# Introgressions of *Lycopersicon pennellii* improve growth and development of greenhouse tomatoes

# Introgressies van *Lycopersicon pennellii* verbeteren groei en ontwikkeling van de kastomaat

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Dit onderzoek is uitgevoerd binnen de onderzoekschool Production Ecology and Resource Conservation

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# Introgressions of *Lycopersicon pennellii* improve growth and development of greenhouse tomatoes

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. M.J. Kropff,
in het openbaar te verdedigen
op vrijdag 14 oktober 2005
des namiddags te vier uur in de aula

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

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**Introgressions of *Lycopersicon pennellii* improve growth and development of greenhouse tomatoes**

Thesis Wageningen University, The Netherlands – with references – with summaries in English and Dutch. Laboratory of Plant Breeding, P.O. Box 386, 6700 AJ, Wageningen, NL

**ISBN** 90-8504-301-8

**Keywords**

Backcross inbred lines, Introgression, Leaf area ratio, Marker assisted selection, Net assimilation rate, Quantitative trait loci, Relative growth rate, *L. pennellii* LA716, Tomato, Transgression, Yield, Plant physiology.

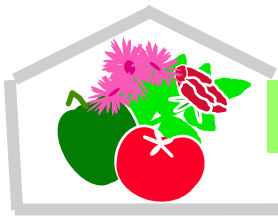
## Contents

*For Antoine Willy and Piet Junior*
*so that you may do better than papa*

*How rough should this Ph.D. period have been……., to call it bumpy?*

# Chapter 1

## General introduction



RASSEN ONDER GLAS MET MINDER GAS

**The research described in this thesis was performed as part of a Dutch research program, entitled “Rassen onder glas met minder gas”, i.e. aiming at breeding more energy-efficient greenhouse crops. This program is financially supported by the Dutch Horticultural Product Board (Productschap Tuinbouw), The Dutch Organization for Energy and Environment (NOVEM), the Department of Agricultural Research (DLO), The Ministry of Agriculture, Nature and Food Quality (LNV) and several private breeding companies.**

## Classical crop improvement requires novel genetic resources

Plant breeding is the art and science of the genetic improvement of crops to produce new varieties. In order to achieve their breeding objectives, plant breeders rely on genetic variability as the key asset to select for important agricultural traits. Selective propagation and new varieties are usually derived from crosses among genetically related modern varieties, meanwhile excluding genetically diverse but less productive primitive ancestors (Tanksley 1997, Tanksley and McCouch 1997). The use of only modern varieties in breeding has led to improved quality and productivity but it has narrowed down the genetic variability hence limiting breeding potentials (Tanksley 1997). The narrow genetic basis of tomato cultivars was illustrated by the level of DNA polymorphism using RFLP marker analysis. The results showed that the cultivated inbred varieties of tomato contain less than 5% of the genetic variation that is available in cross-compatible landraces and wild species (Tanksley and McCouch 1997).

## Molecular markers and Quantitative Trait Loci (QTLs)

The advent of molecular markers and linkage maps has made it possible to find associations between markers and phenotypes. Many of the natural phenotypes are the result of the combined action of several genes; this is the reason that phenotypic values are often seen in a continuous range rather than in discrete classes. If a trait is under the influence of more than one gene the trait is called a quantitative trait and the genetic location of the genes are called quantitative trait loci (QTLs). The complexity of traits showing Gaussian distribution often results from the segregation of several QTLs (Mackay 2004). Another aspect of quantitative traits is their sensitivity to environmental conditions. This characteristic also contributes to the continuous distribution of trait values that is observed in non-uniform environments.

The first molecular genetic linkage map of tomato comprising DNA markers was based on an $F_2$ population derived from an interspecific cross between cultivated tomato, *L. esculentum*, and its wild relative, *L. pennellii* (Tanksley *et al.* 1992). Since then, more advanced maps have been developed (Eshed and Zamir 1995, Haanstra *et al.* 1999) and also maps based on interspecific crosses with other wild species of tomato.

Molecular linkage maps have been successfully used in many applications in plant genetics and breeding, including gene tagging, map-based gene cloning, QTL mapping, and marker assisted selection (MAS). For example in QTL mapping, individual QTLs are identified and characterized in terms of the chromosomal location, phenotypic effect, and gene action with the aid of detailed molecular linkage maps. Molecular linkage maps can also facilitate the introgression of identified QTLs into elite breeding lines and cultivars and minimizing of linkage drag (Tanksley 1993). These characteristics make molecular linkage maps a very useful tool for exploiting quantitative genetic variation in wild species, as has been well demonstrated for many crop plants including tomato (Tanksley and McCouch 1997).

## Biodiversity presents potentials for improving agronomic traits

Interspecific populations have been extensively used for the identification of QTLs for important agronomic or horticultural traits. Therefore comprehensive QTL information is now available for populations derived from several wild species of tomato: *L. pennellii* (Fridman *et al.* 2004, Fridman *et al.* 2002, Gur *et al.* 2004) *L. hirsutum* (Bernacchi *et al.* 1998a, Bernacchi *et al.* 1998b, Bernacchi and Tanksley 1997, Maliepaard *et al.* 1995b, Monforte and Tanksley 2000b), *L. peruvianum* (Bai *et al.* 2004, Fulton *et al.* 1997b, Van Heusden *et al.* 1999) *L. parviflorum* (Bai *et al.* 2003, Fulton *et al.* 2000) and *L. pimpinellifolium* (Doganlar *et al.* 2002, Grandilio *et al.* 1996, Grandillo *et al.* 1996, Grandillo and Tanksley 1996, Van der Knaap *et al.* 2002, Van der Knaap and Tanksley 2001).

One of the most obvious treasures from making distant crosses is the possibility to introgress and to broaden genetic variability. More importantly,

useful QTLs can be readily discovered in otherwise phenotypically inferior wild species. For example, a QTL for increased red pigment in fruit was identified in an accession of the green-fruited species *Lycopersicon hirsutum* Humb., and Bonpl. (Bernacchi and Tanksley 1997) and a QTL for increased fruit size was identified in an accession of the small-fruited species *L*. *pimpinellifolium* Jusl. (Tanksley *et al.* 1996). This shows the importance of favorable wild alleles that can easily be transferred into genetic backgrounds of cultivars.

## Transgressive segregation

The importance of genetic variation as a key asset for plant breeding has been emphasized. The genetic difference between two genotypes or two varieties of a crop species becomes manifest in segregating populations. In such segregating populations transgressive segregation is frequently observed (de Vincente and Tanksley 1993, Rieseberg and Ellstrand 1993). One-sided transgressive segregation can be due to heterosis, which is most pronounced in first generation hybrids ($F_1$). In a survey of 171 studies Rieseberg *et al.* (1999) showed that 91% of them reported at least one transgressive trait, and in total 44% of the 1229 traits showed transgressive segregation. Fulton *et al.* (2000) have analyzed advanced backcross populations derived from the cross between *L. esculentum* and *L. parviflorum* for several agronomic traits amongst which soluble-solid content (Brix), Brix*yield, fruit color, fruit shoulder and fruit shape. A total of 199 putative QTLs have been identified for all traits, ranging from one to 19 QTLs per trait. For 70% of the agronomic traits with favorable effects, at least one positive allele originated from *L. parviflorum* despite the overall inferior phenotype of this wild species. These results suggest that transgressive segregation is very common in hybrid populations derived from interspecific crosses. Therefore, we have good reasons to expect better yielding or growing individuals in the progenies from the interspecific cross between tomato cultivar Moneymaker and *Lycopersicon pennellii* LA716. The occurrence of transgressive segregation also implies that the phenotype of an individual is a poor predictor of its genetic potential (Tanksley 1997, Tanksley and McCouch 1997). In a

QTL analysis, Van der Knaap and Tanksley (2001) revealed a single major QTL controlling early fruit development on Chromosome 7 using an interspecific population derived from *L. esculentum* cv. Sun 1642 (TA491) and *L. pimpinellifollium* LA1589. Alleles from *L. pimpinellifollium* were associated with favorable agronomic effects in 70% (19/30) of the horticultural traits, despite the overall inferior agronomic phenotype of this species.

## Beneficial QTLs identified from wild relatives of tomato

To illustrate the importance of wild *Lycopersicon species* in the tomato breeding as source of beneficial alleles for tomato breeding, some studies are presented below.

### *Wild alleles for biotic stress resistance*

Many QTLs controlling disease resistances have been identified in populations derived from interspecific crosses in the Lycopersicon genus. In some cases the alleles for resistance have been introgressed into modern cultivars. Lindhout *et al.* (1993) screened 127 accessions representing eight wild relative species of tomato for resistance to *O. lycopersicum* and found a large variation in resistance between species as characterized by disease incidence, mycelium growth and lack of sporulation. *L. hirsutum* was the species with the most resistant accessions; the accessions of *L. pennellii* were moderately resistant; species of the subgeneric group of *L. esculentum* and of the 'peruvianum-complex' were all susceptible. *L. parviflorum* was classified separately due to a large variation between accessions. Except for this species, little variation has been found between accessions within species. Overall, high levels of resistance were observed in four accessions of *L. hirsutum*, in one of *L. parviflorum* and in one of *L. peruvianum*. Later, Van der Beek *et al.* (1994) investigated the inheritance of resistance to powdery mildew (*Oidium neolycopersici*) using hybrid populations derived from a cross between tomato and the wild relative *L. hirsutum* G1.1560 and found an incompletely-dominant resistance gene. In another study, Ciccarese *et al.* (1998) screened 146 accessions of *L. pimpinellifolium*, 132 of *L. esculentum*

var. cerasiforme and 53 of *L. peruvianum* for resistance to powdery mildew. They found two plants of *L. esculentum* var. cerasiforme accession LA-1230 to be resistant; one resistant symptom-less plant of accession LA-1230 was designated LC-95. When $F_1$ and $F_2$ progenies were produced with the homozygous LC-95 as one of the parents, they found that the $F_1$ plants were susceptible while the $F_2$ and backcross segregations fitted the hypothesis of a single recessive gene which was designated *ol*-2. In follow up studies Huang *et al.* (2000) fine mapped the *Ol*-1 gene. Moreover, they found an additional and incompletely dominant gene resistance (*Ol*-3); this gene was also located on Chromosome 6. Although *Ol*-1 and *Ol*-3 are mapped in the same chromosomal region, there was some evidence that these two genes are not identical but probably two different functional genes in a cluster of *Ol*-homologues. Bai *et al.* (2003) described three QTLs in *L. parviflorum* G1.1601 which are involved in resistance to *O. neolycopersici*. The three QTLs jointly explained 68% of the phenotypic variation in resistance against powdery mildew in $F_2$ and $F_3$ generations. Later Bai *et al.* (2004) have characterized another dominant gene in *L. peruvianum* LA2172, *Ol*-4, and very recently (Bai *et al.* 2005) *Ol-5* originating from *L. hirsutum* PI247087. They were able to develop Nearly Isogenic Lines (NILs) containing *Ol* genes through repeated backcrossing.

Bas *et al.* (1992) investigated genetic variation for resistance to the greenhouse whitefly (*Trialeurodes vaporariorum*) of four genotypes of tomato (*L. esculentum*) and two subspecies of *L. hirsutum*. Resistance was quantified by the whitefly life history components adult survival, oviposition rate, pre-adult survival and developmental period, measured on plants inoculated with whiteflies in clip-on cages. On *L. hirsutum* f. *glabratum* whiteflies had the lowest adult survival, oviposition rate and pre-adult survival. On *L. hirsutum* these components were intermediate whereas on all *L. esculentum* genotypes they were highest. Maliepaard *et al.* (1995a) used an $F_2$ of an interspecific cross between cultivated tomato (*L. esculentum* cv. Moneymaker) and *L. hirsutum* f. *glabratum* to identify QTLs for greenhouse whitefly resistance. They found two QTLs affecting oviposition rate that mapped to Chromosome 1 (*Tv*-1) and 12 (*Tv*-2). $F_3$ lines homozygous for either the *L. esculentum* allele

or the *L. hirsutum* f. *glabratum* allele at one or both loci confirmed the effects of *Tv*-1 and *Tv*-2.

Several other QTL studies have been performed on tomato. For instance, Huang and Lindhout (1997) screened wild *Lycopersicon* accessions for resistance to the Fusarium wilt disease caused by *Fusarium oxysporum* f.sp. lycopersici (*Fo*l) race 1 and race 2. Most accessions were highly susceptible, some showed intermediate resistance, but one accession of *L. cheesmanii* (G1.1615=PI 266375) and two accessions of *L. chilense* (G1.1556 and G1.1558) were highly resistant to *Fol* Races 1 and 2. The resistance in the latter three accessions was equal or higher than the resistance determined by the known I-genes that have been widely used in breeding programs. Sandbrink *et al.* (1995) identified genes for bacterial canker resistance caused by *Clavibacter michiganensis* ssp. michiganensis in *L. peruvianum* LA2157 (using a population derived from an intraspecific cross) in five regions on Chromosomes 1,6,7,8 and 10. In another study, Van Heusden *et al.* (1999) used an $F_2$ population derived from the interspecific cross between *L. esculentum* cv Solentos and *L. peruvianum* LA2157 to map resistance against *C. michiganensis* ssp. michiganensis. They identified three QTLs for resistance which mapped to Chromosomes 5, 7 and 9 respectively.

Moreira *et al.* (1999) showed that a QTL on Chromosome 2 of *L. hirsutum* f. *glabratum* G1561 was responsible for partial resistance against the arthropod *Liriomyza*.

***Wild alleles for fruit quality***

Wild alleles controlling fruit morphology have also been identified. For instance Kabelka et al. (2004a) used a backcross inbred population derived from *L. esculentum* and *L. hirsutum* LA407 to evaluate the potential for the improvement of red-fruited tomatoes. Two independent *L. esculentum*-derived QTL alleles, associated with improved color, have been identified. Expression of these QTLs required an epistatic interaction with *L. hirsutum* alleles; thus wild alleles were needed to enhance fruit color. In a study to analyze the variation in tomato fruit shape, Van der Knaap and Tanksley (2003) used a segregating population derived from a cross between *L. esculentum* cv.

Yellow stuffer and *L. pimpinellifolium.* The latter produces a small round fruit, typical of most wild species. In a QTL analysis for both fruit size and fruit shape, three QTLs were identified influencing fruit shape. In a study to determine QTLs for extremely elongated tomato fruit, Van der Knaap *et al.* (2002) used a segregating population derived from *L. esculentum* cv Long John (elongated fruit phenotype), and *L. pimpinellifolium* LA1589 (round fruits). Four fruit shape QTLs located on Chromosome 2, 3, 6 and 11 were detected. Fulton *et al.* (2000) have analyzed advanced backcross populations derived from the cross between *L. esculentum* and *L. parviflorum* for fruit color, fruit shoulder and fruit shape and identified several QTLs affecting fruit quality.

***Wild alleles for productivity***

Yield is often analyzed in terms of fresh fruit yield harvested or soluble solid content (Brix) per fruit. In tomato there is usually a negative relationship between total fruit yield and soluble solids concentration (Steven and Rudich 1978). Therefore, Brix*yield is often calculated to provide a better estimate of the amount of processed product that can be expected per plot. In greenhouse tomato this can also be used as a prediction of sweetness. There are several reports on the beneficial exploitation of wild alleles for tomato yield. Over the past decade populations derived from interspecific crosses between tomato and wild relative species have been assayed for yield and associated traits. For instance, a major QTL known as *fw2.2* which controls fruit weight has been identified in a segregating $F_2$ population derived from *L. esculentum* x *L. pimpinellifolium* and later it was shown that an ortholog is present in *L. pennellii* (Alpert *et al.* 1995). *fw2.2* is located on chromosome 2 and *L. pennellii* allele accounted for 30% resp. 47% of the variation for tomato fruit weight respectively. Bernacchi *et al.* (1998b) identified a QTL originating from *L. hirsutum* which was associated with an increase in total yield and soluble solid content up to 15%. Fulton *et al.* (2000) identified several QTLs affecting soluble solid content (Brix) and Brix*yield using an advanced backcross population derived from the cross between *L. esculentum* and *L. parviflorum*. Fridman *et al.* (2002) conducted a high resolution QTL mapping

of a nine cM introgression originating from the wild tomato species *L. pennellii* in indeterminate and determinate growing tomatoes. In the greenhouse or indeterminate tomato background Brix 9-2-5 affects the total soluble content of the fruits. The effect of Brix 9-2-5 was shown to be caused by an apoplastic invertase that plays a role in sugar partitioning into the fruits. An analysis of the same chromosome segment in open field or determinate tomato background revealed two QTLs 0.3 cM apart: the fruit specific Brix 9-2-5 that affects Brix only and another QTL giving an altered growth habit and an increase in plant weight, yield, and Brix. Brix 9-2-5 did not negatively affect fresh fruit yield, thus increasing sugar content and Brix* yield. Fulton *et al.* (2000) evaluated a myriad of agronomic traits including yield, fruit weight, soluble-solid content, Brix*yield, pericarp thickness and a few others using an advanced backcross population derived from *L. esculentum* E6203 and *L. parviflorum* LA 2133 and found for each trait at least one positive QTL originating from the wild species.

***Growth and growth traits***

Growth is the process by which a plant increases in dry weight. Because of the dynamics of growth, that are influenced by many environmental factors it is difficult to assay growth in one parameter. In the present study, we assess growth and development using different traits among which: plant dry weight, rate of appearance of leaves, flowers, leaf area expansion, stem length, and stem thickness. QTLs influencing growth traits have been identified in wild relatives of tomato; for instance, Holtan and Hake (2003) used the publicly available segmental introgression lines of Eshed and Zamir (1995) to detect QTLs controlling differences in leaf area expansion and dissection between *L. esculentum* and *L. pennellii* and identified 22 QTLs which primarily affect leaf area and leaf dissection in tomato. Using inbred backcross lines of *L. pimpinellifollium* LA1589 with a genetic background of *L. esculentum* cv. E6203 Doganlar *et al.* (2002) identified several QTLs of agronomic importance including plant growth. Eshed and Zamir (1995) analyzed a population consisting of 50 introgression lines originating from a cross between the green-fruited species *L. pennellii*

and the cultivated field tomato (cv. M82) and identified 6 QTLs which influenced plant weight.

## Marker assisted selection

Plant breeders can use a known association of molecular markers with a trait or a chromosome segment and further select for the presence of molecular markers rather than the phenotypes. This process is known as marker assisted selection (MAS). MAS is particularly worthwhile for traits of which the determination is difficult or time consuming. MAS can be applied for tagging, selecting and tracking of chromosome segments which influence quantitative traits like yield or other complex traits through several generations of crossings. The genetic mapping of introgressions which carry interesting QTLs has opened new promising opportunities in classical plant breeding. In this thesis we took full advantage of MAS to develop a set of Backcross inbred lines (BILs).

## Backcross inbred lines

Backcross inbred lines (BILs) have a combination of the genetic information of two parents (i.e. a chromosome segment from a wild relative species in the genome of a cultivar). Each BIL contains one homozygous introgression segment and a complete set of BILs ideally covers the complete genome of the related wild species (Eshed and Zamir 1994a). A complete set of BILs has several advantages in genetic studies in comparison to other mapping populations:

1. Differences between an introgression line and the line without the introgression can immediately be attributed to a defined chromosomal region.
2. BILs are homozygous lines and via selfing an infinite number of genetically identical plants can be obtained. Having enough plants allows replication of measurements and experiments, also in different seasons and

environments. In this way, Genotype by Environment (G X E) interactions can be studied.

3. A practical advantage of BILs is that the introduction of an interesting trait into a commercial cultivar can be relatively straightforward and rapid. BILs have also disadvantages such as lack of the detection of traits which are caused by epistatic effects. Moreover, the generation of BILs is quite labor intensive and time consuming.

Several sets of BILs have been developed for wild relatives of tomato. These include *L. pennellii (Eshed and Zamir 1994b)*, *L. hirsutum* (Monforte and Tanksley 2000a), *L. lycopersicoides* (Chetelat and Meglic 2000), and *L. chmieleskii* (http://www.keygene.com/pdf/PO%20Introgression%20Library%20Tomato.pdf). In barley, a set of BILs was constructed between two different spring barley cultivars, ('Scarlett' and 'Thuringia') and *Hordeum vulgare* ssp. *spontaneum* (von Korff *et al.* 2004). In lettuce, a set of BILs was constructed between *Lactuca sativa* cv Olof and *Lactuca saligna* (wild lettuce) (Jeuken and Lindhout 2004). BILs have proven to be a powerful stable permanent mapping population.

In the present study we used the wild relative *L. pennellii* LA716 (http://www.sgn.cornell.edu/help/solanum_nomenclature.html) aimed at identifying alleles with a positive influence on yield and growth characteristics which underlie general crop performance in *L. esculentum* cv. Moneymaker. *L. pennellii* grows in the wild from the north of Peru (Piura) to the north of Chile (Tarapaca) in dry rocky hillsides and sandy areas from sea level to 3000 m. We developed a set of BILs and pre-BILs and investigated the contribution of homozygous chromosome segments of *L. pennellii* LA716 on plant growth physiology in greenhouse, indeterminate growing tomato. A similar set of BILs has been developed by Eshed and Zamir (1995) in the genomic background of determinate growth or field tomato. Although the genetic constitutions of field and greenhouse tomatoes are similar (Miller and Tanksley, 1992); they are not identical. Each variety had been developed for different environments, methods of production, and foods uses and hence have obviously different physiology. The most notorious difference in their physiology is reflected in indeterminate growth (continuous growth) compared to determinate growth,

whereby the plant stem ends in a florescence which results in a bushy plant type. Therefore we developed essentially the same set of BILs as has been developed by Eshed and Zamir (1995), but with the old greenhouse cultivar Moneymaker as recurrent parent.

## Physiological factors underlying growth, development and yield

Plant growth analyses have been performed using many tomato genotypes (Lindhout and Pet 1990, Lindhout *et al*. 1991, Nieuwhof and Dijk Van de 1988, Nieuwhof *et al*. 1989, Nieuwhof *et al*. 1991, Nieuwhof *et al*. 1993). In spite of these studies on plant growth and development, clear associations between physiological parameters and crop performance have not yet been established thoroughly. Insight in the association between yield and physiological parameters is necessary to understand how crop physiology influences productivity. In most cases the authors seek to understand to what extent differences in plant growth are affected by physiological parameters which underlie growth. For instance, Heuvelink (1996) found a correlation between specific leaf area and plant dry weight in juveniles of greenhouse tomato cultivars. In this thesis we explore physiological parameters which affect plant growth, development and yield with the objective to use them as effective selection handles during crop improvement.

Several studies aiming at yield improvement in tomato have reported enormous successes in terms of fresh fruit weight production and soluble solid content (Brix) (Alpert *et al*. 1995, Frary *et al*. 2000, Fridman *et al*. 2004, Gur and Zamir 2004). An increase in fruit weight as high as 47% was reported by Alpert *et al*. (1995). We postulate that yield in tomato has reached a near-maximum by exploiting the genetic variation within the cultivated tomato. This has been achieved by breeders by using classical methods of plant breeding within the cultivated germplasm. To make the next step in improving tomato plant growth and development, alternative approaches by using related wild species are needed. The approach in this thesis is to study in detail the individual characters which underlie plant growth and development and their specific contributions to yield. In addition we seek to associate these

parameters with certain introgression fragments from the wild species *L. pennellii* genome.

## Characterization of BILs for yield and its determining traits

Most of the genetic variation present in wild species has a negative effect on the adaptation of plants to agricultural environment; hence, the challenge is to identify and utilize the advantageous traits (Gur and Zamir 2004). The characterization of BILs for complex traits is relatively straightforward. Phenotypic differences or new traits which emerge in the BILs can be attributed to the presence of alien DNA or to the absence of DNA of the elite cultivar. With this in mind BILs can be characterized for factors influencing yield. Due to the availability of many plants with identical genotypes, destructive measurements can be done on more than one plant and can be repeated in several environments. The number of plants that can be handled is the rate-limiting component in the utilization of the potentials of an exotic genetic resource which is immortalized in BILs. This is particularly true for quantitative traits where the phenotype has to be evaluated in different seasons, environments, and genetic backgrounds. In the present thesis growth, development, and yield of BILs were thoroughly studied in two successive greenhouse experiments. Physiological parameters which underlie crop performance were also analyzed.

## The research objectives

The objective of this thesis can be summarized in a few research questions:

- What are technically optimal assessments of the genetic variation in growth and development in the cultivated tomato?
- Does the wild relative species *L. pennellii* LA716 harbor favorable alleles for growth, development and yield in a genetic background of the greenhouse tomato?

- Are these QTL-alleles the same as those that influence plant growth, development and yield in field tomatoes?
- To what extent do single homozygous chromosome segments of *L. pennellii* LA716 influence growth, development, and productivity in greenhouse tomato?
- What are the physiological parameters that may explain the improved growth, development, and yield of QTLs from *L. pennellii*?
- What are the consequences of the results for breeding of greenhouse tomatoes?

The ultimate aim of the present study is to identify BILs that contain favorable alleles for plant growth, development, or yield. Such BILs can be used as breeding materials for better yield in greenhouse tomato.

## Research methodology

A summary of the research methodology utilized in this thesis is depicted in Figure 1.

### *Outline of the thesis*

The objectives of this thesis are addressed in three experimental chapters. **Chapter 2** reports a growth analysis performed on juvenile tomato plants of a collection of old cultivars representing wide genetic variation from all over Europe. The results show that there is need for more genetic variation.

In **Chapter 3** the development of BILs containing chromosome-segments of *L. pennellii* LA716 is reported. BILs were periodically characterized destructively for traits which determine growth and development. Relative growth rate (RGR) and its physiological components LAR, NAR, LWR, and SLA were analyzed for each BIL. RGR's of BILs were compared with BILs at fixed plant dry weight. A correlation analysis was done between RGR and its physiological parameters in search of an explanation on

how the differences in RGR are affected by genotypic variation in underlying physiological parameters.

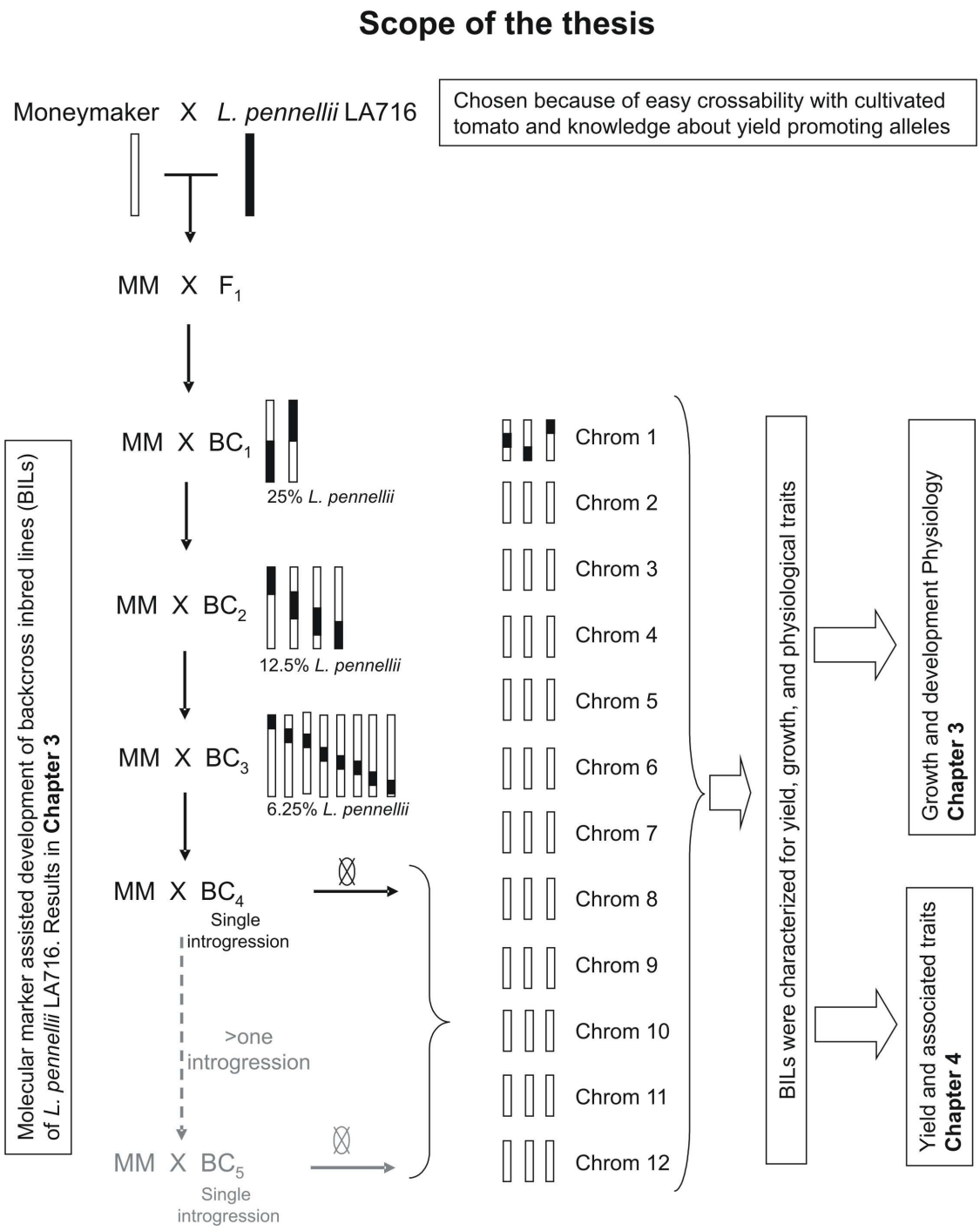



**Figure 1. A schematic representation of the methodology used to investigate *L. pennellii* LA176. A single $F_1$ plant was used to generate a $BC_1$. Marker assisted selection was used to select and track *L. pennellii* chromosome segments through several backcross generations. BILs were characterized for physiological traits determining growth and development as well as yield, and associated traits.**

The economic yield (fresh fruit harvested) of *L. pennellii* LA716 BILs was evaluated and the results are reported in **Chapter 4**. Other yield associated traits such as soluble sugar content (Brix), total biomass production and allocation were also investigated.

The general discussion in **Chapter 5**, establishes the red line through all the results obtained in this thesis. The relevance of understanding the processes that underlie growth and development in order to breed for yield is highlighted. Goals achieved in this study are discussed and some suggestions are given on the perspectives opened by the results of this thesis for the future of tomato breeding programs.

# Chapter 2

## The usefulness of genetic differences in the growth curves of young tomato plants

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**Manuscript under review**

## Abstract

**The growth of eight genotypes of tomato was analyzed. Differences in the relative growth rate (RGR) in dependence of plant development were studied. Plants were harvested at regular intervals and the plant dry weights and leaf areas were measured. Growth curves were fitted according to a second order polynomial with the logarithm of the dry weight data. Small but significant differences in RGR were found over a period of 17 to 74 days after sowing (DAS). Some genotypes initially showed a relatively restricted growth but recovered later on, whereas the opposite pattern was also observed. The changes in RGR during plant development were studied in more detail by analyzing the growth in shorter time intervals. Most genotypes showed a steadily and regularly decreasing RGR. However, the breeding line 'IVT-KT$_1$' had initially a stable level of RGR that sharply declined halfway the growth period. Strong growth was represented by genotypes which combined a high initial value of RGR with a slow but steady decrease in RGR. The differences in RGR were correlated with differences in net assimilation rate (NAR) but not with differences in leaf area ratio (LAR). The results indicate that data of growth analyses are only valid for a specific period of investigation. This implies that the growth of tomato plants should be analyzed over the full growth period to get an impression of the growth potential.**

## Introduction

The purpose of growth analyses in applied plant breeding is to identify genetic differences in growth potential by studying growth traits and physiological parameters which underlie optimal crop performance. Growth models are useful to describe genotypic differences in plant growth, though these are often difficult to assess due to relatively large variations in environmental conditions and consequently, these studies require larger populations of plants that are evaluated periodically under controlled conditions. Numerous studies have been carried out on tomato growth (Causton 1991, Causton 1994, De Groot *et al.* 2001, Fernandez and Miller 1987, Heuvelink 1995e, Hoffmann and Poorter 2002, Hunt and Cornelissen 1997, Lindhout *et al.* 1991, Nieuwhof *et al.* 1991, Smeets and Garretsen 1986a) and the development or improvement of statistical procedures has proven to be essential for these studies (Garretsen and Keuls 1986, Hoffmann and Poorter 2002, Keuls and Garretsen 1982, Lindhout *et al.* 1991, Poorter and Garnier 1996, Royo and Blanco 1999, Venus and Causton 1979). Procedures such as the numerical or the functional approach (Causton 1991, Causton 1994, De Groot *et al.* 2001, Fernandez and Miller 1987, Hoffmann and Poorter 2002, Hudu *et al.* 2002, Hunt 1979, Hunt 1982, Hunt *et al.* 2002, Hunt and Cornelissen 1997, Lindhout *et al.* 1991, Nieuwhof *et al.* 1991, Poorter 1989, Poorter and Garnier 1996, Smeets and Garretsen 1986a) or the sequential analysis (Jolliffe *et al.* 1982) are useful in describing the effect of culture conditions, such as temperature, nutrition, light, $CO_2$ concentration, influence of assimilate supply or sink-source interaction on plant growth or production (Heuvelink 1995d, Heuvelink and Buiskool 1995, Heuvelink and Marcelis 1996, Hoogenboom *et al.* 2004, Krug and Liebig 1995, Lindhout and Pet 1990, Nieuwhof and Van de Dijk 1988).

Most crop models use one or more genotype-specific parameters to identify differences in performance among genotypes (Hoogenboom *et al.* 2004). Genotypic variation for relative growth rate (RGR) and physiological characters in cultivated tomato have been studied extensively (Lindhout *et al.* 1991, Nieuwhof *et al.* 1991, Poorter and Lambers 1991, Smeets and

Garretsen 1986a). For instance, Lindhout *et al.* (1991) studied the genetic differences underlying growth by using 88 accessions of five *Lycopersicon species* and showed that large differences in RGR occurred between and within wild tomato species (5.3 to 11.8 %) and within the cultivated tomato (8.5 to 12.2%). In other studies involving correlation analysis between RGR and its component physiological growth parameters Nieuwhof *et al*. (1991) and Smeets and Garretsen (1986a) found that SLA (specific leaf area), and consequently LAR (leaf area ratio), is most strongly correlated with RGR. Despite the large genetic differences in plant growth, only occasionally were these differences significant, most likely because of the limited numbers of plants per genotype that could be evaluated. Consequently, the validity of the results of these studies was often limited.

The present study aimed at improving the growth analyses by using more replicates and more evaluations in order to more reliably establish genetic difference underlying plant growth. To this end, eight tomato genotypes were chosen based on previous experiments that represented a wide genetic variation in growth. The experiment comprised twelve replicates per genotype and ten harvests in a period of 17 to 74 days after sowing (DAS). In addition to RGR, the crop growth components: net assimilation rate (NAR), and LAR were determined in the same period. A correlation analysis was done to study the relationship of changes in RGR with the crop growth components during the development of young tomato plants.

## Materials and methods

### *Plant material*

The homozygous lines of the cultivated tomato (*Lycopersicon esculentum*) used in this study are from the tomato collection of the Centre for Genetic Resources (http://www.genebank.nl/). The eight genotypes were chosen as representatives of the variation in plant growth and photosynthesis as described in Table 1. ‘Line A’ was from Nunhems Zaden (Haelen, The Netherlands), ‘Line F’ from Rijk Zwaan (De Lier, The Netherlands), and the hybrid cultivar ‘Counter’ from De Ruiter (Bergschenhoek, The Netherlands),

IVT-$KT_1$ was a cold tolerant line from Plant Research International while Bakonycsernyei and Bubjekosoko are true breeding cultivars from eastern Europe, which have previously been used extensively in breeding research (IVT-Annual-report 1985). Premier and 'MXXIV-13' are Dutch lines (Hoek *et al*. 1993).

**Table 1. Some characters of the eight genotypes in this study**

| Genotype | type[1] | $RGR_t$[2] | $RGR_1$[3] | $RGR_3$[4] | Photosynthesis[5] |
|---|---|---|---|---|---|
| IVT-$KT_1$ | BL | - | - | + | -- |
| Line A | PL | + | | | + |
| Premier | TB | - | - | +/- | ++ |
| Line F | PL | - | | | +/- |
| Counter | H | | ++ | ++ | |
| MXXIV-13 | TB | + | | | + |
| Bakonycsernyei | TB | | - | + | |
| Bubjekosoko | TB | | + | - | |

[1] BL: breeding line, PL: parent line of hybrid variety, TB: true breeding cultivar, H: hybrid variety.

[2] $RGR_t$ *(RGR day$^{-1}$)* : - : < 90 ; + : 90-100 mg·g$^{-1}$·d$^{-1}$ (Smeets and Garretsen 1986b)

[3] $RGR_1$ (RGR at *lnW* = 1): - : 90-100 ; + : 100-110 ; ++: >110 mg·g$^{-1}$·d$^{-1}$ (Lindhout *et al*. 1991)

[4] $RGR_3$ (RGR at *lnW* = 3): - : <50; +/-: 50-60; +: 60-70; ++: 70-80 mg·g$^{-1}$·d$^{-1}$ (Lindhout *et al*. 1991)

[5] photosynthesis: -- : <5.0, - : 5.0-5.5, +/- : 5.5-6.0, + : 6.0-6.5, ++ : >6.5 mg$CO_2$·dm$^{-2}$·h$^{-1}$ (Nieuwhof and Dijk Van de 1988)

### *Experimental conditions and design*

Seeds were graded according to weight, and seeds of the fraction containing most seeds were sown in pots of 13 cm diameter, two seeds per pot. The pots were placed in a selektron (a growth room of 100 $m^2$) as described by Smeets and Garretsen (1986b). After germination and

emergence at 23℃, one seedling per pot was maintai ned. Philips 400 W HPI-T lamps at 24 W/m² supplied light for eight hours per day (24 $Wm^{-2}$; 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Temperature was gradually decreased in two days intervals from 19/14℃ (day/night) on day 4, to 19/12℃ on day 6 a nd finally to 19/10℃ on day 8 after sowing. The $CO_2$ concentration was maintained at approx. 340 ppm.

The set up was a randomized block design with twelve complete blocks. Initially, each block contained eight genotypes with 16 plants per genotype and per plot. Ten days after sowing, plants were selected for uniformity and the five least uniform plants per plot were removed. Plants of each block were positioned on a carriage that was replaced twice a week to reduce possible block effects due to light intensity differences. At day 46 after sowing the plants were lifted to bring the tops of the canopies at positions equidistant from the light. For analyses one randomly assigned plant from each plot was harvested at 10, 17, 22, 28, 32, 37, 46, 56, 66, and 74 days after sowing. Dry weights of the plants (W) and the leaf areas (LA) were measured for each of these plants. After each harvest the remaining plants on the carriage were placed at wider distances to avoid shading.

***Statistical analysis***

To avoid large residuals, the values for three blocks were averaged. Growth curves per plot were fitted to the plant weights according a second order polynomial function as described by Lindhout *et al*. (1991):

$$\ln(W) = a + bt + ct^2 \quad \textbf{(1)}$$

By definition RGR ($g \cdot g^{-1} \cdot d^{-1}$) is a function of dry weight (W):

$RGR = \frac{d \ln(W)}{dt}$ (which follows from the definition $\frac{dW}{dt} = RGR \cdot W$).

Using equation (1) RGR can be expressed as a function of time:

$$RGR = b + 2ct \quad \textbf{(2)}$$

or as a function of dry weight:

$$RGR = \sqrt{b^2 - 4c(a - \ln(W))}, \quad \textbf{(3)}$$

RGR can be divided into two components: the net assimilation rate (NAR = RGR x W / LA, where LA is leaf area ($cm^2$)) and the leaf area ratio (LAR = LA / W). NAR is a measure of the amount of photosynthetic product going into plant material while the LAR is the measure of the part of the plant that is engaged in photosynthesis (Poorter and Remkes 1990, Poorter and Van der Werf 1998). Combined they give a relative description of growth over time based upon plant characteristics.

The parameters RGR, NAR and LAR were calculated as follows. First, a second order polynomial was fitted to the data [equation (1)], yielding estimates of the coefficients *a*, *b* and *c*. Next, these estimates were plugged into equation (3) to obtain an estimate of RGR at a given plant weight (W). Subsequently, NAR was calculated as NAR = RGR x W / LA, and LAR was calculated as LAR = LA / W.

The RGR, NAR and LAR were calculated at fixed plant weights that were well within the range of the plant weights during the growth period, and averaged per genotype. ANOVA was applied to these estimates as described by (Payne *et al.* 1987). Our data enabled the calculation of correlations between RGR, NAR and LAR, both across genotypes (i.e. at a fixed dry weight) and within genotypes (i.e. across a range of dry weight values or time intervals).

## Results

### *Developmental changes in* RGR, NAR *and* LAR

The growth of eight genotypes of tomato was recorded from day 10 to day 74 after sowing. Plants harvested at day 10 turned out to be too small for accurate measurements of plant weights and leaf areas, and therefore were excluded from data processing. The data obtained from the remaining nine harvests were used for calculation of growth components.

The mean *lnW* over all genotypes ranged from -4.8 at day 17 to 1.7 at day 74. The mean RGR decreased from 0.166 $g \cdot g^{-1} \cdot d^{-1}$ at *lnW* = -4 to 0.085 $g \cdot g^{-1} \cdot d^{-1}$ at *lnW* = 1. At the middle of the growth period $RGR_{-1}$ (i.e. the RGR

where lnW=-1) ranged from 0.121 $g \cdot g^{-1} \cdot d^{-1}$ for 'Premier' to 0.134 $g \cdot g^{-1} \cdot d^{-1}$ for 'Counter'. These differences were highly significant (Table 2).

**Table 2. Differences in RGR between eight genotypes of tomato, calculated over the period from 17 to 74 DAS.**

| Genotype | $RGR_{-2}$ | $RGR_{-1}$ | $RGR_{0}$ |
|---|---|---|---|
| IVT-$KT_1$ | 0.139 | 0.125 | 0.109 |
| Line A | 0.145 | 0.128 | 0.108 |
| Premier | 0.136 | 0.121 | 0.103 |
| Line F | 0.138 | 0.123 | 0.106 |
| Counter | 0.150 | 0.134 | 0.116 |
| MXXIV-13 | 0.138 | 0.123 | 0.106 |
| Bakonycsernyei | 0.151 | 0.132 | 0.109 |
| Bubjekosoko | 0.145 | 0.126 | 0.104 |
| s.e.d | 0.0035 | 0.0026 | 0.0022 |

$RGR_{-2,}$ $RGR_{-1}$ and $RGR_{0}$ are the calculated RGR values from the fitted functions at ln(W) of -2, -1 and 0 respectively.

The present growth analyses were quite extended as nine evaluations were done over a longer period of time. Usually, fewer evaluations are done over shorter period of time for logistic reasons. We analyzed the deviations from the calculated growth curves, when fewer observations are done by not taking part of the data into account. This was done by dividing the evaluation period into two overlapping shorter periods; one covering the first seven evaluations from day 17 to day 46, and a second one covering the last seven evaluations from day 32 to day 74 after sowing. Each genotype was represented by 120 plants, all thus making a reliable growth analysis possible. The RGR, NAR and LAR were calculated in these periods. In Figure 1 the RGR values over the different growth periods calculated for eight genotypes are presented. Three of the eight genotypes showed variation in RGR during shorter intervals of growth investigation compared to the whole period. ‘IVT-

$KT_1$' (Figure 1a) showed strong growth in the first period but RGR decreased sharply during the second. The RGR of 'Line F' (Figure 1b) decreased moderately in the first period but showed a steeper decrease in the second period. Counter (Figure 1c) registered a slight decrease in RGR in the first period but recorded a much smaller and steady decrease in the second period. The five other genotypes (Figure 1d) did not show significant variation in changes of RGR during the shorter periods of growth investigation compared to the whole period.

The growth curves, calculated over shorter intervals of 'Line A', Premier, 'MXXIV-13', Bakonycsernyei and Bubjekosoko resembled the curve over the entire growth period very much (Figure 1d). Apparently these curves already showed a good fit when part of the data was excluded from the analyses.

The RGR, NAR and LAR, calculated over the three growth periods described above, were compared at $lnW = -1$ for all genotypes (Table 3). The differences in RGR were not significantly different but for two lines: the RGR of 'Counter' over the entire and late growth period (as shown in Figure 1c); the values of the $RGR_{-1}$ of 'IVT-$KT_1$' were significantly different when calculated over the three growth periods (as shown in Figure 1a). NAR and LAR varied largely between genotypes but not within genotypes during growth periods except for IVT-$KT_1$ in which a remarkable decrease in NAR is recorded during DAS 17 – 74.

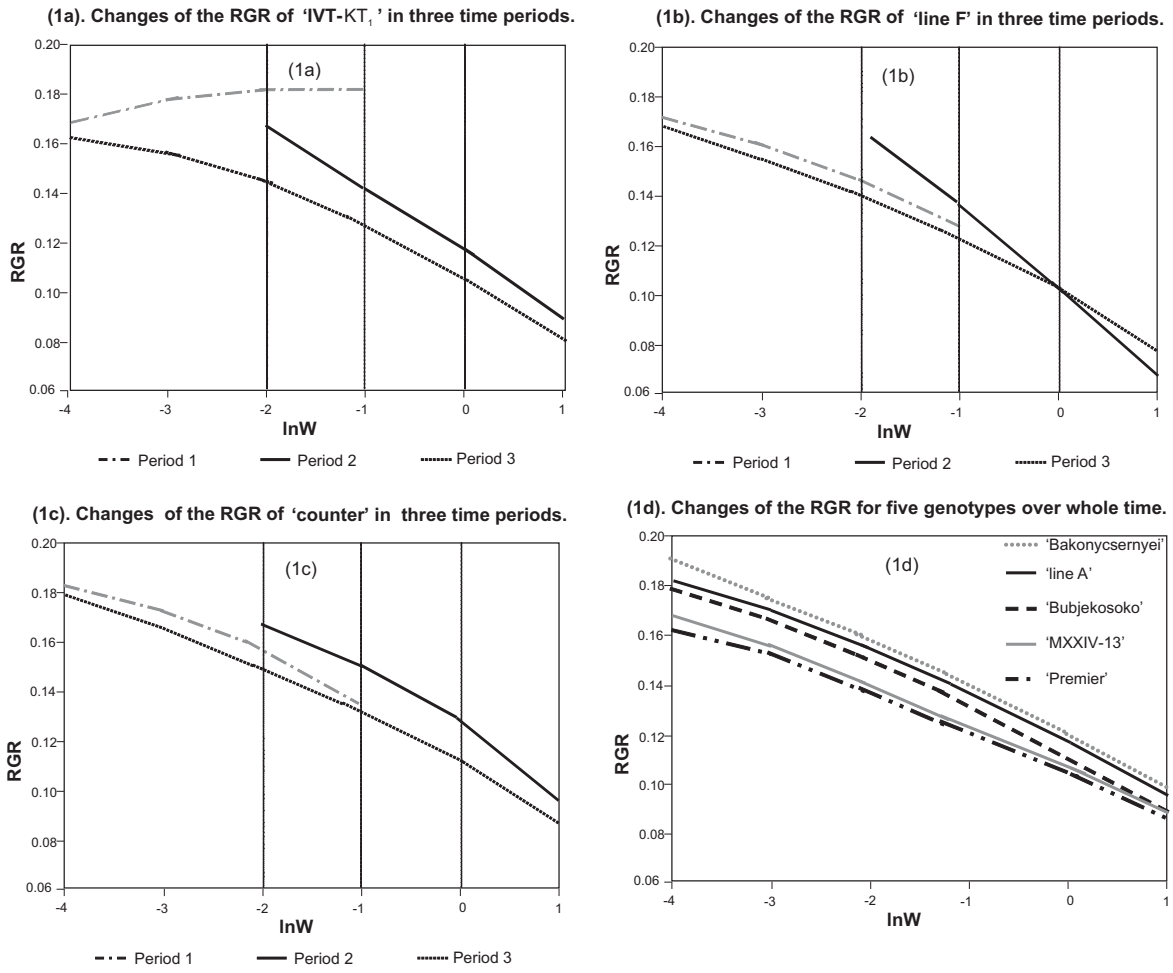


**Figure 1. Predicted values of RGR during plant development investigated during two short period intervals and through out the whole period.**

**Table 3. Growth characters of eight genotypes of tomato calculated over different growth periods.**

| Genotype | $RGR_{-1}$ ($g \cdot g^{-1} \cdot d^{-1}$) growth period (d) | | | $NAR_{-1}$ $g \cdot cm^{-2} \cdot d^{-1}$ ($10^{-3}$) growth period (d) | | | $LAR_{-1}$ $cm^2 \cdot g^{-1}$ ($10^{-3}$) growth period (d) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 17-46 | 32-74 | 17-74 | 17-46 | 32-74 | 17-74 | 17-46 | 32-74 | 17-74 |
| IVT-$KT_1$ | 0.182 | 0.141 | 0.125 | 0.276 | 0.214 | 0.185 | 0.671 | 0.662 | 0.675 |
| Line A | 0.121 | 0.136 | 0.128 | 0.201 | 0.224 | 0.210 | 0.602 | 0.608 | 0.608 |
| Premier | 0.105 | 0.122 | 0.121 | 0.180 | 0.205 | 0.204 | 0.586 | 0.599 | 0.593 |
| Line F | 0.128 | 0.138 | 0.123 | 0.232 | 0.262 | 0.233 | 0.553 | 0.529 | 0.529 |
| Counter | 0.135 | 0.151 | 0.134 | 0.236 | 0.265 | 0.237 | 0.574 | 0.568 | 0.566 |
| MXXIV-13 | 0.113 | 0.124 | 0.123 | 0.173 | 0.189 | 0.189 | 0.663 | 0.657 | 0.650 |
| Bakonycsernyei | 0.128 | 0.140 | 0.132 | 0.214 | 0.244 | 0.229 | 0.598 | 0.574 | 0.577 |
| Bubjekosoko | 0.117 | 0.133 | 0.126 | 0.209 | 0.229 | 0.212 | 0.558 | 0.580 | 0.595 |
| s.e.d. | 0.020 | 0.006 | 0.0026 | 0.037 | 0.012 | 0.005 | 0.023 | 0.013 | 0.014 |

**Correlation between RGR, NAR and LAR**

The correlations between RGR and NAR, RGR and LAR as well as NAR and LAR were studied over three growth periods among the genotypes. The correlations between RGR and NAR as well as RGR and LAR are shown on Figure 2 and Figure 3 respectively. The NAR was significantly correlated with RGR among the genotypes irrespective of the growth period.

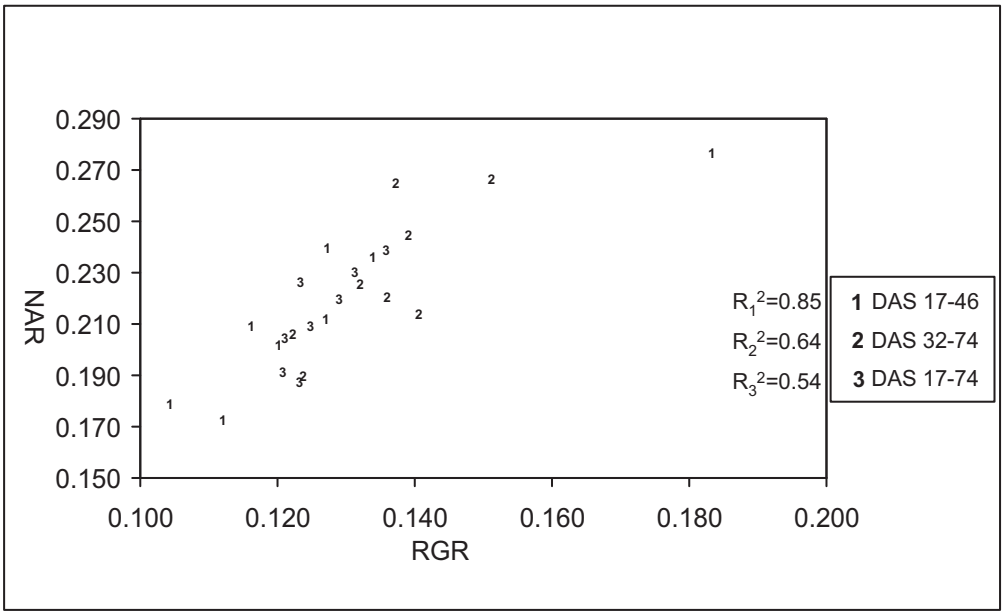


**Figure 2. The NAR versus the RGR per growth period among the eight tomato cultivars investigated in this study.**

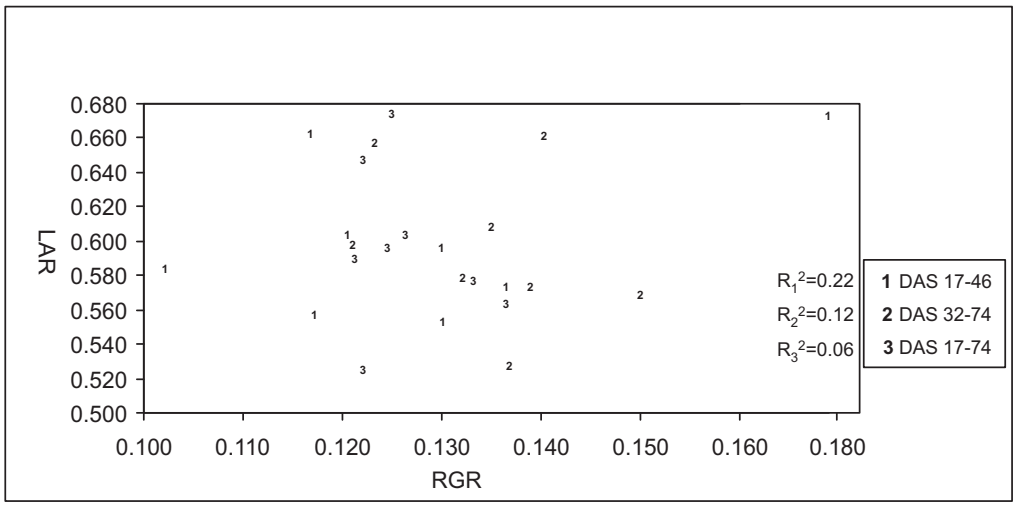


**Figure 3. The LAR versus RGR per growth period among the eight tomato cultivars investigated in this study.**

The genotypes Bakonycseryei, Bubjekosoko, Counter and Line A are characterized with optimal growth. Optimal growth is represented by genotypes which combine relatively high initial values of RGR with a small decrease in RGR (Lindhout *et al.* 1991). Also these four genotypes show strong correlations between NAR and RGR at higher plant dry weight. 'IVT-$KT_1$' and 'Line F' show large variations in patterns of change in RGR during plant development (Figure 1a and 1b). A sharp decrease in RGR for 'IVT-$KT_1$' and 'Line F' coincided with a decrease in correlation between NAR and RGR during growth period DAS (32-74) in both cases.

In contrast to the NAR, no significant correlation was found between LAR and RGR at any time during plant development (Figure 3). As expected, NAR and LAR were correlated through out the growth periods.

## Discussion

In the present study changes in RGR during the development of young tomato plants were analyzed. In previous studies, the RGR of tomato genotypes was calculated at a fixed time point (Nieuwhof *et al.* 1993, Smeets and Garretsen 1986a, Smeets and Garretsen 1986b) or at fixed plant weights (Lindhout *et al*. 1991). According to the latter study, the RGR as a function of weight is more informative, since the authors suggest that changes in RGR during plant development might differ between genotypes. In the present study more plants per genotype were used and more evaluations were done to enable a more accurate study of differences in developmental changes in RGR between eight genotypes of tomato.

Five of the genotypes investigated showed similar growth curves over the whole growth period investigated. It was concluded that the RGR of these genotypes decreased at a constant rate. The other three genotypes showed differences in the pattern of change of RGR during plant development. The breeding line 'IVT-$KT_1$' showed the most deviating growth characteristics. The RGR of this genotype initially decreased very slowly but dropped strongly at higher plant weights. Consequently, a second order polynomial function of the growth curve of this genotype over the entire growth period from 17 to 74 days

was not very accurate. Attempts to correct the model using the approach described by Goudriaan and Monteith (1990), did not improve the fitting of the data for 'IVT-$KT_1$'. An entirely different model might have been suitable for this genotype at this specific phase of plant development.

Generally, the differences between RGR, NAR and LAR calculated over different growth periods were small compared to the differences between genotypes. In calculating RGR, NAR, and LAR; precautions were taken to avoid bias as described by Hoffmann and Poorter (2002). The correlation between the three growth characters was not dependent upon the growth period. In no instance a significant correlation between RGR and LAR was observed, meanwhile a significant correlation between RGR and NAR was observed among genotypes. This is not in agreement with de Jong and Jansen (1992), Dijkstra and Lambers (1989a), Dijkstra and Lambers (1989b), Nieuwhof *et al.* (1991), Poorter and Lambers (1991), and Smeets and Garretsen (1986a), who found that the SLA, and consequently LAR, but not NAR, is most strongly correlated with RGR. Heuvelink (1999) showed that SLA and the developmental stage of a vegetative unit have a large influence on crop growth rate. Perhaps the emphasis on developmental stage also signifies that correlation between SLA and biomass production would not hold true throughout plant development. This at least appears to be true for some physiological parameters such as LAR. Unfortunately the methodology used in the present study does not give us the tool necessary to investigate correlation between RGR and SLA because the plant weight was not measured separately for leaves and the main stem.

The results of the present study concerning the correlation between RGR and NAR were confirmed when the eight genotypes were evaluated at slightly different conditions (not shown). The same tomato genotypes as used in this study were used previously to study the correlation between photosynthesis parameters in relation to growth differences (Nieuwhof *et al*. 1993). According to this study, the net $CO_2$ fixation rates of leaves were correlated with NAR and RGR, but only at night temperatures of 10 and 14 °C but not at 6 $^{o}$C. In the same study positive correlations were found between RGR and LAR at night temperatures of 6 °C but not at 10 and 14 $^{o}$C. It was

suggested that the observed genotypic variation in $CO_2$ fixation rates and growth probably reflected genotypic differences in responses of the stomata to the low relative humidity in the growth room. An additional explanation could be found in the fundamental equilibration of the physiological resources of the crop. It is possible that plants in the above mentioned study used most of the energy in maintenance respiration when night temperatures were set near chilling temperatures of 6 $^{o}$C rather than use it to produce assimilation product building blocks to enhance growth. At such a low temperature the conversion efficiency of photosynthates would also be very low resulting into an overall slow growth. This might explain the lack of correlation between RGR and NAR at night temperatures of 6 $^{o}$C. In a physiological growth analysis of seven greenhouse tomato backcross inbred lines (BILs) containing introgressions of *L. pennellii* LA716 positive correlations were found between RGR and NAR under normal greenhouse growing conditions (Owona *et al*. chapter 3). All the above suggest that the extend to which genetic differences in RGR could be explained by variations in NAR, LAR or other physiological components of growth depend considerably on the genotype, developmental stage of the plant and the environmental conditions under which the study is conducted.

It is not clear why the NAR plays the most important role in the growth differences between some of the genotypes in the present study. On the one hand, it might be that the observed correlation between RGR and NAR is restricted to the plant material investigated or to the present stage of vegetative development. On the other hand, differences in photosynthetic efficiency may explain the differences in NAR and RGR of the eight genotypes.

It is important to notice that the functional approach used to calculate RGR, NAR, and LAR in this paper implies that positive correlations would normally arise between RGR and NAR. This is true if correlation analysis is done within a genotype because such correlations would be enhanced mostly by experimental error, as RGR and NAR are calculated from the same raw measurements or function thereof. However when correlation analysis is done among the genotypes, correlation between RGR and NAR is essentially

determined by inherent genetic and physiological factors within each genotype.

The general tendency in our results show that a genotype that exhibits a relatively higher initial RGR at the juvenile stage combined with small but steady decreases in RGR might present good perspectives in selection for strong growth in later stages of development. In some cases genotypes that exhibited a strong initial growth had a slower growth as older plant and *vice versa*. The results also showed that genetic differences in RGR relate better to NAR in heavier as well as in older plants at a more advanced stage of vegetative growth. As tomato is commercially cultivated in the greenhouse for a period of one year, the influence of physiological parameters on growth and development should be studied throughout the whole year rather than an analysis of plant growth components over a restricted growth period.

# Chapter 3

## Introgressions of *Lycopersicon pennellii* in greenhouse tomatoes may push the yield to unprecedented levels: A physiological analysis

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**To be submitted for publication**

## Abstract

**A set of seven selected backcross inbred lines (BILs) with introgressions of *Lycopersicon pennellii* LA716 in the background of a greenhouse tomato (*L. esculentum* cv. Moneymaker) was developed to study the effect of the introgressions on growth and development of greenhouse tomato. Plants were characterized in the vegetative plant stage for leaf number (LN), leaf length (LL) leaf dry weight (LW), leaf area (LA), stem dry weight (SW), stem length (SL), stem girth (SG) and plant dry weight (W) periodically from 41 to 90 days after sowing under conventional greenhouse conditions. A second order polynomial function provided a good fit with the natural logarithm transformed data for all growth traits investigated. All BILS showed significant differences in at least one of the traits measured like LA, LW, SL, and W. Significant differences were also observed in the dynamic parameters like relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR). Strong correlations were found between RGR and but not between RGR and LAR. Two BILs showed an improved growth in terms of dry biomass production, and another two in partitioning. Overall, 27 QTLs affecting plant growth and development were detected, that offer great potentials for physiological research and for breeding.**

## Introduction

The genetic constitution of crops plays a central role in shaping growth, development and eventually productivity (Wu *et al.* 2004). In addition, different cultivars or wild species can vary widely in their potential growth rate. Dry matter production and partitioning of greenhouse tomato cultivars have been studied extensively (Heuvelink 1995a, Heuvelink 1995b, Heuvelink 1995c, Heuvelink 1995d, Heuvelink 1997, Lindhout and Pet 1990, Lindhout *et al.* 1991, Nieuwhof and Dijk Van de 1988, Nieuwhof *et al*. 1989, Nieuwhof *et al*. 1991, Nieuwhof *et al*. 1993, Nieuwhof *et al*. 1987, Nilsen *et al*. 1983). Such studies have been focused on the genetic variation and relationships of physiological growth components and the contribution of these components to plant growth, expressed as biomass production. To describe the dynamics of plant growth, mathematical equations are used to simulate growth, development and yield as a function of time, weather, radiation, soil conditions or crop management (Hoogenboom *et al*. 2004). This allows the parameters which influence growth and development. For tomato, growth in the vegetative plant stage is best described by second order polynomial functions on a natural log-scale of plant dry weight (Keuls and Garretsen 1982; Lindhout *et al.* 1991, Owona *et al*. chapter 2).

The results of these studies have also been the basis of the design of growth models, like the tomato growth simulation model, TOMSIM (Heuvelink 1996, Heuvelink 1999). This model can be used to study the effect of various growth components as well as external conditions or cultivation practices on growth and development of greenhouse tomato. For example, Heuvelink (1999) described that specific leaf area (SLA) and leaf pruning had a large influence on crop growth rate and also on leaf area index (LAI)

Other studies have focused on the genetic variation in relative growth rates and the crop physiological components that may explain the differences. Poorter and Lambers (1991) described the genetic differences in growth between 24 wild species common to Western Europe and Royo and Blanco (1999) between and among spring and winter triticale. Lindhout *et al.* (1991) showed that the genetic differences in relative growth rates (RGR) of 88 wild

and cultivated accessions of wild and cultivated *Lycopersicon* species ranged from 5.3 to 11.8% and 8.5 to 12.2% per day respectively. Nieuwhof (1993) reported an association between RGR and NAR when tomato genotypes were grown at night temperatures of 10-14 $^0$C and a correlation between RGR and LAR when the same genotypes were grown at night temperatures of 6 $^0$C. In a comparative physiological growth analysis using old tomato cultivars representing the genetic variation of greenhouse tomatoes in Europe, Owona *et al.* (chapter 2) found significant correlations between RGR and NAR. Other studies report variation in RGR and its physiological components between and within tomato varieties or species (Heuvelink and Buiskool 1995, Hunt and Cornelissen 1997, Lindhout and Pet 1990, Lindhout *et al.* 1991, Nieuwhof *et al.* 1991, Nieuwhof *et al.* 1993, Poorter and Lambers 1991, Poorter and Remkes 1990, Royo and Blanco 1999, Smeets and Garretsen 1986a) QTLs associated to plant growth physiology have been identified in field, 'determinate growth' or processing tomato. Doganlar *et al*. (2002) reported several QTLs in backcross inbred lines of *Lycopersicon pimpinellifolium.* Among other traits, they investigated plant growth, and growth associated traits such as flower number, flowering time, and leaf curliness. They found four QTLs for vegetative plant growth with explained phenotypic variation ranging from 5 to 6 %. They mapped two different QTLs on Chromosome 3 and in both cases the *L. pimpinellifolium* alleles were surprisingly associated with an increase in plant growth, while the *L. pimpinellifolium* alleles of the QTLs on Chromosomes 2 (*grw2.1*) and 9 (*grw9.1*), were associated with reduced plant growth. The QTL *grw9.1* was first identified in another population of *L. pimpinellifolium* (Tanksley *et al.* 1996). *Grw9.1* was mapped in the same region as a *Grw*-QTL in a *L. peruvianum* derived advanced backcross population (Fulton *et al.* 1997a). In addition, the *fw2.2*-QTL was identified on Chromosome 2 of *L. pimpinellifolium* and later also in *L. pennellii* (Alpert *et al.* 1995). The QTL *fw2.2*-QTL in both species accounted for 30% and 47% of the variation for tomato fruit weight, respectively. The fruit weight and shape QTLs identified in an advanced backcross population of *L. esculentum* x *L. pennellii* also had orthologs in the related *Solanaceous* species, pepper and eggplant (Frary *et al.* 2004). Holtan and Hake (2003)

identified 30 QTLs that contribute to morphology and characteristics of the mature tomato leaf by using the publicly available introgression line library of *L. esculentum* and *L. pennellii* (Eshed and Zamir 1995). The majority of QTLs showed additive effects while five QTLs showed over-dominance.

The domestication of tomato has led to increased productivity and quality, but at the same time it has narrowed the gene pool (Peel and Rasmusson 2000, Simmonds 1993, Tanksley and McCouch 1997, Vello 1984). Related wild species of cultivated tomato have contributed natural variation to cultivated tomato for many beneficial traits including disease and pest resistance (Bai *et al.* 2004, Bai *et al*. 2003, Moreira *et al*. 1999); yield (Eshed and Zamir 1994b, Eshed and Zamir 1995, Lindhout *et al*. 1994, Monforte and Tanksley 2000b, Nesbitt and Tanksley 2001, Van der Knaap and Tanksley 2001), growth and associated traits (Fulton *et al*. 1997b, Hoogenboom *et al*. 2004, Monselise *et al*. 1978), or fruit quality (Causse *et al*. 2001, Fridman *et al*. 2002, Grandillo *et al.* 1999, Saliba-Colombani *et al*. 2001, Van der Knaap *et al.* 2002, Van der Knaap and Tanksley 2003). Backcross inbred lines (BILs) which contain a single homozygous introgression of a wild species have proven to be an extremely useful tool to study the effects of such introgressions. BILs have already been proven to be useful tools for genetic studies (as described above). In the present study, we are interested in growth and development of greenhouse tomatoes. As the publicly available BIL libraries have an industry tomato genotype as recurrent parent and this genotype is not adapted to the greenhouse conditions in North-West Europe, we developed our own set of BILs.

We developed a set of BILs and investigated the contribution of homozygous chromosome segments of *L. pennellii* LA716 to plant growth physiology in greenhouse tomato.

## Material and methods

### *Development of backcross inbred lines*

The wild relative *L. pennellii* LA716 was used as pollen parent in an interspecific cross with *L. esculentum* cv. Moneymaker. A single $F_1$ was backcrossed with Moneymaker serving as recurrent parent to obtain $BC_4$ (Figure 1).

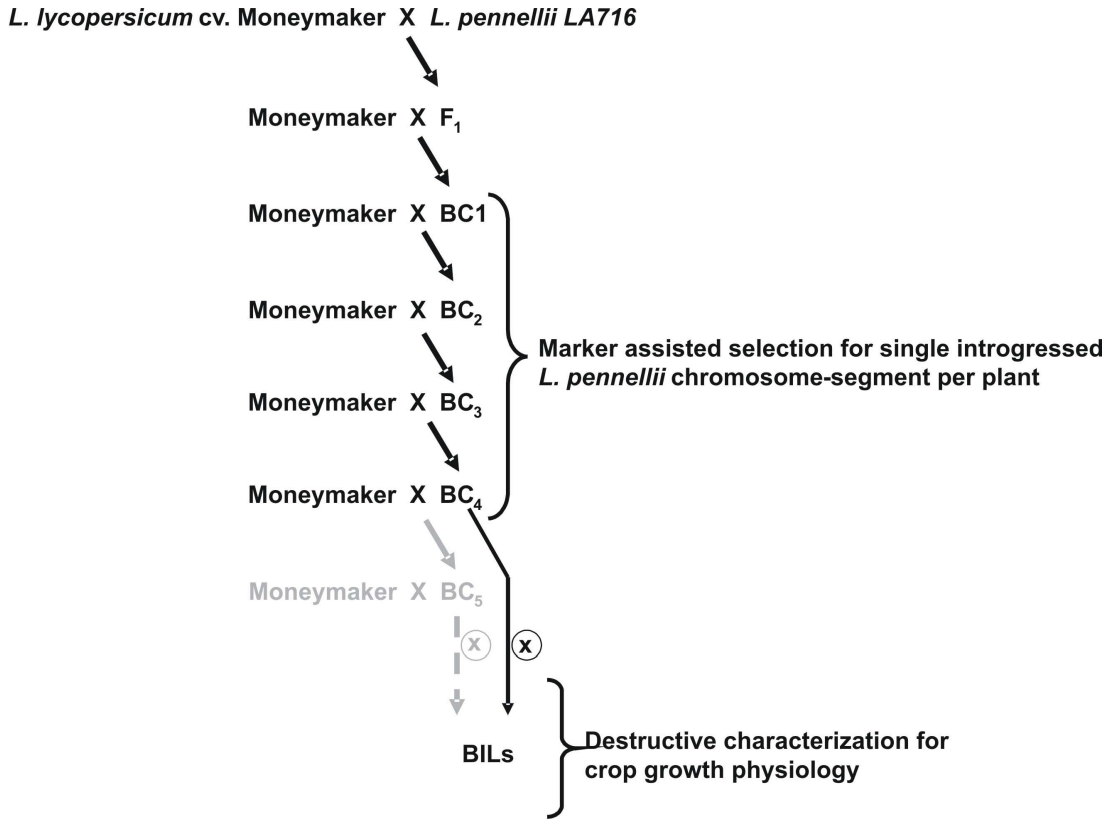



**Figure 1. BILs are obtained through several generations of backcrossing with the recurrent parent starting from a single $F_1$ individual. Plants which are selected to contain only one introgression are selfed to become homozygous. Intentionally, each BIL contains one introgression segment and a complete set of BILs should cover the complete genome of the wild species. The grey pathway is necessary to obtain a full set of BILs.**

Each selection was preceded by marker aided genotyping based on AFLP markers. DNA sampling and AFLP analysis were done as described by Jeuken and Lindhout, (2004). Marker aided selection started at the $BC_1$.

From the $BC_4$, 192 individuals were genotyped taking advantage of the published map of *L. pennellii* (Haanstra *et al.* 1999) using 355 AFLP markers. The Graphical Genotype Software (GGT) (Van Berloo 1999, http://www.dpw.wageningen-ur.nl/pv/) helped to reveal the number and size of introgressions in each plant per generation. Plants with a single introgression were selected for a generation of selfing. Meanwhile plants with more than one introgression (pre-BILs) were selected for backcrossing. Attempts were made to recover any lost chromosome fragments using seeds from earlier generations of backcrosses. The crossing scheme for the development of backcross inbred lines is shown in Figure 1.

***Selection of BILs for growth physiology analyses***

The growth and development of 12 BILs were analyzed in a preliminary study in the period June – November 2003. The seven BILs with the most contrasting developmental patterns, compared to Moneymaker, were selected for a more thorough growth analysis.

***Experimental conditions design***

A homogenous fraction of *L. pennellii* LA716 BILs and Moneymaker seeds were sown on the 29$^{th}$ of December 2003 in a growth chamber at Wageningen University. Seedlings emerged from the soil in the dark at 22 - 25$^{o}$C and 70-80% air humidity 12 to 14 days after sowing (DAS). On 15 DAS the 80 most homogenous useable transplants were potted in 15 x 15 cm rock-wool blocks. Potted seedlings were transferred to a greenhouse compartment and spaced at a plant density of about 3.5 plants per $m^2$. The day to night temperature was set at 19/14$^{o}$C at a relative humidity of 70%. For the following 19 days no artificial light was given to the seedlings. Thereafter, plants were cultured following normal Dutch greenhouse standards. On DAS 38 a homogeneous sample of 64 plants per BIL was placed on tables according to a randomized block design with eight blocks and eight plants of each line per block. At DAS 43, 50, 57, 64, 71, 78, 85, and 92 one randomly assigned plant per line from each plot was harvested and measured destructively.

***Destructive characterization of growth traits***

The LN and the ratio leaf to truss were measured to determine the plastochron index. Leaf and truss appearance rate were used as an estimate of the stage of plant development. Plants were further characterized for LL, LA, LW, SL, SG, SW, and W as described by Heuvelink (1995a). The W was the sum of all the above-ground plants organs including fruits. After each harvest the remaining plants were replaced at equidistant positions to avoid shading effects.

***Statistical analysis and computation of biological variables***

Growth curve parameters for increases in ln(LN), ln(LL), ln(LA), ln(LW), ln(SL), ln(SG), ln(SW) and ln(W) per plant were calculated assuming a quadratic relationship in time. Curve parameters and statistical tests were calculated as described by Owona *et al.,* (chapter 2).

To avoid large residuals, the values for eight blocks were averaged. The growth period of the entire investigation (DAS 43-92) was split into two sub growth periods (DAS 43-71, and DAS 64-92) as described by Owona *et al.,* (chapter 2). Independent growth curves per plot and per growth period were fitted to the plant dry weight data according to a second order polynomial function as described by (Lindhout *et al*. 1991).

LAR can further be decomposed into specific leaf area (SLA), a measure of leaf thickness, and leaf weight ratio (LWR), a measure of the leaf weight fraction of the shoot:

$LAR = SLA * LWR$, or

$$\frac{LA}{W} = \frac{LA}{LW} * \frac{LW}{W}$$

where LW is the leaf weight.

The RGR, NAR, LAR, SLA, and LWR were calculated at a fixed value of W, i.e. [ln(W)=3]; this value was covered by the three overlapping growth periods investigated. ANOVA was applied to these estimates; the genetic and the residual variance were calculated. Correlations between RGR, NAR, LAR LWR, and SLA were calculated between lines at fixed dry weight (i.e. ln(W) = 3).

### *Analysis of QTL effect contributed by L. pennellii introgressions*

The advanced backcross QTL mapping method (Tanksley and Nelson 1996) that tests the QTL effects contributed by alien chromosome-segment was used to characterize BILs for crop growth physiology. The statistical significance of the trait values of each BIL as compared to Moneymaker was determined using the non-parametric Wilcoxon rank sum test. All calculations were done in Genstat (Payne 2004) based on the full data set collected from BILs and Moneymaker control.

## Results

### *Backcross inbred lines (BILs)*

A set of 12 BILs and 31 pre-BILs were selected (Figure 2). The selected pre-BILs cover at least 98% of the exotic genome in overlapping introgressions and contain on average two to five additional non-target introgressions. A chromosome-segment about 8.5 cM long at the top of Chromosome 1 was not represented in this population. The fraction of *L. pennellii* regions in pre-BILs ranged from 12.5% to 4% with an average of 5.1%.

### *Selected BILs for this study*

Twelve BILs were selected for preliminary growth studies. Eleven contained a single homozygous introgression while BIL9.2/10.2 contained two introgressions. A set of seven BILs representing the widest genetic variation was further used in this study. They covered 30 - 35 % of the genome of *L. pennellii* LA716. The introgression lengths were estimated from the reference map of Haanstra *et al.* (1999) and ranged from 31 to 53 cM. The percentage of the *L. pennellii* LA716 chromosome segments in the BILs varied between 2 – 4% (Table 1).

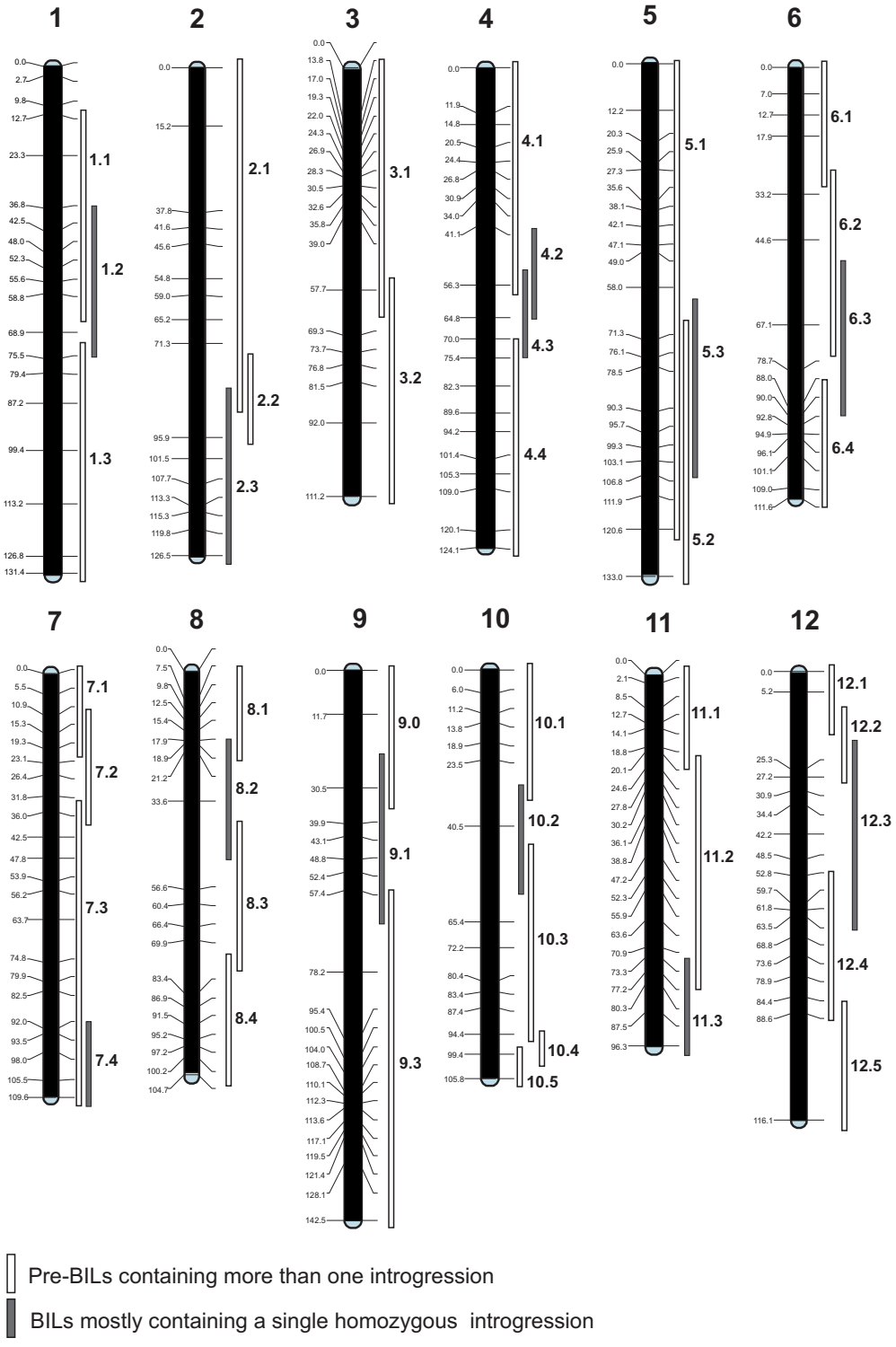



**Figure 2. A set of 12 BILs and 30 pre-BILs. *L. pennellii* LA716 genome coverage is > 98% according to GGT. The black bars represent the 12 chromosomes. Each grey bar represents the introgression segment in each BIL while the white bars represent the targeted chromosome segment in the pre-BILs. The map distances shown on the left of the chromosomes are according to the map of Haanstra *et al*. (1999).**

**Table 1. The selected BILs with specifications of the *L. pennellii* introgressions.**

| Name | Chromosome number | Introgression Locus (cM) | Introgression Size (cM) |
|---|---|---|---|
| BIL1.2 | 1 | 35 – 75 | 40 |
| BIL2.3 | 2 | 80 – 133 | 53 |
| BIL5.3 | 5 | 63 – 103 | 40 |
| BIL6.3 | 6 | 48 – 90 | 42 |
| BIL8.2 | 8 | 18 – 49 | 31 |
| BIL9.1 / 10.2 | 9 / 10 | 20 – 63 / 27 – 56 | 43 / 29 |
| BIL 12.2 | 12 | 16 – 65 | 49 |

***Differences in germination and seedling growth between BILs and Moneymaker***

There were no significant differences in seed weight and size between Moneymaker and the BILs. Small differences in germination time between Moneymaker and some BILs were observed. Seedling emergence from the soil ranged from 12 to 14 DAS. Seeds from all lines germinated 12 DAS except for BIL6.3 and BIL5.3 where it took two days longer. A close observation of phenotypic development of above ground plant organs on 18 DAS revealed no apparent differences in growth between lines. Variation in growth vigor and morphological architecture between lines became increasingly visible from 22 DAS onward (Figure 3).


**Figure 3. Variation in stem length among BILs on 22 DAS**

***Evaluation of differences in growth between BILs and Moneymaker***

The variance within BILs was very low for all the growth traits investigated, while large differences in W, LA, SL, and LW were observed between Moneymaker and BILs (Figure 4). BIL2.3 and BIL8.2 had higher W than Moneymaker while BIL12.2 had a lower W. BIL6.3 had longer SL compared to Moneymaker in contrast to BIL5.3 which had a shorter SL. The widest genetic variation in growth traits was observed in the leaves (i.e. LA and LW). BIL1.2, BIL2.3, BIL8.2 had significantly larger LA, higher LW and produced the highest amount of total biomass (DW).

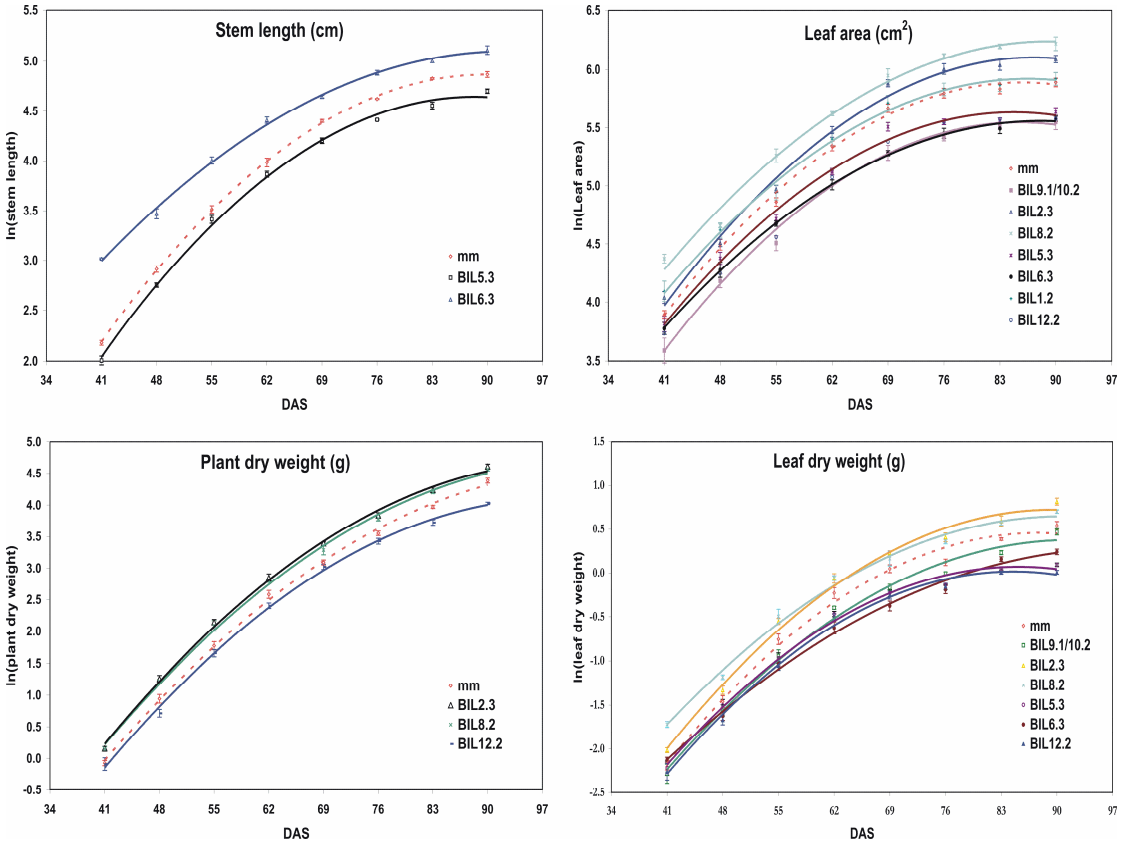



**Figure 4. Curves represent growth as modeled by a quadratic function. The pattern of growth in Moneymaker is represented by dotted lines. The confidence intervals are defined by the standard error of the means. Only curves of BILs which are significantly different from the Moneymaker curve are plotted.**

None of the BILs influenced the rate of appearance of the leaves. Three BILs namely**:** BIL5.3, BIL9.1/10.2, and 12.1 showed phenotypes that were inferior to Moneymaker in terms of growth and development. BIL1.2 contributed alleles which positively influenced LA, W, and RGR. The introgression in BIL2.3 positively influenced LA, LW, LL, W, SG, and RGR. BIL8.2 on its part contributed alleles which promoted W, LW, LA, and RGR but on the other hand was responsible for a reduction in SG and SW (see Figure 4). Overall the latter three BILs were more vigorous in growth as shown in Figure 5.

The quadratic function provided a good fit to the natural logarithm transformed data of growth traits. It explained more than 95% of the variance in the growth process during the period of investigation. Each parameter of the growth curve, i.e. level (a), initial slope (b), and curvature (c), of BILs was compared to those of Moneymaker in a univariate analysis. The slope provides a measure of the growth rate of the trait under investigation. The curvature measures the rate of change in RGR over time. A point where RGR changes fastest indicates a turning point in physiology, where a plant changes from vegetative to generative phase or vice versa.

The F-values from the Wilk's lambda test statistics in the MANOVA procedure indicated that the introgression lines significantly differed with respect to LA, LW, SL, SW and W (Table 2). Significant differences were recorded between BILs and Moneymaker for LA, LW, W and SL.

**Table 2. Analysis of the variances in some growth traits of seven BILs**

| Traits | Wilks' λ | F –calculated (P < 0.05) |
|---|---|---|
| Leaf number (LN) | 0.0521 | 1.58 |
| Leaf length (LL) | 0.1246 | 0.82 |
| Leaf area (LA) | 0.0838 | 54.58*** |
| Leaf dry weight (LW) | 0.4338 | 23.36** |
| Stem length (SL) | 0.2333 | 4.7* |
| Stem dry weight (SW) | 0.2173 | 11.11* |
| Stem girth(SG) | 0.1299 | 1.2 |
| Plant dry weight (W) | 0.1939 | 6.16* |

*, **, *** are significant at P < 0.05, P < 0.01 and P < 0.001 probability levels respectively

***Differences in RGR, NAR, LAR, SLA and LWR***

Large difference in RGR between BILs and Moneymaker were observed, except for BIL5.3. More interestingly, the pattern of change of RGR during plant development, relative to Moneymaker, also differed widely among BILs. These patterns are shown in Figure 5. Basically we observe four patterns, i.e.

(a) Initially the RGR exceeds RGR of Moneymaker ($RGR_{MM}$), but gradually approaches $RGR_{MM}$ over time. BIL2.3 and BIL6.3 show this pattern.

(b) RGR hardly changes over time, but exceeds $RGR_{MM}$ over the whole period. This applies to BIL1.2

(c) RGR equals $RGR_{MM}$ over the while interval of observation. This hold for BIL5.3

(d) RGR is well below $RGR_{MM}$, although a significant change over time may occur. BIL6.3 and BIL12.2 show such a pattern.

Considering these possible patterns, optimal growth is characterized by a high initial value of RGR, followed by a slow decrease as plant development proceeds e.g. BIL2.3, BIL1.2, and BIL8.2. Although the differences in RGR may seem to be small, it is good to realize that an increase as small as 5% in RGR may result in a two fold biomass production over a three month period.

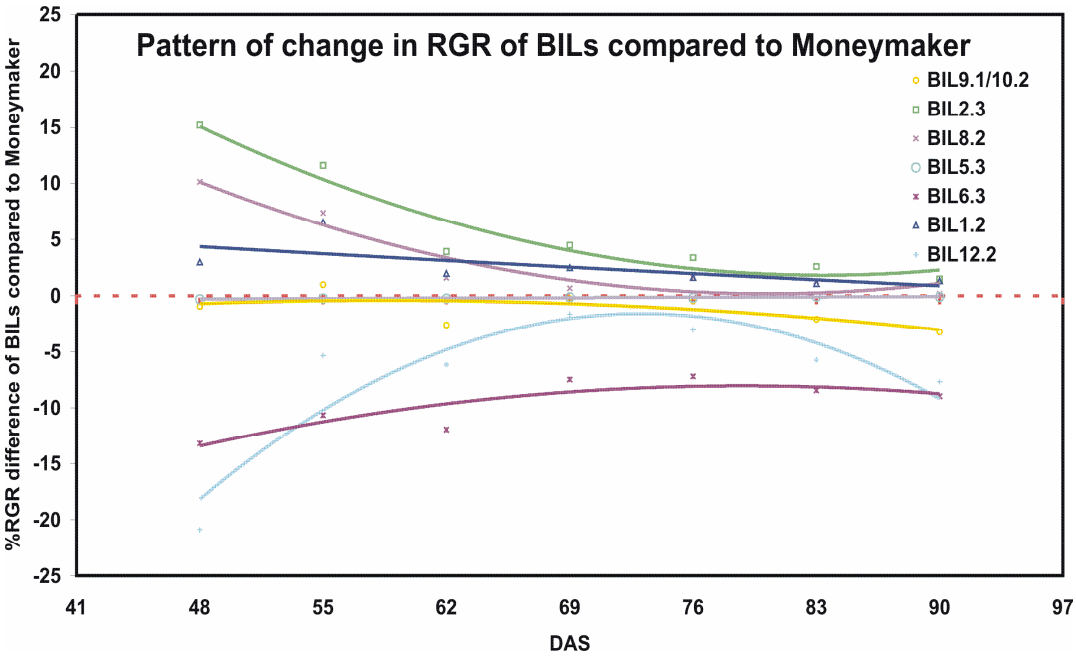



**Figure 5. Differences in RGR during plant development in BILs compared to Moneymaker. The dotted abscissa represents the RGR pattern of MM.**

Large variations in NAR, LAR, SLA, and LWR were observed between BILs and Moneymaker as well as among growth periods (Table 3). LWR did not vary significantly neither between nor within lines in the period earlier than 30 DAS. LWR among lines and between growth periods started to vary from 43 DAS till 92 DAS. These differences increased over time as plants developed and partitioned more dry matter to the stems.

Optimal growth is represented by genotypes which combine a high value of RGR with small decreases in RGR. BILs which have the latter characteristic are shown upper most in Figure 5 above the dotted abscissa which represents the RGR pattern of Moneymaker control. RGR patterns for BILs that grow worse than Moneymaker are found below the dotted abscissa. In a yield analysis BIL1.2, BIL2.3 and BIL8.2 which show optimal growth also produced the highest amount of biomass (Owona *et al*. chapter 4).

**Table 3. Growth characters of seven BILs calculated over different growth periods.**

| Lines | RGR ($g \cdot g^{-1} \cdot d^{-1}$) Growth period (d) | | | NAR $g \cdot cm^{-2} \cdot d^{-1}$ ($10^{-3}$) Growth period (d) | | | LAR $cm^2 \cdot g^{-1}$ ($10^{-3}$) Growth period (d) | | | SLA $cm^2 \cdot g^{-1}$ Growth period (d) | | | LWR $cm^2 \cdot g^{-1}$ Growth period (d) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 43-71 | 64-92 | 43-92 | 43-71 | 64-92 | 43-92 | 43-71 | 64-92 | 43-92 | 43-71 | 64-92 | 43-92 | 43-71 | 64-92 | 43-92 |
| MM | 0.129 | 0.134 | 0.115 | 0.201 | 0.209 | 0.188 | 0.542 | 0.519 | 0.519 | 190 | 150 | 230 | 0.88 | 0.57 | 0.66 |
| BIL1.2 | 0.149 | 0.138 | 0.131 | 0.228 | 0.229 | 0.206 | 0.555 | 0.527 | 0.531 | 200 | 120* | 250* | 0.89 | 0.58 | 0.67 |
| BIL2.3 | 0.187* | 0.159* | 0.127 | 0.282* | 0.274* | 0.215 | 0.548 | 0.535 | 0.543 | 250* | 170* | 250* | 0.88 | 0.57 | 0.68 |
| BIL5.3 | 0.126 | 0.124 | 0.123 | 0.178 | 0.188 | 0.212 | 0.581* | 0.543* | 0.555* | 220* | 120* | 260* | 0.89 | 0.60 | 0.69 |
| BIL6.3 | 0.108 | 0.114* | 0.143 | 0.212 | 0.191 | 0.239* | 0.594* | 0.551* | 0.567* | 210 | 110* | 290* | 0.89 | 0.51 | 0.70* |
| BIL8.2 | 0.161* | 0.148 | 0.124 | 0.223 | 0.216 | 0.195 | 0.677* | 0.559* | 0.579* | 240* | 160 | 240 | 0.95 | 0.62* | 0.71* |
| BIL9.1/10.2 | 0.121 | 0.121 | 0.109 | 0.164 | 0.218 | 0.181 | 0.620* | 0.567* | 0.591* | 220 | 130* | 230 | 0.94 | 0.63* | 0.72* |
| BIL12.2 | 0.121 | 0.119 | 0.128 | 0.161 | 0.228 | 0.236* | 0.542 | 0.672* | 0.681* | 190 | 140 | 290* | 0.94 | 0.63* | 0.74* |
| s.e.d. | 0.013 | 0.008 | 0.016 | 0.023 | 0.022 | 0.019 | 0.017 | 0.011 | 0.013 | 10 | 07 | 09 | 0.04 | 0.02 | 0.02 |

***** Significantly different from Moneymaker (MM)

BIL2.3 has a greater RGR due to a higher NAR meanwhile BIL8.2 has a greater RGR due to a higher LAR. Their SLA's are not significantly different.

### *Correlations between RGR, NAR, and LAR*

The correlation between RGR and its physiological components (NAR, LAR, SLA, and LWR) are shown in Table 4. The NAR was significantly correlated with the RGR among lines during all three growth periods.

**Table 4. The correlations between RGR and its physiological components NAR, LAR, SLA and LWR at fixed plant dry weight using independent growth curves.**

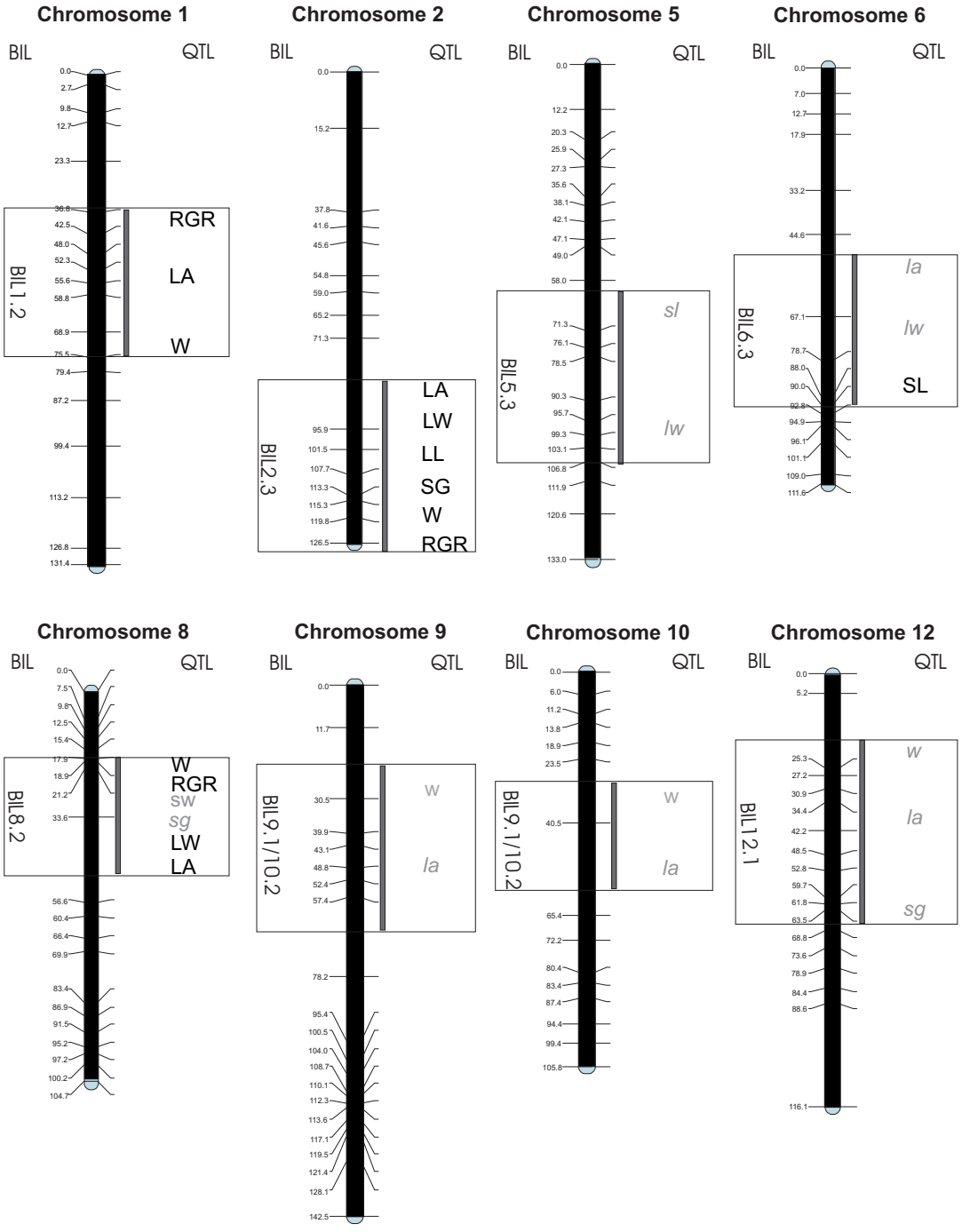
| | RGR | | |
|---|---|---|---|
| | DAS43-71 | DAS64-92 | DAS43-92 |
| NAR | 0.863* | 0.723* | 0.849* |
| LAR | 0.109 | -0.418 | 0.053 |
| SLA | 0.739* | 0.796* | 0.788* |
| LWR | -0.216 | 0.054 | -0.005 |

* = Significance at P<0.05

No significant correlation was found between RGR and LAR at any point in time. Significant correlations over time were found between W and SLA in BIL1.2, BIL2.3, BIL8.2, BIL12.2, and Moneymaker. The correlations between the profiles of SLA and W are in agreement with the findings from the dry matter production sub model of the tomato simulation crop model (Heuvelink 1999).

### *QTL analysis*

In the seven BILs that were analyzed, 27 QTLs were detected (Figure 6). BIL1.2 produced QTL effects on six traits viz. LA, W, SLA, NAR, RGR, and LAR. All the traits influenced by the *L. pennellii* chromosome-segment in BIL1.2 were favorable to growth and development. BIL2.3 positively influenced all the traits LA, LW, SLA, LL, SG, RGR, W, LAR, and NAR. BIL8.2 promoted W, RGR, LW, SLA, LA, LAR, and NAR but caused a marked reduction in SG and SW. BIL6.3 was the only BIL in the set which showed an increase in SL. On the other hand it contributed inferior alleles for LA and SL. Introgressions in chromosomes 5, 9, 10 and 12 only contributed wild alleles with a negative effect to Moneymaker.




QTLs > MM = Capital & regular
QTLs < MM = Small letter & italic

**Figure 6. The QTL effects observed in each BIL is attributed to the presence of the alien chromosome-segment. The locations of *L. pennellii* LA716 introgressions (I) are comparable to positions in the published map of Haanstra *et al*. (1999). Traits with significant (P<0.05) changes are indicated with abbreviations. Bold = *L. pennellii* allele increases the trait value; small, & italics = *L. pennellii* allele decreases the trait value.**

## Discussion

The present study aims at identifying QTLs contributed by alien chromosome-segments of *L. pennellii* LA716 that influence crop growth physiology in BILs and to determine how inherent differences in RGR are influenced by variations in physiological parameters. We elaborated on the past studies pertaining to tomato crop growth physiology (Heuvelink 1996, Lindhout and Pet 1990, Lindhout *et al*. 1991, Marcelis *et al.* 1998, Nieuwhof *et al.* 1991, Nieuwhof *et al.* 1993, Smeets and Garretsen 1986a) and used the same experimental and statistical approaches but now on a different set of genetic lines.

BILs immortalize genetic diversity present in the progenies of the interspecific cross between greenhouse tomato and *L. pennellii* LA716. These lines represent a genetic resource on which repeated measurements can be made to characterize a variety of interesting traits.

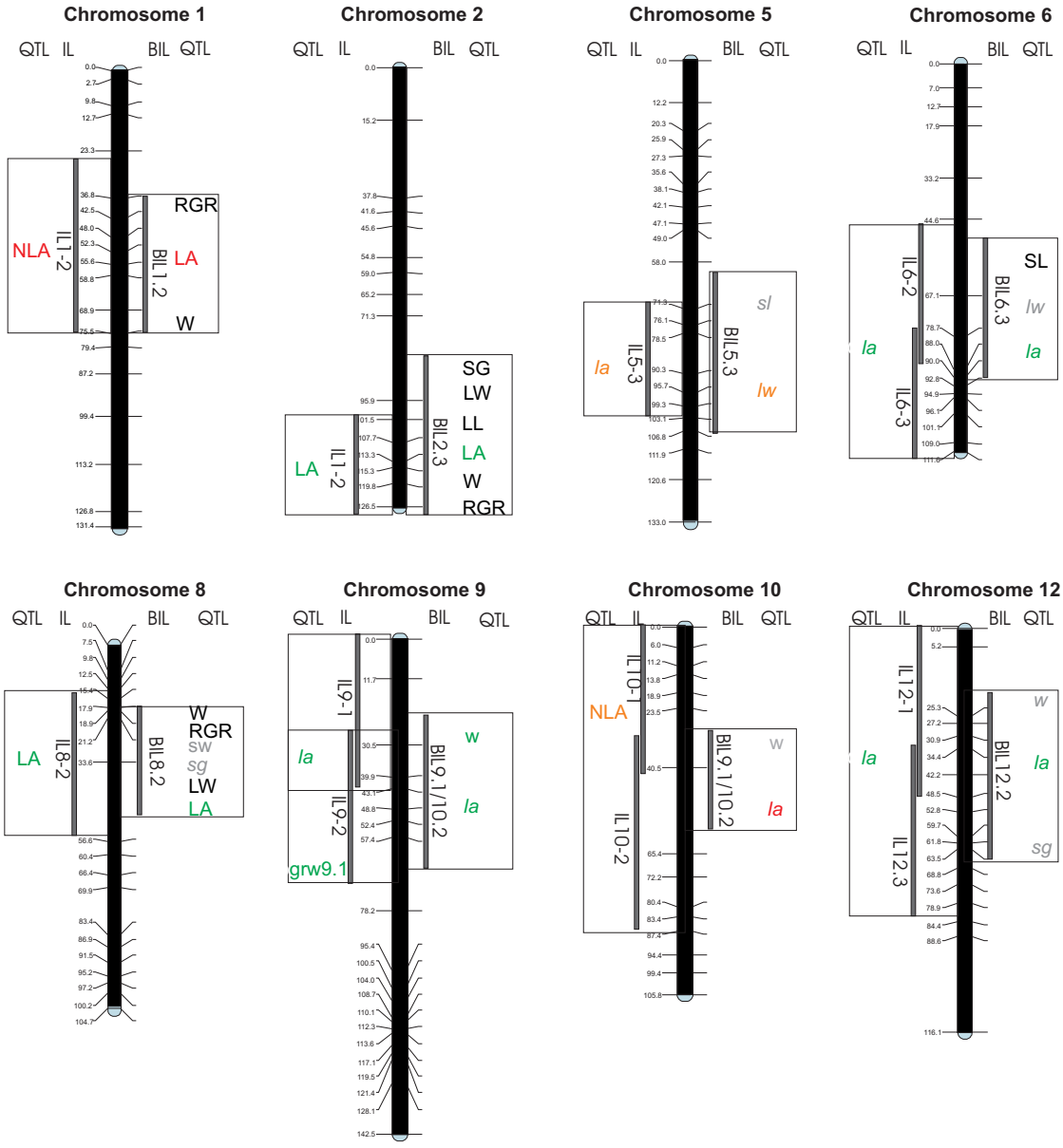
The loss of a small chromosome-segment at the top of chromosome 1 during BIL development may be caused by the absence of viable pollen in plants containing this introgression. However this did not occur on chromosome 1 in the population of Eshed and Zamir (1994a) but rather they were unable to obtain an introgression line containing the *L. pennellii* chromosome segment at the top of chromosome 8. Uniformity in the size of germinating seeds is important for obtaining a homogeneously growing population. Differences in growth occurred among tomato genotypes due to inherent variation in seed weight (Nieuwhof *et al.* 1989).

Although the BILs in the present study have only limited genome coverage, six out of seven showed a change in at least one of the investigated growth traits. All together, they revealed a total of 27 QTLs. These 27 QTLs likely represent only a subset of the real number of loci because of the limited genome coverage and the fact that in a particular BIL more than one locus (loci with the same or opposite effects) could be present. Examples of the latter phenomena was observed by Holtan and Hakes (2003) when they analyzed smaller subdivided introgression lines, QTLs which were not detected in full length lines were revealed. A similar phenomenon was

reported when BILs of *Lactuca sativa* containing introgressions of the wild relative *L. saligna* were analyzed for resistance against *Bremia lactucae* (Jeuken personal communication). Another advantage of BILs which could further be exploited is that, they are ideal breeding material to pyramid introgressions with favorable QTLs. Gur and Zamir (2004) achieved an increase in yield of up to 50% by pyramiding three independent yield promoting genomic regions from *L. pennellii* into *L. esculentum*.

Although QTLs have been frequently reported in the recent past using the advanced backcross inbred line QTL mapping method (Bai *et al.* 2004, Bai *et al.* 2003, Bernacchi *et al*. 1998a, Bernacchi *et al*. 1998b, Doganlar *et al*. 2002, Eshed and Zamir 1994b, Eshed and Zamir 1995, Frary *et al*. 2004, Fridman *et al.* 2004, Fulton *et al*. 2000, Fulton *et al*. 1997b, Gur and Zamir 2004, Kabelka *et al*. 2004a) in tomato, the present study is the first involving plant growth physiology, development and crop performance associated traits.

The introgression on Chromosome 9 of BIL9.1/10.2 in the present study covers the same chromosome region in which *grw*9.1 was identified by Fulton *et al*. (1997a) and is also associated with reduced growth. The QTL *grw*9.1 was identified in a population derived from a cross between *Lycopersicon peruvianum* LA1706 and *L. esculentum* E6203. The fact that a QTL (w) with a similar effect on plant growth as grw9.1 was identified in the present study suggests that w and *grw*9.1 could be orthologs. Orthologs had been found not only with tomato species but also among the related Solanaceous species, pepper and eggplant (Frary *et al.* 2004). Holtan and Hakes (2003) mapped QTLs controlling increments in leaf area in IL-2.6, IL-3.1, IL-8.2, and IL-8.3. The QTL for increase in leaf area in BIL2.3 is in the same regions as IL-2.6 and the one in BIL8.2 is in the same chromosome region as IL-8.2. The QTLs controlling reduced leaf area in BIL6.3, BIL9.1/10.2 and BIL12.2 also fall in the same regions as IL-6.2, IL-9.1.3 and IL-12.3.1 respectively, which are also responsible for a reduction in leaf area in Holtan and Hake (2003). All the loci which are responsible for an increase in leaf area also promote plant growth in this study. Figure 7 shows a comparative illustration of some growth QTLs identified in literature and those detected in the present study.




QTLs > MM = Capital & regular
QTLs < MM = Small letter & italic

**Figure 7. A comparative illustration of all the growth-related QTLs detected in BILs during the present study and some QTLs found in previous studies. QTLs for leaf area were identified using the publicly available introgression lines developed by Eshed and Zamir (1995b) in a study reported by Holtan and Hake (2003). Meanwhile the plant growth QTL (*grw*9.1) was reported by Fulton *et al*. (1997) in study on a population derived from an interspecific cross between *Lycopersicon peruvianum* LA1706 and *L. esculentum* E6203. The QTLs in green were confirmed in this study, those in red could not be confirmed, and meanwhile the orange ones are border and require more investigation. NLA =normal leaf area as in recurrent parent. Both *grw*9.1 and *w* on chromosome 9 represent reduced growth.**

The associations between RGR and NAR found in the present study are in agreement with the results of Nieuwhof *et al*. (1993), when they investigated tomato growth at night temperatures of 10 $^{o}$C and 14 $^{o}$C; as well as with the findings of Owona *et al.* (chapter 2) when growth was analyzed in a selection of genotypes which represented wide genetic variation in old European tomato cultivars.

The functional approach used to calculate RGR and its physiological components NAR, and LAR implies that positive correlations would normally be expected between RGR and NAR. However, when correlation analyses are done among the lines as in this paper, they are essentially determined by inherent genetic and physiological factors within each genotype. Correlation analyses showed that NAR and SLA but not LAR and LWR play a significant physiological role in plant growth within the limitations of plant developmental stages which were investigated.

According to a low temperature growth study by Nieuwhof *et al*. (1993), the dry matter content of leaves were correlated with NAR and RGR, at night temperatures (NT) of 10 - 14 $^{o}$C only, but not at NT of 6 $^{o}$C. The significant correlation found between RGR and SLA confirmed the results of Heuvelink (1999) and Marcelis *et al.* (1998) who reported associations between plant dry weight and SLA in tomato. In the same study mentioned above, Nieuwhof *et al.* (1993) reported that positive correlations were found between RGR and LAR at night temperatures of 6 $^{o}$C but not at 10 and 14$^{o}$C. The caution that these seemingly contradictory findings prompt is that an association between growth and SLA might not necessarily imply that there is a correlation between growth and LAR as well.

In an attempt to decipher this discrepancy, we investigated the variation in LWR (result not shown) which is one of the sub-components of LAR (LAR = SLA * LWR). SLA is a measure of the leaf thickness while LWR gives a ratio of the dry matter which is partitioned into the leaves. A plant which is grown at suboptimal or near chilling conditions generally develops thicker leaves (Heuvelink 1995f). The influence of LWR on the correlation between growth and LAR is minimal in juveniles because leaf to stem dry weight ratio is always close to one. In this case, the only varying factor from

the function LAR = SLA * LWR is the SLA. This might explain why growth relates to LAR in juveniles and in plants that are grown at low energy conditions. It is obvious that plants growing at near chilling night temperatures will use most of their energy in maintenance respiration. In plants that grow under normal condition the LWR is shifting away from one towards zero during plant development. For instance, in the present study the average LWR was 0.91 for 40-70 days old plants and dropped to an average of 0.58 for 60-90 days old plants because the plants have to invest more in stems and supporting tissues. One of the consequences in the shift and variation in LWR among genotypes is that the correlation between plant dry weight and LAR decreases and eventually becomes non-significant as the plants get older although RGR remains correlated with SLA at all times.

It is almost evident that barriers of important agronomic traits such as yield have been reached while using the current breeding methods. Judging from the current rate of discovery of QTLs originating from wild alleles, it is certain that wild relatives comprise huge under-exploited potentials for crop improvement. However, the majority of identified QTLs are not always implemented in applied breeding. The approach and findings of this paper underline the power of diverse BILs for high-resolution perspective on the possibility to exploit growth and physiological traits as handles for indirect selection for crop performance and productivity.

## Chapter 4

# Introgressions of *Lycopersicon pennellii* in greenhouse tomatoes may push the yield to unprecedented levels: A yield analysis

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**To be submitted for publication**

## Abstract

**Seven backcross inbred lines (BILs) derived from the interspecific cross between *Lycopersicon esculentum* cv. Moneymaker and *L. pennellii* LA716 were evaluated for yield and yield-associated traits in replicated greenhouse trials during two successive seasons. BILs were characterized for fresh fruit yield, soluble solid content, and total above ground biomass, biomass allocated to fruits, early fruit development, and fruit maturation in time. Significant variation was observed for all traits between BILs. Also, transgressive BILs were found for most traits. Overall we detected 16 QTLs with an influence on yield or associated traits seven of which gave values superior to the values of Moneymaker. Fruit yield was also evaluated in a spacing experiment viz. 3.5 plants/$m^2$ (normal) and 5 plants/$m^2$ (dense). BILs grown at dense crop spacing showed a yield disadvantage of about 7.5% per plant. In general this study underlines the potential of alleles originating from *L. pennellii* LA716 to enhance yield in greenhouse tomato and demonstrates the usefulness of BILs in genetic analyses.**

## Introduction

Economic yield in tomato is a complex trait which is affected by a combination of factors such as fruit weight, fruit size, total fruit weight and soluble solid contents or Brix (Gur and Zamir 2004). Complex traits such as yield have a continuous phenotypic distribution, implying that many genes with relatively minor effects, termed quantitative trait loci (QTLs), determine in combination with environmental circumstances, the final expression of the trait (Septiningsih *et al.* 2003). Tomato breeders have achieved tremendous gains in yield through conventional breeding (Fridman *et al*. 2004), but decades of domestication and selection have considerably reduced the gene pool in the cultivated tomato.

Wild relative species present a genetic resource that can broaden the genetic base of cultivated tomato and can be the source of useful traits (e.g. Zamir 2001). Studies in tomato have shown that the introgression of chromosome segments can considerably improve yield and sugar content (Gur and Zamir 2004). For instance, 23 QTLs for Brix and 18 QTLs for fruit mass were found in a study using a population of 50 introgression lines covering most of the *L. pennellii* genome (Eshed and Zamir 1995). Other studies identified a major QTL for fruit weight (fw2.2) originating from *L. pimpinellifolium* and *L. pennellii* which accounted for 30% and 47% respectively, of the total phenotypic variance in fruit mass in field tomato (Alpert *et al.* 1995). Again, in another study it was shown that wild alleles from *L. hirsutum* were associated with 15% increases in total yield and soluble solids and 41% improved Brix*yield (Bernacchi *et al.* 1998a, Bernacchi *et al.* 1998b).

Advances in molecular marker technologies have made it easier to identify agronomically important QTLs in related wild species and to follow the introgression into major crop species (Zamir 2001) and also to avoid unfavorable linkage drag (Tanksley and McCouch 1997). Molecular markers offer a faster and more accurate approach to breeding, since selection can now be based on genotype rather than solely on phenotype. Molecular marker maps can also be used to develop backcross inbred lines (BILs) that have a

single wild relative chromosome segment in the background of a cultivar. Examples of wild relative species used for the development of introgression lines are: *L. pennellii* (Eshed and Zamir 1994a, Eshed and Zamir 1995), *L. hirsutum* (Monforte and Tanksley 2000a) and *L. lycopersicoides* (Chetelat and Meglic 2000). In several other crops similar introgression libraries have been constructed. In lettuce Jeuken and Lindhout (2004) developed a set of BILs between *Lactuca sativa* and the wild species *L. saligna*; Von Korff *et al.* (2004) constructed two sets of introgression lines of wild barley, *Hordeum spontaneum*, in two different spring barley cultivars.

BILs are a powerful tool for detecting QTLs for traits that are difficult to evaluate. BILs have a high percentage (mostly higher than 90%) of the recurrent parent genome and a low percentage (mostly less than 10%) of the wild-parent genome. Therefore, all the phenotypic differences between a BIL and the cultivar are primarily attributable to the introgressed segment. BILs present the advantage that the genetic resolution of QTLs is much higher than in transient populations like $F_2$ and backcross individuals or recombinant inbred lines which segregate for multiple QTLs dispersed over the whole genome. Several QTLs can segregate simultaneously in an $F_2$ and this tends to mask individual effects since they introduce high error variances in statistical analyses, as a result of which individual QTLs can be detected only when using advanced statistical tools. Contrarily, the chance that in a single BIL masking effects of QTLs occur is a lot less, although not impossible. Numerous studies on BILs have already shown their usefulness for mapping and characterizing QTLs (Eshed and Zamir 1995, Fridman *et al*. 2004, Gur and Zamir 2004, Kabelka *et al*. 2004a, Kabelka *et al*. 2004b, Monforte *et al*. 2001, Zamir 2001). The advanced backcross inbred line approach used in the present study has earlier been used for the identification (Tanksley and Nelson 1996) and transfer (Fridman *et al.* 2004, Gur and Zamir 2004) of favorable QTL alleles from exotic stocks to elite cultivars.

In the present study the backcross inbred lines QTL analysis was extended to the cross between the wild relative species *Lycopersicon pennellii* LA716 and the greenhouse tomato cultivar *L. esculentum* cv. Moneymaker. The focus was the search for QTLs from *L. pennellii* LA716 that improve yield

in greenhouse tomato. Unlike the *L. pennellii* LA716 introgression lines developed by Eshed and Zamir (1994a) which were developed in the background of field tomato and used in several yield and QTL studies (Eshed and Zamir 1995, Frary *et al.* 2004, Frary *et al.* 2000, Fridman *et al.* 2004, Gur and Zamir 2004, Holtan and Hake 2003), the BILs used in the present study were developed in the background of a greenhouse tomato and a yield QTL analysis performed on them is being reported for the first time. The present paper describes the QTL analysis for fresh fruit yield, soluble solid content (Brix), biomass production and biomass allocation in above ground parts using seven *L. pennellii* BILs.

## Materials and Methods

### *Plant material*

A single plant of *Lycopersicon pennellii* LA716 was used as a pollen parent in an interspecific cross with greenhouse tomato *L. esculentum* cv. Moneymaker. A single $F_1$ plant was used as pollen parent in recurrent crosses with Moneymaker. Greenhouse tomato backcross inbred lines were developed following the method described by Jeuken and Lindhout (2004) with some modifications [chapter 3]. Marker assisted selection started in the $BC_1$ generation. The first BILs were obtained in the $BC_4S_1$ generation. The cumulative yield of 12 BILs was analyzed in an evaluation in the period June – November 2003. Seven BILs representing the variation in yield and interesting associated traits were selected for further analysis.

### *Greenhouse trial*

A homogenous fraction of *L. pennellii* LA716 BILs and Moneymaker seeds were sown on the 29$^{th}$ of December 2003 in a growth chamber. Seedlings emerged from the soil in the dark at 22-25$^{o}$C and 70-80% air humidity 12 to 14 days after sowing (DAS). On 15 DAS the 672 most homogenous transplants per BIL were potted in 15x15 cm rock-wool blocks. Potted seedlings were divided into two sample groups of 288 and 384 plants and transplanted to the greenhouse either at normal Dutch greenhouse

culture crop spacing (3.5 plants $m^2$) for the former group, or at dense crop spacing (5 plants per $m^2$) for the latter group. The plots for each BIL were replicated six times in a randomized block design. No artificial light was given to the seedlings/plants till 34 DAS. And thereafter, all treatments were carried out according to Dutch greenhouse standards.

***Phenotypic evaluations and QTL analysis***

Growth traits such as plant height, leaf length, leaf number, stem length, flower set, flower position, fruit set were analyzed on 43, 50, 57, 64, 71, 78, 85, 92, 118, 131, 140, 148, and 155 DAS as defined in Owona *et al.*, (chapter 3]. The plastochron index was determined at various stages of plant development as rate of appearance of above plant organs. The fresh weight and dry weight of all leaves pruned per plant were recorded. Fresh red fruits were harvested and weighed on 118, 131, 140, 148, 155, 159, 164, 172, 184, 192, 198, 210, and 218 DAS. The soluble solid content or Brix of red fruits was determined according to the method described by Eshed and Zamir (1994b) using a digital 'Minolta refractometer' (Brix, sugar percentage). During each harvest a representative sample of 40 fresh fruits per BIL was analyzed for Brix. The Brix*yield was derived as the product of the Brix reading multiplied by the red fresh fruit yield. The total end point biomass production and its allocation to fruits, leaves, and stem were analyzed on 218 DAS. For subsequent analyzes, data from six replications per BIL were averaged.

Statistical analyses were performed on Genstat (Payne 2004). Average values of the traits measured for the BILs under investigation were compared to Moneymaker. In order to analyze BILs, the statistical significance ($P<0.05$) of the trait values of each BIL as compared to Moneymaker were evaluated using the Wilcoxon rank sum test. Results are presented as percent difference from Moneymaker. The coefficient of variation (CV) for each trait was calculated by dividing the overall standard deviation by the mean. Plots of the dynamic evaluation of the percent fresh fruit yield per BIL over time were used to detect early fruit development in BILs. A QTL was assigned to an introgression segment if the trait value of the corresponding BIL was significantly different from the control (Moneymaker). When a BIL significantly

differed from Moneymaker with respect to more than one trait, the chromosome segment was 'assigned' a QTL for each of those traits.

## Results

### *BILs*

Backcross inbred lines (BILs) were obtained in the $BC_4S_1$ generation. BILs contained mostly one homozygous introgression of *L. pennellii* LA716 in the background of the greenhouse tomato Moneymaker (Figure 1) except BIL9.1/10.2 which has two introgressions (Owona *et al.* chapter 3).

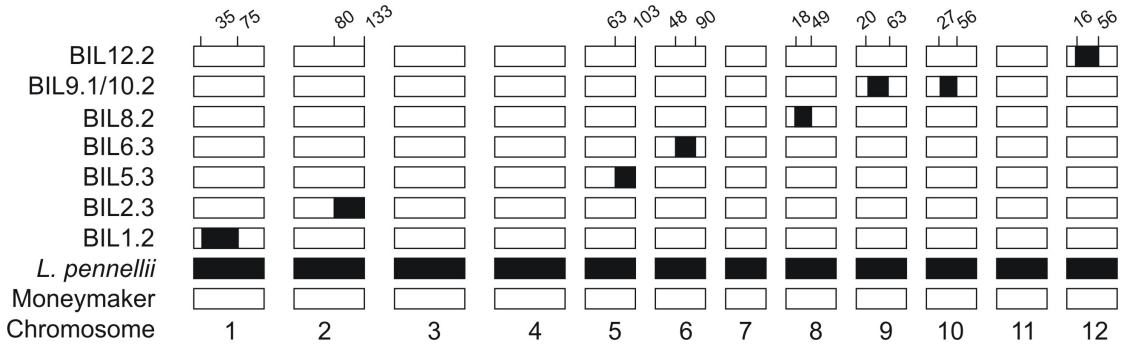



**Figure 1. A schematic illustration for the locations of *L. pennellii* LA716 homozygous introgressions. The black blocks represent segments of *L. pennellii* LA716 whereas the white background is the genome of Moneymaker.**

**Table 1. A summary of the introgression lines (BILs) showing the chromosome assignment, size of introgressions, and the percentage of *L. pennellii* LA716. The position of each introgression on the chromosome is specified in centiMorgan (cM).**

| Name | Chromosome number | Introgression Locus (cM) | Introgression Size (cM) | % of *L. pennellii* DNA |
|---|---|---|---|---|
| BIL1.2 | 1 | 35 – 75 | 40 | 3 |
| BIL2.3 | 2 | 80 – 133 | 53 | 4 |
| BIL5.3 | 5 | 63 – 103 | 40 | 3 |
| BIL6.3 | 6 | 48 – 90 | 42 | 4 |
| BIL8.2 | 8 | 18 – 49 | 31 | 3 |
| BIL9.1 / 10.2 | 9 / 10 | 20 – 63 / 27 – 56 | 43 / 29 | 4 / 2 |
| BIL12.2 | 12 | 16 – 65 | 49 | 4 |

The seven BILs used in this study cover about 35-40% of the *L. pennellii* LA716 genome. BILs contained introgressions in chromosomes 1, 2, 5, 6, 8, 9, 10 and 12 (see Table 1). The introgression length per BIL ranges from 14 to 53 cM. The centiMorgan intervals are in reference to the *L. pennellii* LA716 integrated map of (Haanstra *et al.* 1999).

### *Backcross inbred line QTL analysis*

Fresh fruit yield:

Six of the seven BILs had an influence on fresh fruit yield. The fresh fruit yield of BIL1.2 and BIL2.3 exceeded the yield of Moneymaker by 16% resp. 3% respectively. On the other hand, BIL9.1/10.2, BIL5.3, BIL6.3, and BIL 12.2 showed a decrease in fresh fruit yield relative to Moneymaker (Table 2). At dense crop spacing, BIL1.2 and BIL2.3 exceeded Moneymaker in fresh fruit yield by 15% resp. 8% while BIL9.1/10.2, BIL5.3, BIL6.3, and BIL 12.2 all lag behind Moneymaker (Table 2; Figure 2).

A dynamic plot of the percent weight of fresh fruits in BILs showed that BIL1.2 had 17% higher yield throughout most of the period of the investigation (Figure 3a). The exception was in the growth period covering 184 to 210 DAS when the fresh fruit yield decreased to 7% before rising back to 17% (Figure

3b). Several fruits produced by BIL1.2 during the period 184 to 210 DAS showed symptoms of 'blossom end-rot' (Figure 3b). All the other BILs influenced yield negatively (Table 2)

Brix: BIL2.3 and BIL5.3 had an increase in soluble solid content in mature fully ripe fruits of 20 % and 18% respectively at normal crop spacing. Brix was affected in a similar way at dense crop spacing within the margin of experimental error as measured by the coefficient of variation. The chromosome segments in all the other five BILs negatively affected Brix (Table 2).

Brix*yield: We identified BIL2.3 and BIL1.2 to be associated with an increase of Brix*yield of 25% resp. 17% at normal crop spacing respectively 23% and 16% at dense crop spacing. The rest of the BILs negatively influenced Brix*yield at both crop densities.

Total biomass production:

An evaluation of the above ground organs total biomass production showed that BIL1.2 and BIL2.3 produced 17% resp. 22% higher fresh weight at normal crop spacing. These BILs are also the highest biomass producers at dense crop spacing each producing 33% and 28% more fresh weight. (Table 2).

**Table 2. The average fresh fruit tomato yield, Brix, Brix*yield, and total above ground biomass production per plant per BIL at a normal crop spacing of 3.5 plants/m$^2$ and at a dense crop spacing of 5 plants/m$^2$. The percent mean yield parameters relative to Moneymaker are in parenthesis.**

| Genotype | Normal crop spacing (3.5 plants/m$^2$) | | | | Dense crop spacing (5.0 plants /m$^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| | **Yield in Kg per plant** *(% wrt MM)* | **Brix per plant** *(% wrt MM)* | **Brix*yield g per plant** *(% wrt MM)* | **Total biomass Kg per plant** (*% wrt MM*) | **Yield in Kg per plant** (*% wrt MM*) | **Brix per plant** (*% wrt MM*) | **Brix*yield g per plant** (*% wrt MM*) | **Total biomass Kg per plant** (*% wrt MM*) |
| MM | **8.1** | **4.5** | **368** | **11.2** | **6.6** | **4.4** | **296** | **8.9** |
| BIL1.2 | **9.4** (*17*)* | **4.4** (*-2*) | **432** (*17*)* | **13.1** (*17*)* | **7.6** (*15*)* | **4.4** (*0*) | **352** (*19*)* | **11.8** (*33*)* |
| BIL2.3 | **8.3** (*3*) | **5.4** (*20*)* | **460** (*25*)* | **13.6** (*22*)* | **7.1** (*8*) | **5.4** (*23*)* | **395** (*33*)* | **11.4** (*28*)* |
| BIL5.3 | **6.6** (*-19*)* | **5.3** (*18*)* | **352** (*-4*) | **8.6** (*-23*)* | **6.4** (*-3*) | **5.1** (*16*)* | **330** (*11*)* | **7.1** (*-20*)* |
| BIL6.3 | **6.8** (*-16*)* | **4.0** (*-11*)* | **278** (*-24*)* | **11.1** (*-1*) | **6.5** (*-2*) | **4.0** (*-9*)* | **267** (*-10*)* | **9.4** (*6*) |
| BIL8.2 | **8.1** (*0*) | **4.3** (*-4*) | **354** (*-4*) | **11.5** (*3*) | **6.7** (*2*) | **4.3** (*-2*) | **296** (0) | **8.9** (0) |
| BIL9.1/10.2 | **6.3** (*-22*)* | **4.2** *(-6*) | **274** (*-25*)* | **9.3** (*-17*)* | **6.1** (*-8*)* | **4.3** (*-2*) | **273** (*-8*)* | **7.7** (*-13*)* |
| BIL12.2 | **7.4** (*-9*)* | **4.3** (*-4*) | **333** (*-10*)* | **10.2** (*-8*) | **7.1** (*8*) | **4.3** (*-2*) | **315** (*6*) | **7.9** (*-11*)* |
| CV (%) | 13.7 | 7.2 | 13.3 | 12.1 | 14.0 | 6.9 | 19.9 | 10.4 |

wrt = relative to; MM = Moneymaker; CV = Coefficient of variation

* = Significant diff. (P<0.05)

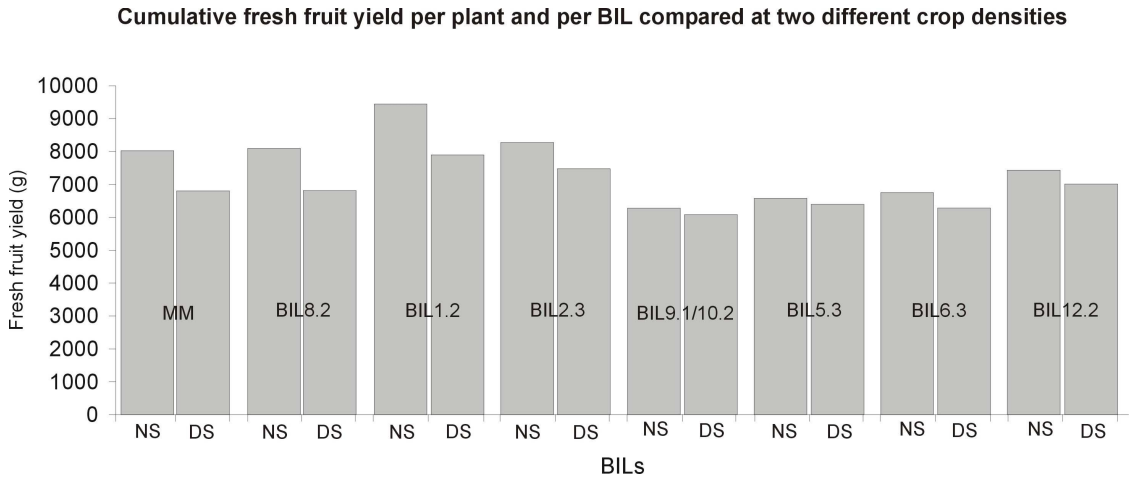



**Figure 2. The cumulative fresh fruit yield (g) per plant of BILs at normal crop spacing (NS) compared to yield at dense crop spacing (DS).**

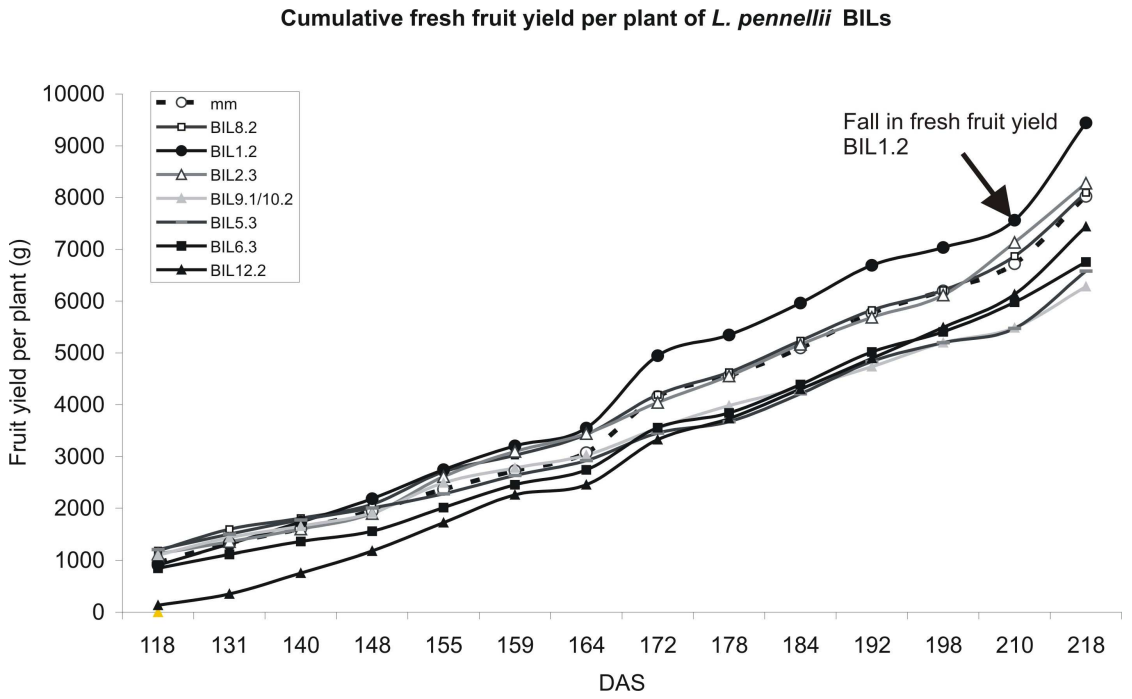



**Figure 3a. Graphs illustrating the dynamic cumulative yield of BILs. Of all BILs analyzed, only BIL1.2 yielded significantly more than Moneymaker. The increase in yield for BIL1.2 was 17% while BIL2.3 showed a non-significant 3% rise in yield. All the other BILs were inferior to Moneymaker. All the fruits harvested during the period of decrease in yield (shown by the arrow) showed various degrees of severity of 'blossom end-rot' symptoms.**

Comparing fruit yield to the total biomass produced in above ground organs:

A comparative analysis between fresh fruit yield and total biomass accumulation in the above ground organs showed that BIL1.2, which is the highest fresh fruit producer (16% more than Moneymaker), is not the highest in total above ground fresh weight (17% more biomass). BIL2.3 which yields 3% more fresh fruits than Moneymaker produces 22% more above ground biomass. The comparison between fruit yield and total above ground biomass production in BIL1.2 is depicted in Figure 4.

**Figure 3b. A sample of fruits affected by blossom end-rot harvested from BIL1.2. Decay is visible on the blossom end of the fruits. Symptom of the physiological disorder is a small darkened or water-soaked area around the blossom end of the fruit, appearing at the time the fruit begins to ripen.**

## Comparative analysis of crop performance at high crop density

Spacing BILs at a high crop density of 5 plants / $m^2$ caused an average fresh fruit yield reduction of 7.5% per plant. Plant growth was strongly influenced by plant density, the highest plant growth rate occurring at normal crop density of 3.5 plants / $m^2$. On the other hand, plant development was not influenced by plant density. Although fresh fruit yield produced per plant was lower at high crop spacing, the total biomass produced was higher. Biomass allocation to the above ground organs was unaffected by crop spacing.

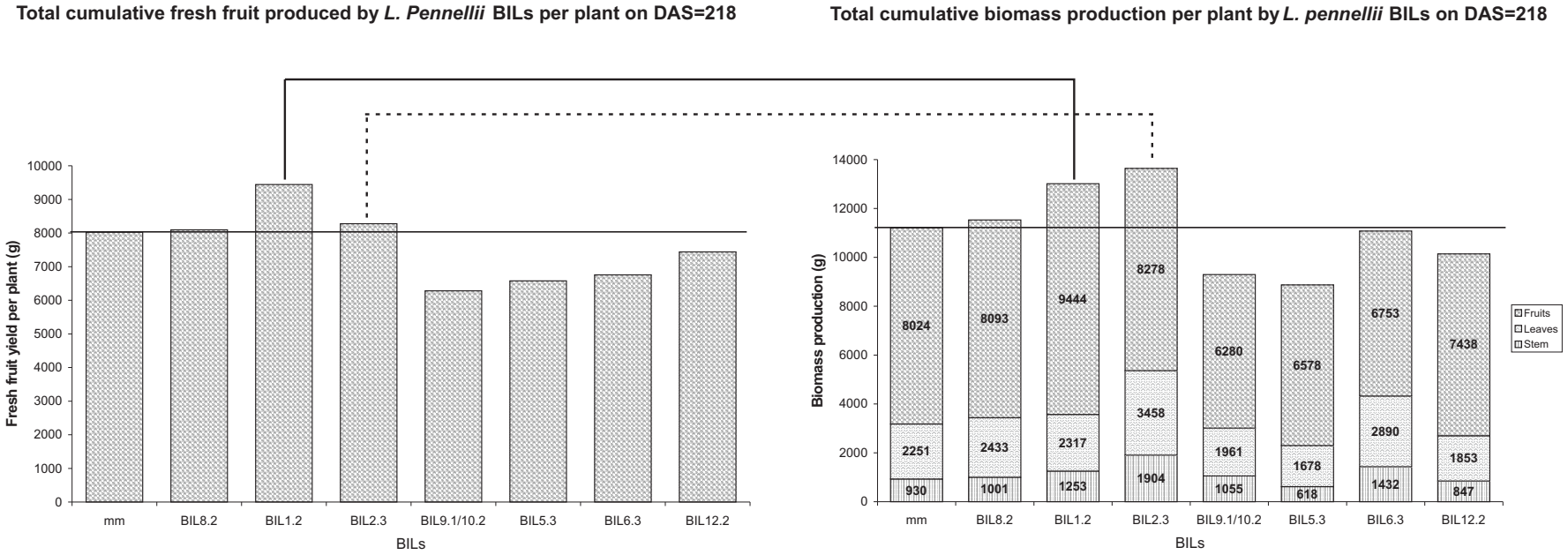
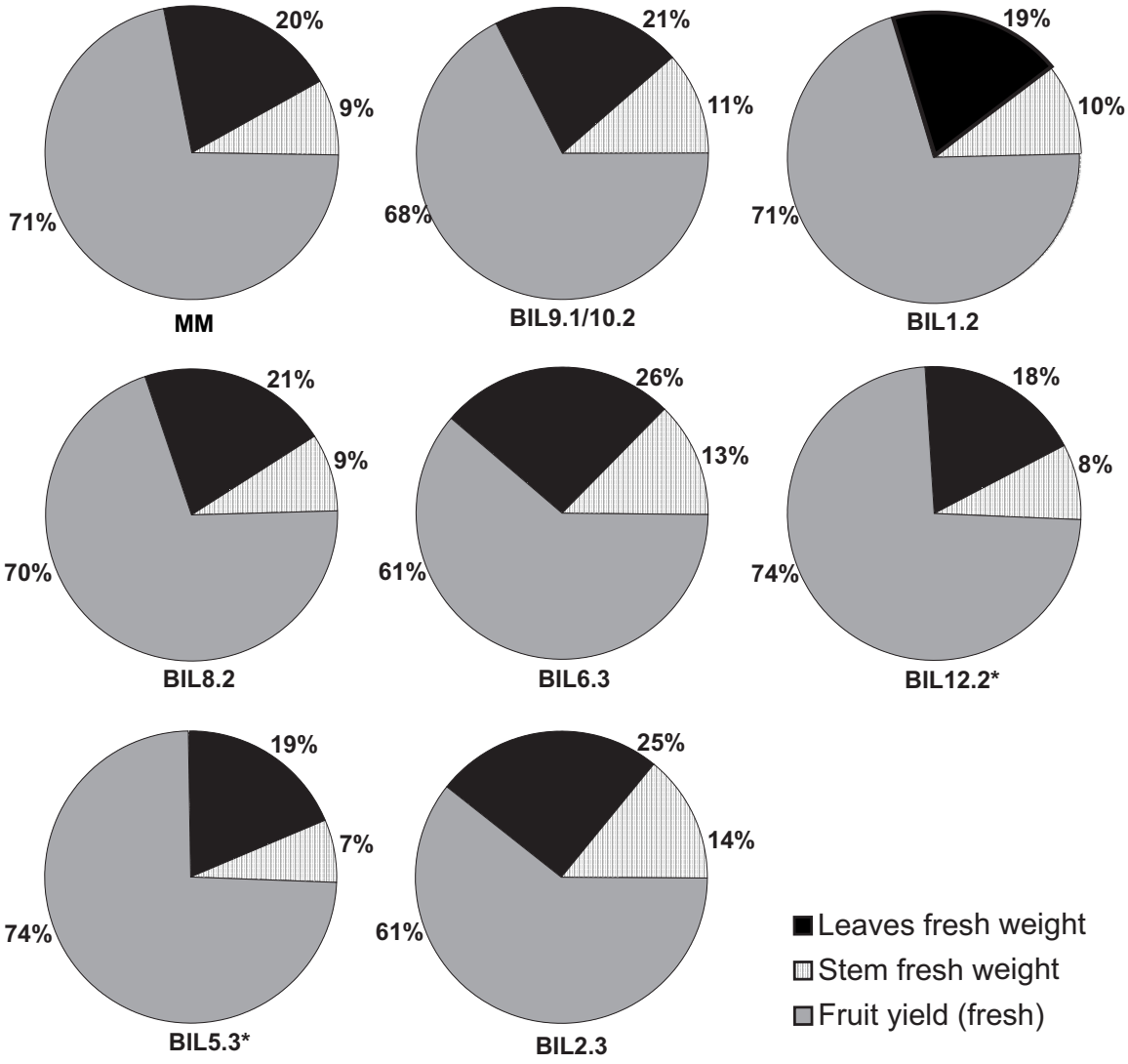



**Figure 4. This comparative figure depicts the relationship between fresh fruit yield and total above ground organs biomass production per plant per BIL. The lines bridge the cumulative fresh fruit yield (left) to the cumulative total biomass produced in the above ground organs (right) in BILs on 218 DAS. The bar charts for total biomass production (right) are segmented into biomass (g) allocated to fruit, leaves, and stem.**

Total biomass allocation:

BILs vary also in the endpoint biomass partitioning into fruits, leaves and stems. Figure 5 shows pie charts indicating different percentages of biomass allocated to fruits, leaves and stem. BIL12.2 and BIL5.3 allocate the highest level of biomass to the fruits. BIL1.2 and BIL8.2 show an allocation pattern similar to Moneymaker, while all the other BILs allocate less to the fruits. Allocation to the fruits is significantly higher ($p < 0.05$) in BIL5.3 and BIL12.2 and lower in BIL6.3 and BIL9.1/10.2.




* =Significantly higher than Moneymaker

**Figure 5. Allocation patterns of biomass to the fruits, leaves and stem in seven BILs and Moneymaker**

Earliness:

A study over time revealed that some BILs yield earlier than others. Although BIL8.2 is not the highest fresh fruit producer throughout the entire growth period, it produces the highest yield in earlier phases of harvest [i.e. from 118 DAS (25%) till about 145 DAS (14%)] before dwindling down to Moneymaker level at 184 DAS. BIL5.3 showed early fresh fruit yield but its yield fell sharply between 118 and 140 DAS. BIL12.2 was late in producing fruits and that BIL6.3 steadily produces on average 18% less fresh fruit.

**Overview of QTLs revealed in *L. pennellii* LA716 BILs**

Six out of seven BILs under investigation in this study differed in at the least one yield or yield-associated trait from the control variety Moneymaker. Only in BIL8.2 this was not the case, here a QTL controlling earliness was inferred. So, most introgression segments harbored more than one QTL. The chromosome segments corresponding to BIL1.2, BIL2.3, BIL9.1/10.2 and BIL12.2 each harbored two QTLs and those of BIL5.3 and BIL6.3 even three QTLs. The QTLs per chromosome-segment of *L. pennellii* LA716 are shown in Figure 6.

An important aspect of our analysis, as revealed by Table 2 and Figure 6, is that some *L. pennellii* genome fragments have an increasing effect on a particular trait, whereas other such segments have a deceasing effect. In particular, for the traits fresh fruit yield and biomass allocation to fruits we see this dispersion of favorable alleles over the parents (cf. Figure 6). Even more interestingly, we see that two introgression fragments may have similar effects on one trait, whereas the same two segments have opposite effects on another trait (e.g. BILs 6.3 and 12.1 with respect to fruit yield and biomass allocation to fruits).

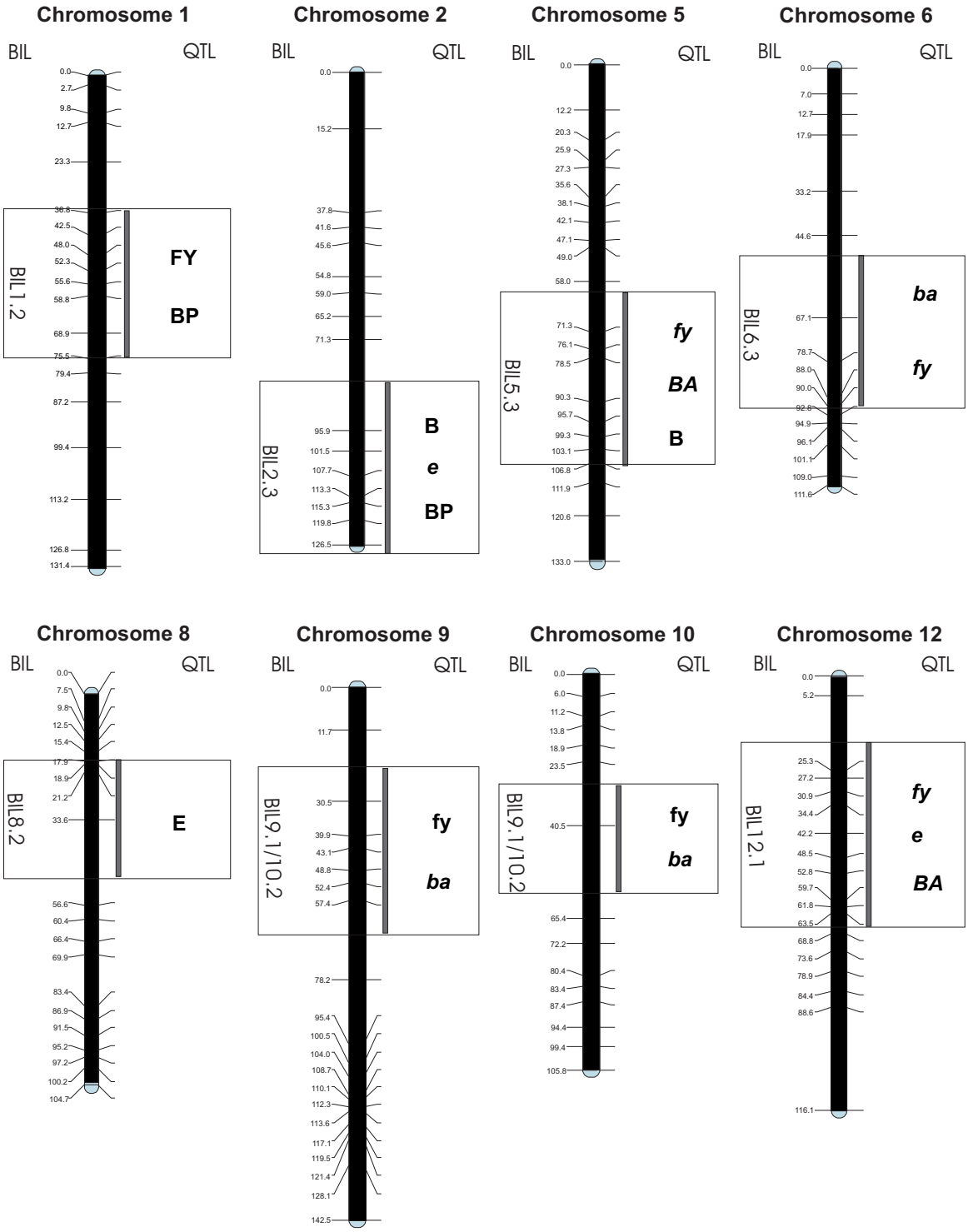
**Discussion**

This study made a characterization possible of genetic factors (QTLs) that control yield and yield-associated traits in *L. pennellii* LA716 BILs. The comparison of the mean trait values in BILs with the recurrent parent

Moneymaker revealed that some of the introgressions significantly influenced several of the traits evaluated in this study. The QTL analysis showed a total of 16 QTLs with an effect on yield and yield-associated traits (Figure 6). This is a relative high number considering the limited *L. pennellii* LA716 genomic coverage by the introgression lines and the possibility that some chromosome segments might contain QTLs with opposite allelic effects. On the other hand, when a chromosome segment had an effect on more than one trait, we counted this as separate, multiple QTLs. Because of the possible pleiotropic effects of a QTL on related traits, this way of 'assigning' QTLs may have resulted in an overestimation of the true number of QTLs.

In a QTL analysis using the publicly available introgression lines of *L. pennellii*, Holtan and Hakes (2003) detected QTLs for leaf dissection in tomato when using smaller subdivided introgression lines which were not detected in full length introgression lines. Since our introgression segments are (still) fairly large, we speculate that subdivision of the BILs into smaller chromosome segments might also reveal more QTLs.

The *L. pennellii* chromosome-segment in BIL1.2 which accounted for a 17% increase in fresh fruit yield had earlier been associated with a significant increase in percent green fruit weight and a non significant increase of Brix in IL-2 (Eshed and Zamir 1995). A comparative analysis of markers flanking IL-2 with marker positions on the published map of Haanstra *et al*. (1999) showed that the IL-2 introgression is smaller than our BIL1.2 introgression. The QTLs detected in BIL2.3 were also in agreement with those identified in the studies of Eshed and Zamir (1995). Alleles of *L. pennellii* in IL-2-6, which is a smaller introgression on chromosome 2 than BIL2.3, were also associated with a significant increase in Brix. The introgression containing *fw*2.2 which is flanked by the molecular markers CD66 and TG361 (Nesbitt and Tanksley 2001) is part of BIL2.3. However, the tremendous increases in fruit size and weight associated with *fw*2.2 were not observed in the present study. This might be caused by the fact that introgression in BIL2.3 is about 10 times larger and might contain many donor genes which mask the effect of *fw*2.2. It is interesting to note that this BIL, which will most likely contain *fw*2.2, harbored a QTL for total above ground organs biomass production.




QTLs > MM = Capital & regular
QTLs < MM = Small letter & italic

**Figure 6. An overview of the QTLs. FY=Fresh fruit yield; BP=Biomass production in above ground organs; B=Soluble solid content (Brix); BA=Biomass allocated to fresh fruits; E=Earliness.**

The chromosome-segment in BIL5.3 corresponds to IL5-3, IL5-4, and the top part of IL5-5 (Eshed and Zamir 1994a, Eshed and Zamir 1994b, Eshed and Zamir 1995). BIL5.3 gave a significant increase in Brix, like IL5-4 did. A negative relationship was found in tomato between fruit yield and soluble solid content (Steven and Rudich 1978). This association was confirmed in an advanced backcross QTL analysis of a cross between *L. esculentum* and *L parviflorum* (Fulton *et al.* 2000). We found a similar relationship in this study (see Table 2). Although several QTLs which were detected in the population of Eshed and Zamir (1994a) were also detected in our BILs, it is evident that each introgression is unique and might reveal new or different QTLs

The two QTLs for biomass production (BP) revealed by BIL1.2 and BIL2.3 as well as the two biomass allocations (BA) QTLs of BIL5.3 and BIL12.2 could be of importance in reaching better yielding cultivars. It will be interesting to combine the introgressions enhancing BP and BA and to study the combined effect of high biomass production and efficient allocation to fruits. Gur and Zamir (2004) achieved an increase in Brix*yield of up to 50% by pyramiding three independent yield promoting genomic regions from *L. pennellii* into *L. esculentum*. While evaluating the beneficial perspectives for fresh fruit yield using a pyramiding approach to combine QTLs for BA in BIL2.3 and BP in BIL12.2 into the same genome, a tempting calculation revealed that it would be possible to achieve as much as a 47% increase in fresh fruit yield in greenhouse tomato.

The fact that we were able to confirm QTLs contributed by *L. pennellii* into cultivar M82 (field tomato) in our study shows that these QTLs most likely have the same effects in different tomato backgrounds (field vs. greenhouse). Introgressions from *L. pennellii* LA716 which significantly improved soluble solid contents in red fruits of *L. esculentum* cv M82 acted similar in the genetic backgrounds of two other varieties viz. *L. esculentum* cv A7 *and L. esculentum* cv A8 through a two years testing period (Eshed and Zamir 1994b).

Plant density influences light interception per plant and therefore source strength. Reduced fresh fruit yield and plant growth at dense crop spacing as observed in the present study could be explained by reduced light

interception. In the present study the sink strength measured by the number of fruits was similar at both plant densities. All our results are in agreement with previous findings. For instance, De Koning and De Ruiter (1991), and Heuvelink (1995d) also found that plant growth is strongly reduced at dense crop spacing. De Koning and De Ruiter (1991) reported higher dry matter production per unit area at higher densities despite a decline in the performance of individual plants. In a study to assess the effect of plant density on biomass allocation to the fruits in tomato at different plant densities, Heuvelink (1995d) found that biomass allocation to the fruits was not influenced by plant density. Heuvelink (1999) found similar results while simulating dry matter distribution, based on the sink strength of the plant organs, which was quantified by their potential growth rate.

Of all the BILs investigated, only BIL1.2 showed symptoms of blossom-end rot (BER). BER is a physiological disorder that occurs not only in tomatoes, but also in peppers, eggplant, and some melons. This non-parasitic disorder can be very damaging, with losses in fresh fruit yield of 50% or more (http://ohioline.osu.edu/hyg-fact/3000/pdf/3117.pdf). BER is widely assumed to be a symptom of calcium (Ca) deficiency in the fruit. However, the induction of BER in modern glasshouse tomato production is rarely caused by insufficient Ca (Ho and White 2005). More often, BER occurs in plants with an adequate Ca supply when grown under conditions that either (a) reduce the transport of Ca to rapidly growing distal fruit tissue or (b) increase the demand of the distal fruit tissue for Ca by accelerating fruit expansion (Ho *et al.* 1998). These two reasons seem to be the best explanation for the occurrence of BER in BIL1.2. BIL1.2 was also one of the fastest growing BIL; In a growth analysis using the same set of BILs analyzed in the present study, BIL1.2 was the second fast growing one (chapter 3).

The present study has enabled us to see that a subset of BILs with limited genome coverage and consisting only of first generation BILs positively influenced several important agronomic traits such as FY, BP, B, E, and BA. This shows that wild alleles from *L. pennellii* LA716 present very promising perspectives for the agronomic improvement of greenhouse tomato.

# Chapter 5

## General discussion

**Biodiversity: A prerequisite for crop improvement**

*Lycopersicon pennellii* LA716 is a wild relative of the domesticated tomato and presents enormous potentials for genetic improvement of tomato. Its easy crossability to tomato and the knowledge that it contains several yield enhancing genetic factors made it an interesting candidate as a source of yield promoting alleles for this study.

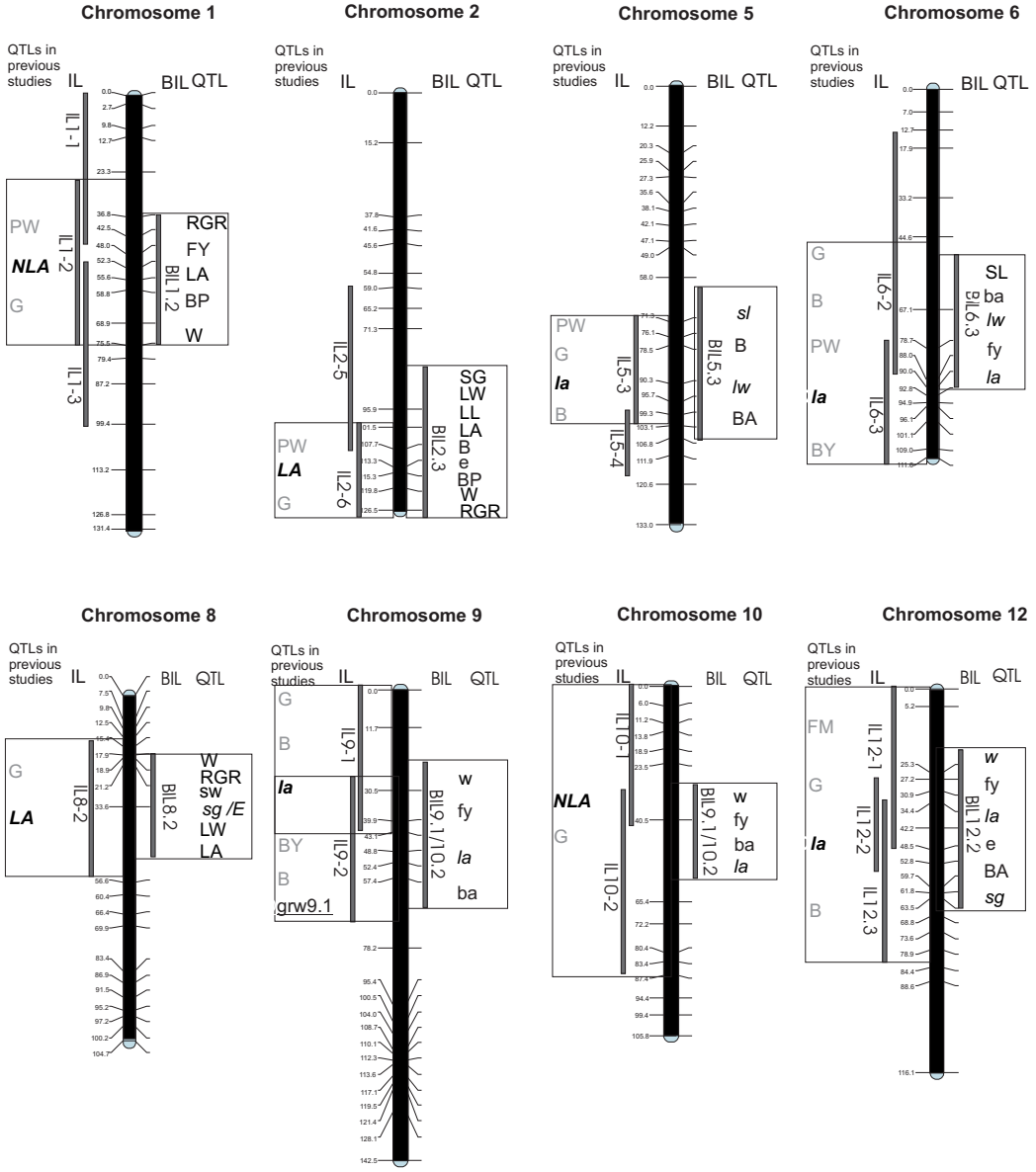
In this study we developed introgression lines containing 40% of the *L. pennellii* genome. About two thirds of these lines have been thoroughly characterized for growth physiology, yield, and yield associated traits. To obtain introgression lines for the other 60% of the *L. pennellii* genome a few more generations of backcrosses and selfing will be necessary. A full set of BILs can lead to the discovery of QTLs which were not detected in segregating populations. BILs which have been studied so far ranged in length between 31-53 cM, and each harbored four to nine QTLs

Alleles from *L. pennellii* have played an important role in the improvement of several other yield associated traits in tomato (Alpert *et al*. 1995, Baxter *et al*. 2005, Eshed and Zamir 1994b, Eshed and Zamir 1995, Fridman *et al*. 2004, Fulton *et al*. 1997a, Gur and Zamir 2004, Holtan and Hake 2003, Zamir 2001). In the present study several chromosome-segments from *L. pennellii* have been detected and associated with genetic factors (40 QTLs in total) pertaining to yield, yield-associated traits, growth and/or physiological traits. Figure 1 shows the chromosomal location of the QTLs which were identified and compares them with those identified in previous studies which utilized a similar approach or mostly used *L. pennellii* as a source of wild alleles.

Of the most relevant 28 QTLs known in literature to contribute towards yield and associated from *L. pennellii*, we were able to confirm 10 (36%) of them in this study. A few interesting examples are *PW* and *G* which where identified in introgression line (IL1-2 & IL1-2) by Eshed and Zamir (1994a) and maps in the same region as *W* and *FY* respectively in BIL1.2 which covers the same chromosome region as the former introgressions. The locus *grw9.1* which is associated with reduced plant growth on Chromosome 9 in the

cultivar E6203 was introgressed from *L. peruvianum* (Fulton *et al*. 1997a) and maps in the same chromosome region as *w* in BIL (9.1/10.2). The QTL *grw9.1* could be an ortholog of *w* but further investigations are required to confirm this hypothesis. In previous studies a major QTL known as *fw2.2* which controls fruit weight was identified in a segregating $F_2$ population derived from *L. esculentum* x *L. pimpinellifolium* and later it was shown that an ortholog is present in *L. pennellii* (Alpert *et al*. 1995). Also, several QTLs identified in different *Solanaceous species* including pepper and eggplant (Frary *et al*. 2004) have been shown to have orthologs in tomato. Therefore, the large numbers of agronomic important QTLs found in *Solanaceous species* in general represent an enormous genetic resource which could be used to improve greenhouse tomato.

The BILs used in this study contain relatively long *L. pennellii* chromosomal segments which range in length from 29 cM to 50 cM. Long introgressions may contain undetected allele(s) at different locations influencing different traits but which are masked by other alleles with opposite effects. An example of an introgression containing more than one QTL is IL1-1 which contains two independent QTLs influencing *PW* and *G* at distinct locations (Eshed and Zamir 1994b). BILs containing longer introgressions are often split into lines containing smaller chromosome segments harboring different QTLs. Often, new QTLs are revealed only after segmenting a larger introgression into near isogenic lines. Evidence if the latter phenomenon was seen when Holtan and Hake (2003) detected QTLs for leaf dissection in subdivided introgression lines which were not detected in the full length introgression line. Another phenomenon was observed in a study using BILs derived from *Lactuca saligna* (Jeuken and Lindhout 2004), where resistance QTLs were not detectable in a transient segregating $F_2$ population but became manifest in BILs. These findings show that BILs developed and used in this study may present more potential and might eventually reveal new QTLs including not only QTLs for yield and associated traits but possibly also QTLs for quality, biotic and abiotic stress




PW=Plant weight W=plant dry weight NLA=Normal leaf area SL=Stem length FY=Fruit yeild

SG=stem girth G=Green fruit weight FM=Fruit mass BA=Biomass allocation BY=Brix*Yield

E=Early fruit development RGR=Relative growth rate BP=Biomass production LL=Leaf length

LW=leaf dry weight SW=Stem dry weight GRW=Plant growth

Small letters represent QTLs for traits whose phenotypes are inferior to the recurrent parent

**Figure 1. An overview of QTLs identified in seven *L. pennellii* BILs are shown on the right of the chromosome bar compared to QTLs from previous studies shown on the left of the chromosome bar. QTLs in gray letters were identified by Eshed and Zamir (1994a). QTLs in black have been identified by Holtan and Hake (2003) using the publicly available introgression lines of Eshed and Zamir (1994a). The QTL on Chromosome 9 was identified by Fulton *et al.* (1997a) and originates from *L. peruvianum*.**

Fridman *et al.* (2004) have used a pyramiding approach to introgress short chromosome segments containing complementary alleles in to a single genome. Using the latter approach they achieved a 50% increase in Brix*yield by pyramiding three *L. pennellii* chromosome segments into cultivated variety M82. The latter approach will be tempting to use with BIL2.3 which is a high biomass producer but allocates poorly to the fruits and complement it with either BIL5.3 or BIL12.2 which allocate biomass efficiently to the fruits but yield less. A tempting calculation in terms of the fresh fruit yield for a cultivar resulting from a pyramiding of BIL2.3 and BIL5.3 or BIL12.2 was done based on the assumptions that both QTLs will fully contribute their individual effects in the same Moneymaker genome. The results showed that such a cultivar could achieve over 40% fruit yield increase.

**Yield determining traits in greenhouse tomato**

In this thesis we also studied physiological traits which underlie crop performance in terms of growth physiology and development. The objective was to identify the genetic factors which underlie physiological traits such that the latter could be used as selection criteria during tomato breeding. We started by investigating the validity of genetic differences in growth curves with respect to plant growth and its physiological component (RGR) as well as subcomponents (NAR and LAR) using old cultivars representing genetic variation in domesticated tomato throughout Europe. The results of this study (Chapter 2) show that differences and pattern of change in relative growth rate (RGR) during plant development are genotype specific. Correlation analyses revealed that genetic differences in RGR are directly affected by variations in net assimilation rate (NAR) which is a measure for the efficiency of assimilation of photosynthates to biomass. The practical implication of the correlation analysis is that NAR is an interesting selection criterion for crop performance. This finding is a stepping stone for further investigations on the possibilities to use genetic variation in NAR as a selection criterion for strong growth.

The results of the study in Chapter 2 concerning the correlation between RGR and NAR were confirmed when the eight genotypes were evaluated at slightly different conditions. The same tomato genotypes as used in Chapter 2 were used previously to study the correlation between photosynthesis parameters in relation to growth differences (Nieuwhof *et al*. 1993). According to this study, the net $CO_2$ fixation rates of leaves were correlated with NAR and RGR, but only at night temperatures of 10 and 14 $^oC$ but not at 6 $^oC$. In the same study positive correlations were found between RGR and LAR at night temperatures of 6 $^oC$ but not at 10 and 14 $^oC$. It was suggested that the observed genotypic variation in $CO_2$ fixation rates and growth probably reflected genotypic differences in responses of the stomata to the low relative humidity in the growth room. An additional explanation could be found in the fundamental equilibration of the physiological resources of the crop. It is possible that plants in the above mentioned study used most of the energy in maintenance respiration when night temperatures were set near chilling temperatures of 6 $^oC$ rather than use it to produce assimilation product building blocks to enhance growth. At such a low temperature the conversion efficiency of photosynthates would also be very low resulting into an overall slow growth. This might explain the lack of correlation between RGR and NAR at night temperatures of 6 $^oC$. In a physiological growth analysis of seven greenhouse tomato backcross inbred lines (BILs) containing introgressions of *L. pennellii* LA716 (Chapter 3) positive correlations were also found between RGR and NAR under normal greenhouse growing conditions. All the above suggest that the extend to which genetic differences in RGR could be explained by variations in NAR, LAR or other physiological components of growth depend considerably on the genotype, developmental stage of the plant and the environmental conditions under which the study is conducted.

The general tendency in our results of Chapter 2 and Chapter 3 showed that a genotype that exhibits a relatively higher initial RGR at the juvenile stage combined with small but steady decreases in RGR might present good perspectives in selection for strong growth in later stages of development. In some cases genotypes that exhibited a strong initial growth had a slower growth as older plant and *vice versa*. The results also showed

that genetic differences in RGR relate better to NAR in heavier as well as in older plants at a more advanced stage of vegetative growth. As tomato is commercially cultivated in the greenhouse for a period of one year, the influence of physiological parameters on growth and development should be studied throughout the whole year rather than an analysis of plant growth components over a restricted growth period.

Growth analysis in Chapter 3 enabled the identification of three BILs which are responsible for stronger growth. BILs 1.2, 2.3 and 8.2 showed higher RGR up to 15% (BIL 2.3). Overall the RGR decreased gradually over time. These increased RGR's effected end point biomass production up to 22%. While comparing BIL1.2 and BIL8.2, it became obvious that the pattern of change (delta RGR) during plant development played an important role in crop performance rather than just the high relative values of RGR. This was a confirmation of the observation in Chapter 2 where it was shown that a strong growth is to be found in genotypes which combine a high initial RGR value with a slow decrease of RGR over time. We expect that the introduction of a higher RGR value in combination with the rate of decrease as present in modern cultivars will result in a better growing cultivars.

In Chapter 4 the introgression lines were analyzed for yield and biomass allocation. BIL1.2 contained a locus responsible for a 17% increase in fresh fruit yield. Yield was also evaluated as total biomass produced per plant (for above ground organs). Interestingly, BIL2.3 which had a non-significant higher fresh fruit yield (only 3% higher) showed the highest increase in total biomass: 22% compared to 17% for BIL1.2. These findings suggested that there is variation for biomass allocation; an investigation of allocation patterns into fruits, stem, and leaves revealed that this was true. Figure 5 in Chapter 4 shows the biomass allocation patterns in BILs. While BIL1.2 partitioned similar as Moneymaker (see Chapter 4, Figure 5), BIL2.3 allocated significantly less biomass to the fruits. Two other BILs viz. BIL5.3 and BIL12.2 allocated a higher percentage biomass to the fruits although they are overall poor yielding lines. Pyramiding the high biomass production (BP) in BIL2.3 with a high allocation of biomass to the fruits (BA) in BIL5.3 and/or BIL12.2 gives interesting perspectives. Such an approach has already been

described by Fridman *et al.* (2004). A tempting calculation based on the BP QTL in BIL2.3 and biomass allocation (BA) QTL in either BIL5.3 or BIL12.2 shows that such a pyramiding effect might increase yield by more than 40%. Recent partitioning studies show that there is no variation in biomass allocation amongst old and modern tomato cultivars (Van der Ploeg personal communication).

**Evolution and perspectives of yield**

Tremendous improvements in productivity have been achieved in greenhouse tomato using classical and modern approaches in plant breeding. The most illustrative example is the evolution of the average fresh fruit yield per square meter in Dutch greenhouses, which increased from 38 kg in 1990 to 44 kg in 1999 (CBS 2005, De Bont and Van der Knijff 2004). At this moment a modern cultivar can produce up to 55 kg/$m^2$ of fresh fruits (anonymous personal communication). The greenhouse tomato production is one of the main heat energy sinks in glasshouse horticulture in the Netherlands. In general, better yielding varieties and improved production methods have reduced the total greenhouse area exploited for greenhouse crops by 40% from 2167 ha in 1980 to 1301 ha in 2004 (CBS 2005). On 30% of this area tomato is cultivated. Additionally, yield improvement as high as 50% might be achieved by introgressing yield-enhancing alleles from *L. pennellii* (Fridman *et al*. 2004, Gur and Zamir 2004). The wild relatives of tomato and the genetic diversity they represent will also play an important role in future developments and in fulfilling demands on new cultivars especially due to the changing greenhouse conditions.

The heat energy input is not the only cause for concern because of the cost of fossil fuels but combustion also results in production of large amounts of carbon dioxide ($CO_2$). Emission of $CO_2$ contributes to global warming (Grubb *et al*. 2002). This is the reason that open energy consuming greenhouses must change to a closed greenhouse concept that consumes less energy. The introduction of cultivars that still grow well at a wider range of temperatures as well as at higher relative air humidity will make the

implementation of a new greenhouse concept more feasible. Overall this means that there will be other demands for future cultivars and for this the use of wild relatives of tomatoes for broadening the gene pool will be inevitable. From a plant breeding perspective, the BIL approach is the best for the development of future greenhouse tomato cultivars not only because of the wide genetic diversity which they represent but also owing to the statistical power and simplicity in trait evaluation methods accompanying this approach.

In conclusion, the research started in this thesis lays down the ground work for the exploration and exploitation of useful alleles from *L. pennellii* for improvement of greenhouse tomato. Further more, it ensures the development of plant material which could be used to perform more scientific investigations to detect new QTLs, identify or clone the underlying gene(s) as well as for studying their functions and structure. These may allow an understanding of the relationship between genes underlying the QTLs. The latter will particularly be achievable when the Solanaceae Genome Project (SOL) for sequencing tomato is completed.

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## Summary

The domestic tomato has a very narrow genetic base and this makes breeding for better performance a difficult task. The wild, crossable relatives of tomato like *Lycopersicon pennellii*, *L. peruvianium*, *L. hirsutum*, *L. parviflorum* and *L. cheesmanii* present the possibility to introduce more variation in the gene pool of the cultivated tomato. An example of such an accession is *Lycopersicon pennellii* LA716. This accession is known to harbor many favorable genes, amongst them genes for yield improvement. The crossability of this accession to cultivated tomato made it an obvious candidate to use as a source for yield promoting alleles. In this thesis we present a classical approach for crop improvement while exploiting modern technological tools. The approach consists of introducing genetic variation by making crosses with related species and selecting for interesting traits in the hybrid progenies. The traditional method used for introgressing chromosome segments of *L. pennellii* LA716 is sexual hybridization using *L. pennellii* LA716 as a male parent followed by recurrent backcrossing to greenhouse tomato (*Lycopersicon esculentum* cv. Moneymaker).

In this thesis, the focus was not only on economic yield which by definition is the biomass allocated to the harvestable organs (fruits). But yield was also examined in a broader biological sense as total above ground biomass produced though out the period of growth. Another aspect of this study was to seek an understanding of growth, development and physiological processes which underlie yield. We analyzed relative growth rate (RGR) and its major physiological components; net assimilation rate (NAR) and leaf area ratio (LAR). The rationale behind this was that yield itself is only an end-product and therefore to seek out and to understand the processes which determine yield will give a better handle on which genotypes to select and by doing this it will be possible to combine positive factors for different aspects of yield.

The validity of genetic differences in growth and its components was pre-established in a growth, development, and physiological analysis conducted on old cultivars representing genetic variation in domesticated tomato cultivars from Europe. The results of this study (**chapter 2**) show that genetic differences and the pattern of change in relative growth rate (RGR)

during plant development are genotype specific. Correlation studies showed that genetic differences in RGR are directly affected by variations in net assimilation rate (NAR) which is a measure for the efficiency of assimilation of photosynthates to biomass. This finding created the possibility for further investigations in order to use genetic variation in NAR as a selection criterion for strong growth.

In **chapter 3**, the objective was to immortalize the genetic variation of the interspecific cross between *L. pennellii* LA716 and Moneymaker. This can be done by creating a backcross inbred line library (BIL-population). A backcross inbred line library is a group of genotypes with single introgressions which gives together a total coverage of the *L. pennellii* LA716 genome. To achieve this several generations of backcross selections have to be genotyped using AFLP molecular markers. BILs were developed following the standard approach as described in Jeuken and Lindhout (2004). The chromosomal locations of *L. pennellii* LA716 introgressions are in reference to the map distances in Haanstra *et al.* (1999). The first backcross inbred lines (BILs) with a single heterozygous introgression of *L. pennellii* LA716 were obtained in the $BC_4$ generation. Two generations of selfings were needed to produce enough seeds of completely homozygous lines with only a single introgression. This line is called immortal because a selfing will generate the identical genotype over and over again. Our set of 12 BILs covered 40 – 45 % of the *L. pennellii* LA716 genome.

The genetic contribution to growth and yield of chromosome segments of *L. pennellii* LA716 in BILs were evaluated using Moneymaker as a control. The results of the growth and yield analyses are also presented in **chapter 3** and **chapter 4**. Because the only difference between BILs and MM is a single introgression it is logical that differences between BILs and Moneymaker were due to the presence of the alien chromosome segment or the absence of the replaced Moneymaker segment.

Growth analyses in **chapter 3** enabled the identification of three BILs giving better growth in greenhouse tomato. BIL1.2, BIL2.3 and BIL8.2 contain homozygous introgressions in chromosomes one, two, and eight respectively. BIL1.2, BIL2.3 and BIL8.2 showed an increase in RGR in the range of 5-3%,

15-4%, and 10-3% respectively. In general, the RGR decreases gradually over time. The faster RGR's observed in BIL1.2, BIL2.3 and BIL8.2 effected end point biomass production with respective increases of 16%, 22% and 3%. Additionally the results of BIL1.2 and BIL8.2 showed that the pattern of change (delta RGR) during plant development plays also a strong role in crop performance. This was a strong confirmation of the finding in chapter 2 which showed that "strong" growth is represented by genotypes which show high initial RGR values and combine it with slow decreases in RGR values over time. We think that lines with relative high values of RGR in the beginning of plant development and which do not have a fast decrease during plant development are the most promising to select for.

Yield analysis in **chapter 4** showed that BIL1.2 and BIL2.3 produced 17-20% respectively 3% more fresh fruits. BILs 1.2, 2.3 and 8.2 produced about 16%, 22% and 3% more biomass (dry matter). Also interesting trends in biomass allocation were observed, such as BIL1.2 which produced 20% more fresh fruits but only 16% more biomass. This was clearly different in BIL2.3 which produced 3% more fresh fruits but an amazing 22% more biomass. Finally BIL8.2 with 0.5% more fresh fruits produced 3% more biomass than Moneymaker. This shows that different BILs have different biomass allocation patterns and prompted us to execute a thorough analysis of biomass allocations to the above plant organs such as fruits, stem and leaves. It was seen that the biomass partitioning patterns were genotype specific and were controlled by either the presence of homozygous introgressions of *L. pennellii* LA716 or the absence of the replaced Moneymaker segment. The results suggested that BIL5.3 and BIL12.2 have a more efficient biomass allocation to the fruits. A tempting calculation shows that BIL2.3 in combination with the introgression of BIL5.3 or BIL 12.2 can give a potential 20-41% yield advantage.

## Samenvatting

De gecultiveerde tomaat heeft een erg smalle genetische basis, wat het veredelen voor betere eigenschappen tot een moeilijke taak maakt. De wilde, voor kruisingen te gebruiken soorten zoals *Lycopersicon pennellii*, *L. peruvianum*, *L. hirsutum*, *L. parviflorum* en *L. cheesmanii* bieden de mogelijkheid om meer variatie in de genenpool van de gecultiveerde tomaat te introduceren. Een voorbeeld van een accessie van een dergelijke wilde soort is *L. pennellii* LA716. Van deze accessie is bekend dat er veel bruikbare genetische informatie in aanwezig is, waaronder genen voor verbetering van de opbrengst. De kruisbaarheid van deze soort met tomaat maakt het een voor de hand liggende kandidaat voor de introductie van factoren die zorgen voor opbrengstverbetering. In dit proefschrift presenteren we een klassieke aanpak voor plantenveredeling, waarbij echter gebruik wordt gemaakt van moderne technologieën. De aanpak bestaat uit het introduceren van genetische variatie uit wilde verwanten en het selecteren op interessante eigenschappen in de hybride nakomelingen. De methode die is gebruikt voor het introduceren van chromosoomsegmenten van *L. pennellii* LA716 zijn kruisingen waarbij *L. pennellii* LA716 als mannelijke ouder is gebruikt, waarna de hybride meerdere keren terug gekruist is met een kastomaat (*L. esculentum* cv. Moneymaker).

In dit proefschrift ligt de focus niet alleen op opbrengst, die gedefinieerd is als de biomassa die is toebedeeld aan de te oogsten vruchten. Opbrengst is ook geanalyseerd vanuit een breder biologisch standpunt; in het bijzonder is gekeken naar de totale bovengronds geproduceerde biomassa gedurende de gehele groeiperiode. Een ander aspect van deze studie was om de groei, ontwikkeling en fysiologische processen die ten grondslag liggen aan de opbrengst te begrijpen. We hebben de relatieve groei ratio (RGR) en zijn belangrijkste fysiologische componenten, netto assimilatie ratio (NAR) en blad oppervlakte ratio (LAR) geanalyseerd. De redenering hierachter is dat de opbrengst zelf slechts een eindproduct is, en dat daarom het uitzoeken en begrijpen van de processen die de opbrengst bepalen een beter handvat zullen zijn voor het selecteren van genotypen, en door dit te doen zal het mogelijk zijn om positieve factoren voor verschillende aspecten van de opbrengst te combineren.

De validiteit van genetische verschillen in groei en zijn componenten was vooraf onderzocht in een groei-, ontwikkelings- en fysiologische analyse op enkele oude rassen. De resultaten van deze studie (**hoofdstuk 2**) laten zien dat genetische verschillen en het patroon van veranderingen in relatieve groei ratio (RGR) tijdens de ontwikkeling van de plant genetisch bepaald zijn. Correlatiestudies wezen uit dat genetische verschillen in RGR direct beïnvloed worden door variaties in netto assimilatie ratio (NAR). Deze vondst creëert de mogelijkheid voor verder onderzoek en de genetische variatie in NAR te gebruiken als selectiecriterium voor sterkere groei.

In **hoofdstuk 3** was het doel om de genetische variatie te fixeren. Dit kan worden gedaan door een “backcross inbred line” bibliotheek (BIL populatie) te maken. Een dergelijke bibliotheek is een groep van genotypen ieder met maar één enkele introgressie, maar die gecombineerd het totale genoom van de donor vertegenwoordigen. Om zulke lijnen te verkrijgen moeten verscheidene generaties van terugkruisingen geanalyseerd worden met behulp van moleculaire markers (bv AFLPs). Er wordt dan geselecteerd op de aanwezigheid van de introgressie en de afwezigheid van verder genetisch materiaal van de wilde verwant. Dergelijke backcross inbred lines (BILs) met maar één enkele heterozygote introgressie werden verkregen in de $BC_4$ generatie. Hierna dient een zelfbestuiving plaats te vinden en zal ¼ van de nakomelingen de introgressie homozygoot hebben. Om genoeg zaden te produceren kan een dergelijke homozygote lijn nogmaals zelfbestoven worden. De door ons gegenereerde set van 12 BILs beslaat 40 – 45% van het genoom van *L. pennellii* LA716.

Acht van de BILs zijn geanalyseerd voor groei en opbrengst en vergeleken met de zaadvaste kastomaat Moneymaker. De resultaten hiervan worden gepresenteerd in **hoofdstuk 3** en **hoofdstuk 4**. Omdat het enige verschil tussen BILs en MM één enkele introgressie is, is het logisch dat verschillen tussen BILs en Moneymaker worden veroorzaakt door de aanwezigheid van het vreemde chromosoomsegment of door de afwezigheid van het vervangen Moneymaker segment.

Groeianalyses in **hoofdstuk 3** maakten de identificatie mogelijk van drie BILs die een betere groei geven. BIL 1.2, BIL 2.3 en BIL 8.2 lieten een toename zien in RGR van respectievelijk 5-3%, 15-4% en 10-3%

respectievelijk. Meestal daalt de RGR geleidelijk in de loop der tijd. Snellere RGR's zoals in BIL 1.2, BIL 2.3 en BIL 8.2 beïnvloeden de uiteindelijke biomassaproductie met toenames van 16%, 22% en 3%. Daarnaast lieten de resultaten van BIL 1.2 en BIL 8.2 zien dat het patroon van verandering gedurende de ontwikkeling van de plant ook een sterke rol speelt in de prestatie van het gewas. Dit bevestigde de vondst uit **hoofdstuk 2** dat "sterke" groei zich voordoet in genotypen die een hoge initiële RGR waarde combineren met een langzame afname van de RGR waardes in de tijd. Lijnen met deze eigenschappen zijn de meest veelbelovende in selectieprogramma's met het doel beter groeiende planten te hebben.

De opbrengstanalyse in **hoofdstuk 4** laat zien dat BIL 1.2 en BIL 2.3 respectievelijk 20% en 3% meer tomaten produceerden en dat de BILs 1.2, 2.3 en 8.2 respectievelijk 16%, 22% en 3% meer biomassa maakten. Ook zijn interessante trends in biomassa allocatie waargenomen; bijvoorbeeld in BIL 1.2, die 20% meer verse vruchten produceerde, maar slechts 16% meer biomassa. Dit was duidelijk anders dan in BIL 2.3, die 3% meer verse vruchten produceerde, maar een verbazingwekkende 22% meer biomassa. Als laatste produceerde BIL 8.2 met 0,5% meer verse vruchten, 3% meer biomassa dan Moneymaker. Dit laat zien dat verschillende BILs verschillende biomassa allocatie patronen hebben. Deze biomassa verdelingspatronen zijn genetisch bepaald en hangen af van de aanwezigheid van bepaalde introgressies. De resultaten tonen dat BIL 5.3 en BIL 12.2 een meer efficiënte biomassa allocatie naar de vruchten hebben. Een verleidelijke berekening toont dat de introgressie van BIL 2.3 in combinatie met de introgressie van BIL 5.3 of BIL 12.2 een potentiële toename in opbrengst van ongeveer 20% kan geven.

## Acknowledgements

It is with an exceptionally overwhelming feeling of satisfaction and gratitude to God that I write this final section of my Ph.D. thesis. If intellectually a Ph.D. represents the elevation of the academic capabilities of an individual to be a certified self-dependent researcher, for me it is also an apotheosis of the hopes and dreams nourished by a whole family for decades, many of whom did not live long enough to witness it happen. And it is with a very painful pinch in my heart that I regret not to have this achievement witnessed by my late father, late mother and most importantly my late brother Michel Philippe Bengono who worked relentlessly in his 42 years of life on earth to help me succeed. Now I can only say thank you.

The most important persons behind the success of this thesis are first of all those who gave me the opportunity to do it and provided their unconditional guidance towards its accomplishment:

- My Promotor Piet Stam was the stabilizing factor each time I faced what seemed to be “no way out situations”. You were always ready to make short term appointments and took the time and patience to teach and explain the things right from the fundamental principles. Your excellent experience in teaching and supervision made things so much easier after our discussions.
- To my Co-promotor Pim; your attentiveness to details, efficiency in working methods, clear comments, suggestions and the continuous insistence to aim higher for every thing we did has been of great help not only for making this thesis, but I will also like to copy these qualities from you. I further appreciate the attention which you paid to difficulties which I encountered in my private life despite the very limited time at your disposal.
- Sjaak, I will like to thank you from the bottom of my heart. This project was turned around for the better the day you stepped into it. Your involvement remains the best thing that happened to me and to this project. I acknowledge your efforts and input for the writing of each and every single one of the chapters in this thesis. Without your patience, criticisms, suggestions, encouragements and certain factors in your personality, lots of things would have been a lot more difficult for me. I am also grateful for

your help in the greenhouse trials. I even remember you helping me pick and weigh fruits during the yield evaluations wearing your regular cloths in sunny afternoons even though you were just passing through the greenhouse by coincidence. This simplicity and humility from you marked me a lot in your personality. I will also like to acknowledge you for the translation of the summary into Dutch.

- I will like to thank Theo who wrote the proposal for which funds were granted to realize this project. Theo you also went through the difficulty of making those repeated phone calls to the immigration police in order to have my work permit granted and supervised me during the early phases of this work before leaving for your appointment as Professor in France. I cannot forget all the efforts you put into this project while you were involved. You also showed your human side to me through the expeditious arrangements you made for me when I had to rush to Cameroon for the funerals of my mother.
- In addition, I will like to thank Dr. Andreas Mordhorst my M.Sc.supervisor who guided me through the first trembling steps of independent research. Your teachings made a part of what I built this thesis on. Thanks also for giving me the chance to experience research in a company set up at Nunhems Zaden.

I will like to thank all the technicians in the laboratory of plant breeding, especially Fien and Petra for their help in the ‘momela’ laboratory; and Marian for a great attitude around the laboratory although we did not work together per see. My gratitude also goes to all the people I collaborated with in UNIFARM. André Maassen and his tomato team composed of Teus van den Brink (my good friend), the Maarten’s Peters and Baan-Hofman always did their best for me. It was also enjoyable to joke with Alex, Pieter, and Bert and to sometimes talk with Gerda, Hanneke and to many others of you. I thank the powerful team assembled by Sjaak to help with the destructive characterization of BILs. I might not remember all the names but I do acknowledge your help. The other person I will like to thank for helping especially with the setting up of the growth analysis experiments as well as the destructive measurements is my very humble Chinese collaborator Zhu

Weimin. Your readiness to help and the way you did it leaves me with no words to thank you. I will also like to acknowledge Ohdja, Irin, Stefano and Junxin; all of them students who worked along at one time or another on this project. My gratitude to Marcos who helped me out several times with the implementation of growth models in Genstat.

To my great colleague and roommate Jaap (Jhappy), I want to thank you for the warmth, support, and a great time in jokes during our regular “coca cola” afternoon breaks (alongside with Marieke and Adillah), and above all, letting us be ourselves. Just being in the same office with you was a positive factor. To my other room mate Marieke, we shared a lot in sincerity, straight forwardness and fun as well. Thanks for the home baked cookies and candies you shared with us from time to time. Other people who made my time great are Ralph (and his family), Asun, Hans, Nelleke, Wole, Adillah, Christian and Luisa. But this does not mean that I enjoyed every other person less.

I extend a special and big thank you to Annie Marchal. I will not even attempt to enumerate how much you helped me during my stay in this department. Things you helped me out with also happen to coincide with the most difficult experiences I have gone through during my entire life. I appreciate you being there at those moments. Through Annie, I will like to thank the entire Laboratory of Plant Breeding.

The road to this achievement has been long, laborious, and punctuated by several stone marks in events and people. I could best express my gratitude by working some of them through time in a chronological order.

To my parents I would like to express my gratitude for making my education such a priority and above all for inculcating in to me the idea that education was the best investment in life. To Michel my late brother I will simply thank you for taking over the responsibility for paying for my school fees from our father. I simply regret that you did not live two little more years longer to witness this promotion. To the rest of my family and friends in Cameroon, I know you upheld me in your prayers and good wishes.

I cannot mention the way to this success with out mentioning my stay in Nigeria and the way it influenced my life. Ayo Odewumi, one of my lecturers in Ahmadu Bello University remains a reference of excellence to me. I thank Louis, Richard, Ivo and Willy for seeing me through all the difficult times and

above all for the warmth and support we brought to one another in the quest to excel to more lucrative grounds.

Life in Holland would not have started so smoothly for me with out the help of many people. I will like to start with the van Schaik family who widely opened their doors for me right away after my arrival in the Netherlands. Their diversified view on the world buffered my adaptation in Holland greatly. Their home remains the only house in the Netherlands where I can invite myself; even for dinner without prior appointment and still be, and feel welcome. My very good friend and classmate Esther van Beek showed me the most pertinent things to know in the Dutch society from my first week in Holland. She remains my best friend today in Holland. Then there is the group of fellow M.Sc. classmates viz, Yuling, Rays, Esther and Pankaj whom I must thank for friendship and solidarity. Dear Pankaj, we particularly felt strong together at several occasions. Thanks for being there at those moments. To Mia Simons my good friend in Nunhem, with your courage, you helped me realize further that no situation can be classified as impossible. I admire you very much and thanks for your friendship. I will also like to thank my family-in law in Friesland for all the experiences they have seen me through during the past six years.

The ultimate step in this thesis was the writing phase which requires absolute concentration. I will like to thank my niece Edwige Bebeme for coming all the way from Cameroon to take care of my sons Piet and Antoine on fulltime scale so that I could totally focus on writing this thesis. I will reward you for ever for what you sacrificed during these last seven months.

And finally to my lovely wife Saskia, we did this journey together and I am happy that you stood by me in these difficult and often uncertain times. Your sacrifice in accepting my absence from the house during several week-ends has finally paid off. I will also like to thank you for meticulous editing and formatting of the final script along side with Antoinette Van Schaik and Oom Bernard. I thank you from my heart for our family and all other things we have achieved together.

To Antoine Willy, papa will talk more now and be less tired. And to Piet Jr., papa will become the “ballenbak” again.

## About the author

Sylvestre Manga Owona (Manga) was born on the 31st of August 1970 (at 8:20 am) in Yaoundé, Cameroon. After primary education in Government Bilingual Primary School Yaoundé, he attended the "Collège Bilingue d'Application de Yaoundé" where he obtained the General Certificate of Education Ordinary Level eight subjects. Later on he proceeded to the Lycée Bilingue de Yaoundé and passed the General Certificate of Education Advanced Level with specialization in Mathematics, Physics, Chemistry, and Biology during the session of June 1991. Manga was awarded a Cameroon Government Scholarship to study pulp and paper engineering in Nigeria. After two weeks of studies he decided to ask the Cameroon High Commission in Nigeria for an authorization for a change of course. He was then admitted to study Microbiology in Ahmadu Bello University, Zaria. The period of these studies coincided with a particularly political troublesome time in Nigeria. During the multiple interruptions of the academic school years, Manga was regularly involved as an assistant in a research project to study the epidemiology of Scab in cowpea debris. Most of the activities involved field work such as designing and laying out field experiments, disease inoculation, scoring lesions, and data processing. After obtaining his B.Sc. (Honors) Microbiology in 1995, he traveled to the South of Nigeria to study cellular and molecular parasitology in the University of Ibadan. He obtained a Master of Science degree (M.Sc.) in Cellular Parasitology in July 1997 and returned to Cameroon to confront a particularly difficult job market. From August 1997 to August 1998, he worked in a medical analysis laboratory (Laboratoire d'Analyses Médicales du Centre) in Yaoundé as senior medical analyst before leaving Cameroon with a scholarship from the Netherlands Organization for Cooperation in Higher Education (nuffic) to study plant and microbial biotechnology in Wageningen. He did his M.Sc. thesis project in Nunhems Zaden on a topic which combined the use of both cellular and molecular biology approaches in plant breeding. After obtaining another M.Sc. in Biotechnology in March 2000, he was appointed for a Ph.D. position in the Laboratory of Plant Breeding at Wageningen University. The outcome of this work is outlined in this thesis.

## The C.T. De Wit Graduate School for Production Ecology and Resource Conservation Ph.D. Education Statement

With the educational activities listed below the Ph.D. candidate; **Sylvestre Manga Owona** has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 22 credits (= 32 ECTS = 22 weeks of activities)

**Review of Literature (4 credits)**

- Biodiversity presents potentials for improving agronomic traits in greenhouse tomato (2000-2004)

**Post-Graduate Courses (4.6 credits)**

- Basic statistics (2000)
- Advanced statistics (2001)
- Critical reflection on science/technology, values & sustainability (2001)
- A future in science: writing a grant proposal (2002)
- Introduction to plant breeding II- genetic variation (2002)

**Deficiency, Refresh, Brush-up and General Courses (4 credits)**

- Basic practicals in plant breeding (2001)
- Selection methods (2001)
- Scientific writing and oral presentations (2001)
- Quantitative methods in plant breeding (2001)

**Ph.D. Discussion Groups (4 credits)**

- Regular presentations and discussions in the multidisciplinary research programme “Rassen onder glas met minder gas” (2000-2005)
- Keygene-AFLP workshop (2002)
- Genetic resources and diversity group (2000-2001)

**PE&RC Annual Meetings, Seminars and Introduction Days (5.5 credits)**

- Genetically modified organisms; benefits and risks, desirable or redundant (2000)
- Ph.D. presentation to the PE&RC panel (2000)
- Food insecurity (2001)
- Ethics in science (2002)
- EPS theme meeting, Lunteren (2002)
- Global climate change & biodiversity (2003)
- Biological disasters (2004)

**International Symposia, Workshops and Conferences (2 credits)**

- Plant Genomic European Meeting, Berlin (2002)
- Plant and Animal Genomic Conference, San Diego (2004)

This work was carried out at Wageningen University, Laboratory of Plant Breeding, P. O. Box 386, 6700 AJ Wageningen, The Netherlands

The research described in this thesis was performed as part of a Dutch research program, entitled “Rassen onder glas met minder gas”, i.e. aiming at breeding more energy-efficient greenhouse crops. This program is financially supported by the Dutch Horticultural Product Board (Productschap Tuinbouw), The Dutch Organisation for Energy and Environment (NOVEM), the Department of Agricultural Research (DLO), The Ministry of Agriculture, Nature and Food Quality (LNV) and several private breeding companies.