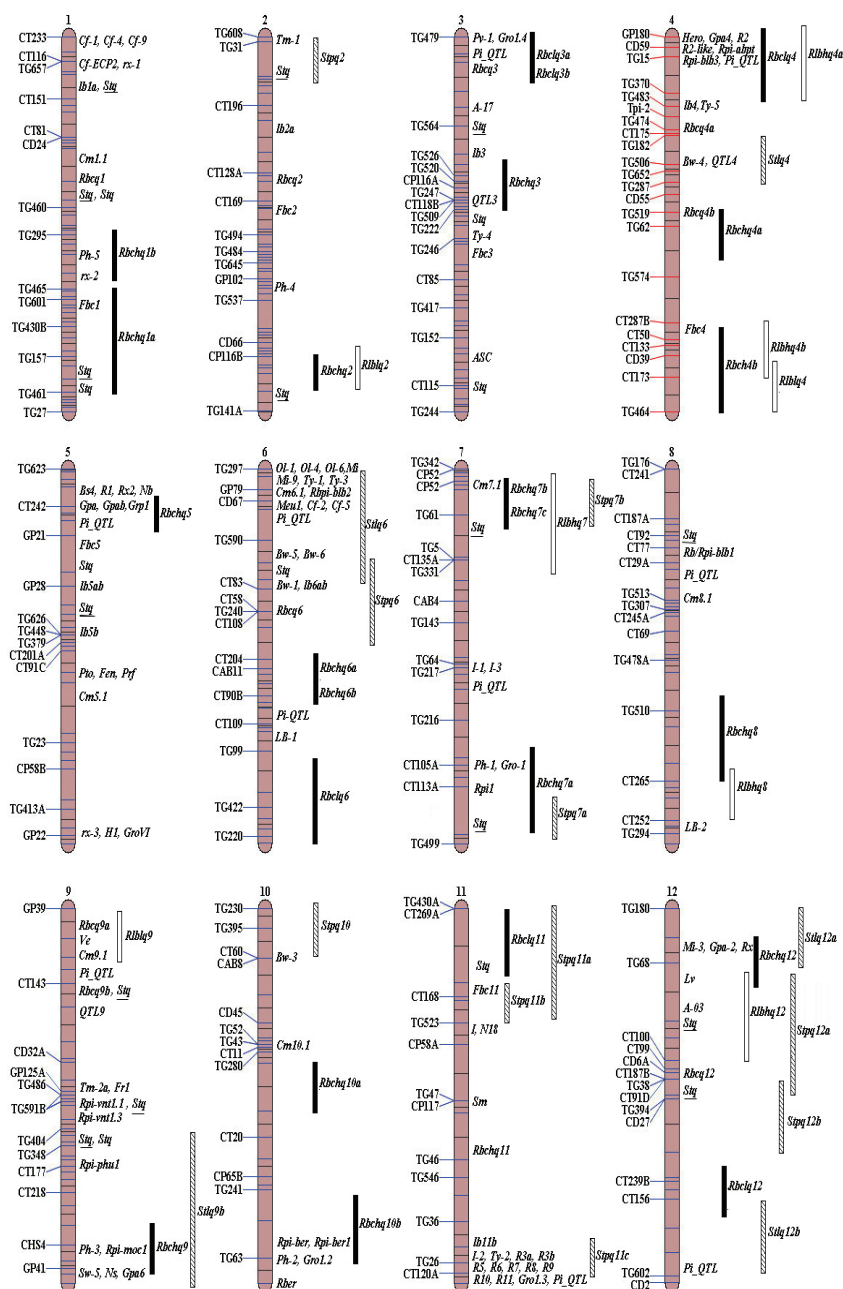
Fig. 1 Integrated QTL map for resistance to *P. infestans*, *B. cinerea* and *salt tolerance*. Tomato map was based on Tomato-EXPEN 2000 (http://solgenomics.net/). Solid vertical bars indicated the approximate locations of QTLs (*Rbchq1b, Rbchq1a, Rbchq2, Rbclq3a, Rbclq3b, Rbchq3, Rbclq4, Rbchq4a, Rbchq4b,*

Rbchq5, Rbchq6a, Rbchq6b, Rbclq6, Rbchq7a, Rbchq7b, Rbchq7c, Rbchq8, Rbchq9, Rbchq10a, Rbchq10b, Rbclq11, Rbclq12) for resistance to gray mold (*B. cinerea*). Hollow vertical bars indicated the approximate locations of QTLs (*Rlblq2, Rlbhq4a, Rlbhq4b, Rlblq4, Rlbhq7, Rlbhq8, Rlblq9 and Rlblq12*) for resistance to tomato late blight (*P. infestans*). Diagonal vertical bars indicated the approximate location s of QTLs (*Stpq2, Stlq4, Stlq6, Stpq6, Stpq7a, Stpq7b, Stlq9b, Stpq10, Stpq11a, Stpq11b,Stpq11c, Stpq12a, Stpq12b, Stpl12a and Stpl12b*) for salt tolerance at the seedling stage. Following references assign remaining resistance loci in tomato: *Asc*, resistance to stem canker (*Alternaria alternata*) (van der Biezen et al. 1995); *Bw* (*Bw-1, Bw-3, Bw-4, Bw-5, Bw-6*), QTLs for resistance to bacterial wilt (*Ralstonia solanacearum*)(Mangin et al. 1999); *Cf* (*Cf-1, Cf-2, Cf-4, Cf-5, Cf-9, Cf-ECP2*), resistance to leaf mould (*Cladosporium fulvum*)(Haanstra et al. 1999;Thomas et al. 1998); *Cm* (*Cm1.1, Cm5.1, Cm6.1, Cm7.1, Cm8.1, Cm9.1, Cm10.1*), QTLs for resistance to bacterial canker (*Clavibacter michiganensis*) (Sandbrink et al. 1995); *Fen*, sensitivity to fenthion(Martin et al. 1994); *Fbc* (*Fbc1, Fbc2, Fbc3, Fbc4, Fbc5 and Fbc11*), leaf resistance or susceptibility to gray mold (*Botrytis cinerea*)(Davis et al. 2009); *Fr1*, resistance to Fusarium wilt (*Fusarium oxysporum* f.sp. *radicis-lycopersici*)(Vakalounakis et al. 1997); *Hero*, resistance to potato cyst namatode (*Globodera rostochiensis*) (Ganal et al. 1995); *I* (*I-1, I-2, I-3*), resistance to different races of Fusarium wild (*Fusarium oxysporum* f.sp. *lycopersici*)(Ori et al. 1997); *LB-1,LB-2 and lb* (*lb1a, lb2a, lb3, lb4, lb5ab, lb5b, lb6ab and lb11b*), QTLs for resistance to tomato late blight (*P. infestans*) (Brouwer et al. 2004;Farary et al. 1998); *Lv*, resistance to powdery mildew (*Leveuillula taurica*)(Chunwongse et al. 1994); *Mi, Mi-3*and *Mi-9*, resistance to root knot nematodes (*Meloidogyne* spp.) (Veremis et al. 1999;Yaghoobi et al. 1995); *N18*, resistance to tobacco mosaic virus (Whitham et al. 1994); *OI-1, OI-4 and OI-6*, resistance to powdery mildew (*Oidium lycopersicum*) (Vanderbeek et al. 1994); *Ph* (*Ph-1, Ph-2, Ph-3, Ph-4 and Ph-5*), resistance to late blight (*Phytophthora infestans*) in tomato(Chunwongse et al. 2002;Conover and Walter 1953;Foolad et al. 2006;Labate et al. 2007;Moreau et al. 1998); *Pto* and *Prf*, resistance to bacterial speck (*Pseudomonase syringae* pv. *tomato*)(Martin et al. 1993;Salmeron et al. 1996); *Py-1*, resistance to corky root rot (*Pyrenochaeta lycopersici*)(Doganlar et al. 1998); *QTL* (*QTL3, QTL4 and QTL9*), stem resistance to gray mold (*Botrytis cinerea*)(Finkers et al. 2008); *rx* (*rx-1, rx-2, rx-3*), resistance to bacterial spot (*Xanthomonas campestris*) (Yu et al. 1995); *Rbcq* (*Rbcq1, Rbcq2, Rbcq3, Rbcq4a, Rbcq4b, Rbcq6, Rbcq9a, Rbcq9b, Rbcq11, Rbcq12*), QTLs for stem resistance to gray mold (*Botrytis cinerea*)(Finkers et al. 2007a;Finkers et al. 2007b); *Sm*, resistance to *Stemphilium* (Behare et al. 1991); *Sw-5*, resistance to tomato spotted wilt virus (Stevens et al. 1995); *Stpq* with the under line, QTLs tolerance to salt during seed germination; *Stpq* without the under line, QTLs for tolerance to salt during the vegetative stage(Foolad 2004); *Tm-1* and *Tm-2a*, resistance to tobacco mosaic virus(Tanksley et al. 1989); *Ty-1, Ty-2 Ty-3, Ty-4 and Ty-5*, resistance to yellow leaf curl virus (Anbinder et al. 2009;Hanson et al. 2006;Ji et al. 2007;Ji et al. 2009;Zamir et al. 1994); *Ve*, resistance to *Verticillium dahliae*(Diwan et al. 1999). R (*R1, R2, R2-like, R3, R4, R5, R6, R7, R8, R9, R10* and *R11*)and QTL (*Pi_QTL, Rpi-abpt, Rpi-blb3, Rpi1, RB/Rpi-blb1, Rpi-mcq1* and *Rber*) for resistance to late blight (*P. infestans*) in potato (Hein et al. 2009;Park et al. 2009). Other genes or QTLs (*Gro4, Bs4, Nb, Gpa, Gpab, Grp1, H1, GroVI, Ns, Gpa6, Gro1.2, Gpa-2 and A-03*) to different pathogens in potato and tomato (Park et al. 2009).

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SUMMARY

Tomatoes are grown world-wide as a fruit vegetable crop. Abiotic and biotic stresses are the main factors to restrict production, and these factors are becoming more and more serious due to human activities. The history of tomato breeding has resulted in a narrow genetic basis and the loss of many valuable genes for resistance or tolerance respectively to abiotic and biotic stresses. Wild tomato species are still adapted to different climates and abiotic and biotic stresses and therefore provide a rich resource for resistance/tolerance to abiotic and biotic stresses. Introgression line (IL) populations provide several advantages over other populations for searching quantitative trait loci (QTL). In this thesis, three introgression line (IL) populations derived from *S. habrochaites*, *S. pennellii* and *S. lycopersicoides* were employed to explore the potential for tolerance to biotic and abiotic stresses.

Among these biotic stresses, both *B. cinerea* and *P. infestans* can have devastating effects by destroying entire crops. Quantitative resistance to *B. cinerea* in wild relatives of tomato (Egashira et al. 2000;Guimarães et al. 2004;Nicot and Moretti 2002;ten Have et al. 2007) has been described, and a QTL study for leaf and stem resistance has been performed in *S. lycopersicoides* LA2951, *S. habrochaites* LYC4 and *S. neorickii* G1.1601 (Davis et al. 2009;Finkers et al. 2007). In Chapter 2 twenty two wild accessions were screened for tolerance to *B. cinerea* and three accessions, *S. lycopersicoides* LA2951, *S. habrochaites* PI134417 and LA1392, were more resistant to our isolate of B. cinerea which was collected in a greenhouse in China. One introgression line population based on *S. habrochaites* LA1777 was used for further analysis due to the availability of this population, although LA1777 had a little less reduction of Lesion Size (LS) and Disease Incidence (DI) compared to S. habrochaites PI134417 and LA1392. The other IL population that was used to identify QTLs for leaf, stem, green fruit and ripe fruit resistance to *B. cinerea* was *S. lycopersicoides* LA2951. Three QTLs were identified from *S. habrochaites* LA1777, which are located on chromosome 1 and 8, and respectively reduce lesion size (LS) on leaves and disease incidence (DI) on stems. Three QTLs, for smaller LS and DI on leaves, were identified from *S. lycopersicoides* LA2951. The evidence from *S. lycopersicoides* LA2951 IL lines hinted

that tomato leaf might present isolate specific resistance to different isolates as found in Arabidopsis (Katherine et al. 2004;Rowe and Kliebenstein 2008) since the loci were not co-localized with the previously identified QTLs (Davis, 2009). Furthermore, many QTLs were found for ripe fruit resistance in the *S. habrochaites* LA1777 IL population. Twelve QTLs are responsible for reducing LS and three QTLs for lower DI. Three QTLs located on chromosome 3, 6 and 12 were identified in the *S. lycopersicoides* LA2951 IL population and all of them are associated with reduced LS on ripe fruit. We showed that loci derived from wild species *S. habrochaites* LA1777 increase the susceptibility of green fruits and that loci from *S. lycopersicoides* LA2951 decrease the susceptibility in green fruits. Ripening-regulated susceptibility of tomato fruit to *B. cinerea* without ethylene requirement (Cantu et al. 2009) might hint to different infection mechanisms in leaf and fruit. A few QTLs responsible for resistance in different tissues are possibly co-localized. This might be the reason for the low correlation of resistance levels between the different tissues (Nicot and Moretti 2002;ten Have et al. 2007). This co-localization will make tomato breeding for complete resistance to *B. cinerea* more complicated.

Qualitative resistance to *P. infestans* from *S. pimpinellifolium* and quantitative resistance from *S. habrochaites* LA2099 and *S. pennellii* LA716 has been described (Brouwer et al. 2004;Brouwer and St Clair 2004;Chunwongse et al. 2002;Moreau et al. 1998;Smart et al. 2007). In Chapter 3, *S. habrochaites* LA1777 had a good level of resistance to several isolates of *P. infestans*. Five introgression lines showed unambiguous higher levels of resistance. All QTLs co-localize with previously described QTLs from *S. habrochaites* LA2099 except QTL *Rlbq*4b. The introgression line population based on the related nightshade *S. lycopersicoides* LA2951 was screened and three QTLs (*Rlblq*2, *Rlblq*4 and *Rlblq*9) for resistance to *P. infestans* were found.

Among abiotic stresses, salinity is becoming more and more a constraint in many crops. In this study, both *S. pennellii* LA716 and *S. lycopersicoides* LA2951 IL populations were used to identify QTLs for salt tolerance in the seedling stage. Tomato seedlings are more vulnerable to salinity as plants in the stage of flowering and fruit setting. Ten QTLs were identified from the wild species *S. pennellii* LA716 and five QTLs were identified in the *S. lycopersicoides* LA2951 (Chapter 4). A pilot indicated that some QTLs might be semi-dominant. The interaction of QTLs for salt tolerance showed a clear non-additive or epistatic effect concerning yield (Eshed and Zamir 1996b). Some of our QTLs co-localize with previously identified loci from *S. pimpinellifolium* (Foolad 2004) and the QTL localized on chromosome 6 is possibly conserved in three tomato species.

Tomato high density maps make it possible to compare accurately map positions of QTL/genes on tomato chromosomes. In total, we identified forty four QTLs for abiotic and biotic stresses in this thesis (Chapters 2, 3 and 4). Most QTLs co-localized and this gives some indication that cross talk between coordinating pathways for abiotic and biotic stress might play a role. Other QTLs are more specific.

In general, low to mid correlations between experiments were observed. This shows that large variation existed between the individual disease or stress assays (Chapters 2, 3 and 4). Differences in interaction between the identified loci and environments might be present and single experiments are not enough to identify all QTLs and replicated experiments are necessary to come to valid conclusions. IL populations provide a convenient tool for replicated trait evaluation. It also can be easily used to test the interaction of QTLs.

The results from this thesis provide the basis to combine QTLs with tolerance to abiotic and biotic stresses and for further narrowing down the introgression size. Some introgressions from these wild relatives are involved in tolerance to multiple stresses and are therefore very interesting for tomato breeders.

中文摘要

番茄作为鲜果蔬菜作物广泛栽培世界于各地。生物及非生物胁迫是限制番茄生产的主要因素，而且由于人类频繁活动，致使这些因素的危害也日益加重。番茄育种的历史原因，也导致其遗传背景变窄，从而丧失了许多抗或耐生物和非生物胁迫的有用基因。野生番茄一直生活在不同的气候环境条件下，需要应对各种生物及非生物胁迫，因此为抗或耐生物和非生物胁迫提供了丰富的资源材料。在挖掘数量性状位点(QTL)方面，相对于其他不同类型的群体，渐渗系(Introgression line, IL)群体展示了多个方面的优势。本论文，利用来自多毛番茄 *S. habrochaites*、潘那利番茄 *S. pennellii* 和类番茄茄 *S. lycopersicoides* 的三个渐渗系群体，开发了它们抗生物和非生物胁迫的潜力。

在这些生物胁迫因素中，番茄灰霉病(*B. cinerea*)和晚疫病(*P. infestans*)可以摧毁整个番茄作物，导致毁灭性灾害。番茄野生近缘种对灰霉病的数量抗性已有报道(Egashira et al. 2000;Guimarães et al. 2004;Nicot and Moretti 2002;ten Have et al. 2007)，而且分别从 *S. lycopersicoides* LA2951, *S. habrochaites* LYC4 和 *S. neorickii* G1.1601 开发了叶部及茎部抗灰霉病的 QTLs。在第二章，22 个野生种番茄用于筛选叶部抗灰霉病，其中 *S. lycopersicoides* LA2951, *S. habrochaites* PI134417 和 LA1329 等 3 份材料表现较好地抗从中国（北京）温室收集的一个分离小种。相对于多毛番茄 *S. habrochaites* PI134417 和 LA1329，LA1777 表现略为减少的病斑大小(Lesion Size, LS)和发病率(Disease Incidence, DI)，基于该野生种开发的渐渗系群体，本论文对其抗性进行了进一步的分析；而另外一个用于鉴定叶部、茎部、绿熟果实及红熟果实的渐渗系群体是来自类番茄茄 *S. lycopersicoides* LA2951。从 *S.*

habrochaites LA1777 群体，鉴定出 3 个 QTL，它们位于番茄第 1 和第 8 条染色体上，分别可以减少叶部病斑面积和茎部的发病率。从 *S. lycopersicoides* LA2951 群体，鉴定出 3 个 QTL，它们可分别减少叶部病斑面积和发病率。正如拟南芥研究报道(Katherine et al. 2004;Rowe and Kliebenstein 2008)，来自 *S. lycopersicoides* LA2951 结果暗示了番茄叶部抗灰霉病也可能存在小种特异抗性，因为本研究鉴定出的位点与前人研究结果不同位(Davis, 2009)。从 *S. habrochaites* LA1777 渐渗系群体鉴定出系列成熟果实抗灰霉病的 QTL，包括 12 个 QTL 可有效减少成熟果实的病斑面积，3 个 QTL 降低发病率；从 *S. lycopersicoides* LA2951 渐渗系群体鉴定出分别位于第 3、6 和 12 染色体上的 3 个 QTL，这些 QTL 均可有效减少成熟果实病斑面积。来自 *S. habrochaites* LA1777 的位点提高绿熟果实的感病性，而来自 *S. lycopersicoides* LA2951 的位点减少感病性。成熟果实抗灰霉病的抗性不需要乙烯的参与(Cantu et al. 2009)，暗示了灰霉病病菌对叶部和果实的侵染可能存在不同的机制。在不同组织抗病 QTL 中，只有很少的 QTL 同位，这可能是导致不同组织抗性水平相关性低的原因(Nicot and Moretti 2002;ten Have et al. 2007)，这也使番茄抗灰霉病育种变得更为复杂。

醋栗番茄 *S. pimpinellifolium*、多毛番茄 *S. habrochaites* LA2099 及潘那利番茄 *S. pennellii*LA716 业已报道分别对晚疫病具有质量和数量抗性 (Brouwer et al. 2004;Brouwer and St Clair 2004;Chunwongse et al. 2002;Moreau et al. 1998;Smart et al. 2007)。在第三章，多毛番茄 *S. habrochaites* LA1777 表现对不同几个生理小种较好的抗性。5 个来自 LA1777 群体的渐渗系呈现较高水平的抗性。除 QTL *Rlbq*4b 外，其余 QTL 均与前人报道的来自 *S. habrochaites* LA2099 的 QTL 同位。另外，利用来自茄属类植物 *S. lycopersicoides* LA2951 的渐渗系群体，鉴定出 3 个抗晚疫病的 QTL (*Rlbq*2，*Rblq*4 和 *Rlbq*9).

在非生物胁迫因子中，盐害已成为限制许多作物生长的因素。本研究利用来自潘那利 *S. pennellii* LA716 和类番茄茄 *S. lycopersicoides* LA2951 的两个渐渗系群体，鉴定了苗期耐盐 QTL，因为番茄苗期相对与开花坐果期更容易受到盐害的威

胁。从野生潘那利 *S. pennellii* LA716 群体鉴定出 10 个 QTL，从类番茄茄 *S. lycopersicoides* LA2951 群体鉴定出 5 个 QTL(第四章)。初步试验指出，一些苗期耐盐 QTLs 呈现半显性遗传；耐盐 QTL 互作如影响产量的 QTL 一样，呈现非加性或者上位效应 (Less than additive effect) (Eshed and Zamir 1996b)。一些 QTL 与前人鉴定的来自醋栗番茄 *S. pimpinellifolium* 的位点同位 (Foolad 2004)，而且位于第六条染色体上的位点在番茄作物中可能是保守的。

番茄高密度遗传连锁图谱为在番茄染色体上准确比较已定位的 QTL 或基因提供了可能。本论文，总共鉴定出 43 个抗生物或非生物胁迫的 QTLs (第二、三和四章)。大多数 QTL 同位显示了交叉对话(cross talk)在协调生物胁迫和非生物胁迫中可能扮演重要角色，而另外一些 QTL 则是特异性的。

通常而言，试验间的相关性呈现低或中等水平的相关性，这一现象表明在抗病或耐胁迫分析中存在较大的差异(第二、三和四章)。这些差异可能是 QTL 与环境互作的结果。单个试验不足以鉴定全部 QTL，重复试验是获得有效数据结果所必需的，而渐渗系群体为重复多点试验提供了方便，也为研究 QTL 互作提供了可能。

本论文结果为番茄抗或耐生物和非生物胁迫 QTL 聚合及进一步缩小渐渗系片段的大小奠定了基础。一些来自野生种的渐渗系表现抗或耐复合胁迫，这些品系将会引发番茄育种者极大的兴趣。

Samenvatting

De tomatenteelt is wereldwijd van grote economische betekenis. Abiotische en biotische stress zijn de belangrijkste factoren die het productieniveau bedreigen en deze factoren nemen in belangrijkheid toe. De geschiedenis van de tomatenveredeling heeft geresulteerd in een smalle genetische basis van het veredelingsmateriaal en daardoor een verlies van vele waardevolle genen die resistentie of tolerantie tegen abiotische en biotische stress zouden kunnen bewerkstelligen. Tomatensoorten, die nog in het wild voorkomen, zijn in verschillende klimaten aangepast aan abiotische en biotische stress en zijn daarom een rijk reservoir van eigenschappen die zorgen voor resistentie/tolerantie tegen abiotische en biotisch stress. Introgressielijn (IL) populaties bieden verscheidene voordelen bij het zoeken naar chromosoomgebieden (quantitative trait loci of QTLs) in de wilde verwanten die de effecten van abiotische en biotische stress verminderen of zelfs te niet doen. In dit proefschrift zijn drie introgressielijnpopulaties gebruikt om zulke chromosoomgebieden te zoeken. De populaties zijn gebaseerd op accessies van drie wilde verwanten van tomaat: *Solanum habrochaites*, *Solanum pennellii* en *Solanum lycopersicoides*.

Zowel *Botrytis cinerea* als *Phythophtora infestans* kunnen grote productieverliezen veroorzaken en zelfs de oorzaak zijn dat de complete productie in een bepaalde teelt verloren gaat. In wilde verwanten van tomaat heeft onderzoek laten zien dat er kwantitatieve resistentie tegen *B. cinerea* aanwezig is (Egashira et al. 2000;Guimarães et al. 2004;Nicot and Moretti 2002;ten Have et al. 2007). Er zijn QTL studies voor het vinden van blad en stengel resistentie uitgevoerd met accessies van wilde verwanten: *S. lycopersicoides* LA2951, *S. habrochaites* LYC4 en *S. neorickii* G1.1601 (Davis et al. 2009;Finkers et al. 2007). In hoofdstuk 2 van dit proefschrift zijn 22 accessies van wilde verwanten getest op tolerantie tegen *B. cinerea*. Drie van deze accessies, *S. lycopersicoides* LA2951, *S. habrochaites* PI134417 en *S. habrochaites* LA1392, gaven tolerantie tegen het door ons gebruikte, uit China afkomstige, isolaat van *B. cinerea*. Omdat van geen van deze drie accessies een introgressielijnpopulatie bestaat hebben we de introgressielijnpopulatie gebaseerd op de accessie *S. habrochaites* LA1777 gebruikt voor het identificeren van QTLs. De reductie in

lesiegrootte (lesion Ssze, LS) en in het percentage doorzettende infecties (disease incidence, DI) was in *S. habrochaites* LA1777 maar iets minder dan in *S. habrochaites* PI134417 and LA1392. Naast bovengenoemde introgressielijnpopulatie is ook een dergelijke populatie gebaseerd op *S. lycopersicoides* LA2951 gebruikt om QTLs tegen *B. cinerea* te vinden in blad, stengel, groene vruchten en rijpe vruchten. Twee QTLs zijn geidentificeerd afkomstig uit *S. habrochaites* LA1777, deze zijn gelocaliseerd op Chromosoom 1 en 8 en zorgen voor een verminderde lesiegrootte (LS) op bladeren en een lager aantal doorzettende infecties (DI) op stengels. Drie QTLs voor kleinere lesiegrootte of doorzettende infecties op bladeren zijn gevonden in de populatie gebaseerd op *S. lycopersicoides* LA2951. Omdat deze QTLs niet co-localiseren met andere beschreven QTLs (Davis, 2009) denken we dat de tolerantie in de bladeren isolaat specifiek is. Een dergelijk resultaat was al eerder beschreven in *Arabidopsis thaliana* (zandraket; (Katherine et al. 2004;Rowe and Kliebenstein 2008). Ook zijn er veel QTLs (twaalf voor een reductie in lesiegrootte en drie voor een lager aantal doorzettende infecties) gevonden voor tolerantie in rijpe vruchten in de *S. habrochaites* LA1777 IL populatie. In deze populatie zijn drie QTLs voor een verminderde lesiegrootte gevonden en wel op Chromosoom 3, 6 en 12. In dit proefschrift tonen we aan dat chromosoomgebieden uit *S. habrochaites* LA1777 de gevoeligheid van groene vruchten voor *B. cinerea* verhogen en dat chromosoomgebieden afkomstig uit *S. lycopersicoides* LA2951 de gevoeligheid voor *B. cinerea* in groene vruchten verlagen. Cantu et al. (2009) beschreven dat de rijpheidsafhankelijke *B. cinerea* gevoeligheid van tomatenvruchten duidt op verschillen in infectiemechanisme in bladeren en vruchten. Enkele introgressies zijn verantwoordelijk voor zowel resistentie in het ene weefsel als in het andere (co-localisatie). Dit is waarschijnlijk de reden voor de lage corellatie tussen resistentienivo's in verschillende weefsels (Nicot and Moretti 2002;ten Have et al. 2007). Dit en de co-localisatie maken het veredelen voor complete resistentie tegen *B. cinerea* complex.

Kwalitatieve resistentie tegen *P. infestans* afkomstig van *S. pimpinellifolium* en kwalitatieve resistentie afkomstig van *S. habrochaites* LA2099 en *S. pennellii* LA716 is al eerder beschreven (Brouwer et al. 2004;Brouwer and St Clair 2004;Chunwongse et al. 2002;Moreau et al. 1998;Smart et al. 2007). In hoofdstuk 3 tonen we aan dat *S. habrochaites* LA1777 een hoog niveau van resistentie heeft tegen verschillende isolaten van *P. infestans*. Vijf introgressielijnen hadden zonder twijfel hogere resistentieniveau's en de chromosoomlocaties van vier van de vijf komen overeen met eerder beschreven QTLs van *S. habrochaites* LA2099, de uitzondering is QTL *Rlbq4b*. In de populatie gebaseerd op *S. lycopersicoides* LA2951 zijn drie QTLs (*Rlblq2*, *Rlblq4* and *Rlblq9*) voor resistentie tegen *P. infestans* aangetoond.

Naast de biotische bedreigingen wordt abiotische stress, zoals de gevoeligheid voor hogere zoutconcentraties in de bodem, meer en meer een probleeem voor veel gewassen en ook voor tomaat. In dit proefschrift zijn *S. pennellii* LA716 en *S. lycopersicoides* LA2951 IL populaties gebruikt om QTLs te identificeren in het zaailingstadium. Tomatenzaailingen zijn gevoeliger voor verhoogde zoutniveau's dan volwassen planten die bloeien en vruchten maken. In hoofdstuk 4 worden tien QTLs geidentificeerd in *S. pennellii* LA716 en vijf in *S. lycopersicoides* LA2951 die verantwoordelijk zijn voor een een hoger niveau van zouttolerantie. Het in één plant samenbrengen van verschillende QTLs toonde aan dat sommige QTLs volledig dominant kunnen zijn. De interactie van zouttolerantie QTLs laten voor opbrengst een minder effect zien dan de som van de individuele effecten (Eshed and Zamir 1996b). Sommige van onze QTLs co-localiseren met eerder geidentificeerde QTLs in *S. pimpinellifolium* (Foolad 2004) en de QTL op Chromosoom 6 is mogelijk geconserveerd omdat deze in drie verschillende tomatensoorten gevonden wordt.

Genetische kaarten van tomaat maken het mogelijk kaartposities van QTLs of genen op de tomatenchromosomen te vergelijken. In totaal, hebben we 43 QTLs geidentificeerd in dit proefschrift (Hoofdstukken 2, 3 en 4). Vele QTLs co-localiseren en dit geeft misschien een indicatie dat dezelfde metabolische routes een rol spelen bij zowel tolerantie tegen abiotische als biotische stress. Andere QTLs zijn meer specifiek.

Er werden maar lage tot middelmatige correlaties tussen de experimenten gevonden. Dit bevestigt de grote variatie tussen de individuele ziekte- of stressbepalingen. (Hoofdstukken 2, 3 en 4). Er zijn mogelijk verschillen in de interactie van de door ons geidentificeerde loci en de omgeving. Daarom zijn losstaande, enkele experimenten niet voldoende om alle QTLs te identificeren en herhalingen zijn nodig om goede conclusies te kunnen trekken. IL populaties bieden de mogelijkheid meerdere herhalingen met veel planten uit te voeren en zijn ook het startmateriaal om de interactie tussen QTLs te onderzoeken. De resultaten van dit proefschrift vormen de basis voor het combineren van QTLs met tolerantie tegen abiotische and biotische stress en voor het verkleinen van de grootte van de introgressies. Sommige introgressies van de wilde verwanten zijn betrokken bij tolerantie tegen meerdere bedreigingen en zijn daardoor erg interessant voor tomatenveredelaars.

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I would like to deeply thank my daily supervisor, director **Du Yongchen.** You provided me a great gift so that I could go to Wageningen University to be a PhD study and continue to finish my PhD research. You do not realize how important your support was to me in obtaining the scholarship from China Scholarship Council. Without it I could not have the chance to finish my PhD study after I moved to the Laboratory of Plant Breeding. I also would like to say many thanks to many staff members working in my institute as they gave me much help whenever I stayed in the institute or abroad. I also gratefully acknowledge the help of **Dr. Wang Xiaowu** for setting up the experiment and critical comments for research and **Prof. Liu Guanfshu** for arranging many things with foreign affairs.

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Curriculum Vitae

Junming Li was born on October 1st, 1968 in Inner Mongolia municipality, China. He obtained his BSc as a Horticultural major in Inner Mongolia Agriculture University in September 1990 and MSc in Shenyang Agriculture University in September 1993.

From 1993 till now, he is involved in the team of breeding processing tomatoes in the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences. As a visiting scholar from 1999 to 2000, he worked on potato and tomato subjects for one year supervised by Dr. Barone Amalia in Research Institute for Vegetable and Ornamental Plant Breeding, National Research Council, IMOF, Portici, Italy.

From 2003 to 2009, he joined the Sandwich PhD program in the Laboratory of Plant Breeding, Graduate School of Experimental Plant Sciences (EPS) of Wageningen University and the Graduate School of Chinese Academy of Agricultural Sciences (CAAS). This thesis is sponsored by the Royal Dutch Academy of Sciences (KNAW) and the Asian Facility (project AF01/CH/8 Sino-Dutch Genomic Lab and Vegetable Research Center), Hi-Tech Research and Development Program (2006AA10Z1A6), Key Laboratory of Horticultural Crops Genetic Improvement (China) and Ministry of Agriculture and National Basic Research and Development Program (2009CB119000).
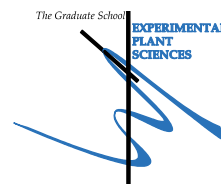
List of publications

Li J, Liu L**,** Bai Y**,** Finkers R**,** Du Y**,** Yang Y, Xie B, Visser RGF**,** van Heusden AW Identification of QTLs for resistance to *Botrytis cinerea* using *S. habrochaites* LA1777 and *S. lycopersicoides* LA2951 introgression line populations. Euphytica (To be submitted)

Li J, Liu L**,** Bai Y**,** Zhang P**,** Finkers R**,** Du Y**,** Visser RGF**,** van Heusden AW Investigating seedling salt tolerance in two tomato introgression libraries**.** Euphytica (To be submitted)

Li J, Liu L, Bai Y, Finkers R, Wang F, Du Y, Yang Y, Xie B, Visser RGF, van Heusden AW (2009) Identification and mapping of quantitative resistance to late blight (*Phytophthora infestans*) in *Solanum habrochaites* LA1777. Euphytica (Submitted)

Sun W, Xu X, Liu A, Zhu H, Liu L, **Li J**, Hua X (2009) Comparative transcriptomic profiling of salt tolerant wild tomato species and salt sensitive tomato cultivar. Plant and Cell Physiology (submitted)

Ercolano MR, Carputo D, **Li J**, Monti L, Barone A, Frusciante L (2004) Assessment of genetic variability of haploids extracted from tetraploid (2n= 4x= 48) *Solanum tuberosum*. Genome 47:633-638

Chen L, **Li J**, Wang Y, Qian H, Li T (2008) Establishment and optimization of resistant gene codominant-SCAR in tomato. Molecular Plant Breeding 6(5): 1000-1004 (in Chinese)

Wu Y, Yang J, **Li J**, Liu L (2008) Fingerprint analysis of *L. hirsutum* and *L.pimpinellifolium* wild species and cultivated tomato. Chinese Agricultural Science Bulletin 24(4): 80-84 (in Chinese)

Su C, **Li J**, Huo X (2007) Activation tagging and its application in tomato functional genomics. Acta Agriculturae Boreali-Sinica 22(B08):172-176 (in Chinese)

Li J, Song Y, Yang Y, Du Y, Feng L, Xie B, Xu H (2007) Age-related resistance to main strains T1 of late blight (*Phytophthora infestans*) in china during tomato seedlings. Journal of Plant Genetic Resources 8(2):231-233 (in Chinese)

Chen L, Li T, **Li J**, Song Y, Wang Y, Wang X, Xu H (2006) Construction of CAPS marker for highly resistant gene *Cf-9* to tomato leaf mould. Plant Protection 32(3):21-24 (in Chinese)

Song Y, Chen L, **Li J**, Zhang Z, Li T, Xu H, Zhou Y (2006) Identification of the genes with resistance to leaf mold and tomato mosaic virus by PCR reaction in tomato. Journal of Plant Genetic Resources 7(2):165-169 (in Chinese)

Song Y, **Li J**, Xu H (2006) A processing tomato F1 hybrid -'Hongza No.35'. China Vegetables5:26-27(in Chinese)

Li J, Zhu S, Song Y, Zhang Z, Atanassova B, Zhang S, Xu H (2006) Development of AFLP molecular markers tightly linked with *Ps-2* locus in tomato. Journal of Plant Genetic Resources 7(1):35-38 (in Chinese)

Li J, Song Y, Zhu T, Chi Q, Xu H, Zhou Y (2006) Reviews about molecular breeding of salt tolerance on tomato. Molecular Plant Breeding 4(1):111-116 (in Chinese)

Li J, SongY, Chen L, Yang Y, Xu H, Fen L, Zhang Z, Zhou Y (2005) Simultaneous identification of multi-genes with resistance to root-knot nematode and tomato mosaic virus respectively by multiplex PCR markers in tomato. Journal of Agricultural Biotechnology13(6):698-702 (in Chinese)

Li J, Yang Y, Song Y, Xu H, Feng L, Zhou Y, Mo L (2005) Identification of tomato wild species resistant to late blight (*Phytophthora infestans*) and primary introgression into cultivars in China. Acta Horticulturae Sinica 32(5):892-894(in Chinese)

Zhang Z, **Li J**, Song Y, Yun X, Xu H, Zhou Y (2005) An overview on the gray mold (*Botrytis cinerea*) and the control of it in tomato. Journal of Inner Mongolia Institute of Agriculture and Animal Husbandry 26(2):125-128 (in Chinese)

Chen L, **Li J**, Song Y, Li T, Xu H, Zhou Y (2004) Simultaneous identification of multi-genes with resistance to tomato mosaic virus and tomato spotted wilt virus by PCR markers in tomato. Scientia Agricultura Sinica 37(7):982-986 (in Chinese)

Xing H, Song Y, Yun X, **Li J**, Zhou Y, Xu H (2004) A review of tomato male sterility. Chinese Agricultural Science Bulletin 20(2):157-160(in Chinese)

Li J, Song Y, Xu H, Zhou Y, Carole C, Feng L (2003) Simultaneous identification of multi-genes with resistance respectively to root- knot nematode and TSWV by PCR markers in tomato. Acta Horticulturae Sinica 30(6):678-682 (in Chinese)

Li J, Song Y, Xu H, Zhou Y, Atanassova B (2003) The study on *ps*-2 male sterility lines 01222 for hybrid production in tomato. Chinese Agricultural Science Bulletin 19(2):16-18(in Chinese)

Li J, Zhou Y, Xu H, Yang B (2003) The linkage analysis of *ss* gene and its effect on seed germination in tomato (*Lycopersicon esculentum* Mill.). 30(1):34-38(in Chinese)

Education Statement of the Graduate School
Experimental Plant Sciences

The Graduate School
EXPERIMENTAL PLANT SCIENCES

Issued to: Junming Li
Date: 19 Janurary 2010
Group: Laboratory of Plant Breeding, Wageningen University

1) Start-up phase	*date*
► **First presentation of your project**	
Toward prodcution of an activation population in tomato	Jun 25, 2003
► **Writing or rewriting a project proposal**	
► **Writing a review or book chapter**	
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*1.5 credits**

2) Scientific Exposure	*date*
► **EPS PhD Student Days**	
PhD day 2003, Utrecht	Mar 27, 2003
► **EPS Theme Symposia**	
EPS Theme 3 Symposia, 2006, Amsterdam	Nov 10, 2006
► **NWO Lunteren days and other National Platforms**	
Vegetable molecular breeding symposium in China, 2004	Jun 17-18, 2004
► **Seminars (series), workshops and symposia**	
The Dutch-Chinese life science forum, Utrecht University	Oct 07, 2006
Workshop on WU-CAAS project 2005, China	May 09, 2005
Workshop on WU-CAAS project 2005, China	Jan 11, 2005
Workshop on WU-CAAS project 2004, Wageningen	Nov 22, 2004
Workshop on WU-CAAS project 2004, Wageningen	Feb 23, 2004
Workshop on WU-CAAS project 2003, Wageningen	Oct 26, 2003
Workshop on WU-CAAS project 2003, Wageningen	Jun 25, 2003
Sem. Frontiers in Plant Science, Botanical centre, Wageningen (2x)	2003
► **Seminar plus**	
► **International symposia and congresses**	
27th International Horticultural Scientific Symposium (in Korea)	Aug 13-19, 2006
The Second National workshop for Plant Functional Genomics(in China)	Aug 08-13, 2006
Congress of tissue culture and molecular biology (in China)	May15-18, 2005
► **Presentations**	
27th International Horticultural Scientific Symposium (in Korea) (Oral)	Aug 13-19, 2006
The Second National workshop for Plant Functional Genomics(in China) (Poster)	Aug 08-13, 2006
Congress of tissue culture and molecular biology (Poster)	May15-18, 2005
Poster presentation in autumn school (in CAAS) 2003	Nov 10-15, 2003
► **IAB interview**	
► **Excursions**	
Subtotal Scientific Exposure	*10.2 credits**

3) In-Depth Studies	*date*
► **EPS courses or other PhD courses**	
Molecular Genetics (CAAS)	Sep 2002
Bioinformatics (Wageningen)	Sep 2003
Modern Scientific & Technological Revolution and Current Society - CAAS	Sep 2002
Progress in Agricultural Science and Technology - CAAS	Sep 2002
Plant Functinal Genomics	Sep 2005
Colligation examination	Sep 2005
► **Journal club**	
Literature discussion in group meetings	2003-2006
► **Individual research training**	
Biological chemical experiments training, 2003	May 01-15, 2003
Subtotal In-Depth Studies	*25.5 credits**

4) Personal development	*date*
► **Skill training courses**	
English training course for reading, listening and wirting (CAAS), 2003	2002-2003
How to write an application grant (CAAS)	2002-2003
Scientific paper writing(CAAS)	2002-2003
Endnote Course(Wageningen)	Oct 2003
► **Organisation of PhD students day, course or conference**	
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*10.1 credits**

TOTAL NUMBER OF CREDIT POINTS*	**47.3**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 credits

* A credit represents a normative study load of 28 hours of study